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Pharmacokinetics of the Monoclonal Antibody MHAA4549A Administered in Combination With Oseltamivir in Patients Hospitalized With Severe Influenza A Infection

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Abstract

MHAA4549A is a human anti-influenza A monoclonal antibody developed to treat influenza A. We report MHAA4549A serum, nasopharyngeal, and tracheal aspirate pharmacokinetics from a phase 2b study in hospitalized patients with severe influenza A. Patients were randomized 1:1:1 into 3 groups receiving single intravenous doses of 3600 mg (n = 55) or 8400 mg (n = 47) MHAA4549A or placebo (n = 56). Patients also received oral oseltamivir twice daily for ≥5 days. Serum, nasopharyngeal, and tracheal aspirate pharmacokinetic samples were collected on days 1-60 from MHAA4549A-treated groups. Day 5 plasma samples from all groups were collected for assessing the pharmacokinetics of oseltamivir and its active metabolite, oseltamivir carboxylate. Noncompartmental pharmacokinetic analysis was performed using Phoenix WinNonlin. Data were collected during a preplanned interim analysis that became final when the trial terminated because of a lack of efficacy. Serum MHAA4549A concentrations were dose-proportional and biphasic. Mean MHAA4549A clearance was 288-350 mL/day, and mean half-life was 17.8-19.0 days. Nasopharyngeal MHAA4549A concentrations were non-dose-proportional. We detected MHAA4549A in tracheal aspirate samples, but intersubject variability was high. MHAA4549A serum and nasopharyngeal exposures were confirmed in all MHAA4549A-treated patients. Serum MHAA4549A had faster clearance and a shorter half-life in influenza A-infected patients compared with healthy subjects. MHAA4549A detection in tracheal aspirate samples indicated exposure in the lower respiratory tract. Oseltamivir and oseltamivir carboxylate exposures were similar between MHAA4549A-treated and placebo groups, suggesting a lack of MHAA4549A interference with oseltamivir pharmacokinetics.

Keywords

antiviral agents, influenza A virus, MHAA4549A, monoclonal antibody, pharmacokinetics and drug metabolism

Influenza infection is an upper and lower respiratory disease with a broad spectrum of presentations that can result in fever, shortness of breath, pneumonia, respiratory failure, secondary respiratory infections, and even death. Approximately 200 000 to 278 000 patients are hospitalized with severe influenza infections annually in the United States.[1,2] Hospitalization because of severe influenza is associated with high mortality, intensive care unit (ICU) admission,[3] mechanical ventilation support in an ICU,[4] and prolonged hospital stays.[3] During a pandemic season, outcomes may be more serious, with up to 34% of patients requiring ICU care and a mortality rate as high as 15%.[3] During the season from October 1, 2016, to April 30, 2017, 78% (14 185 of 18 184) of laboratory-confirmed, influenza-related hospitalizations in the United States were associated with influenza A infection.[5]

Standard-of-care therapy for patients hospitalized with influenza involves supportive measures and administration of neuraminase inhibitors such as oseltamivir, zanamivir, laninamivir, and peramivir.[6] However, this vulnerable population still experiences a considerable degree of morbidity and mortality, indicating that more effective treatments are needed.[7-9]

MHAA4549A is a human anti-influenza A monoclonal antibody developed to treat patients hospitalized with influenza A infection. It was cloned from a single, human plasmablast cell isolated from an

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influenza-vaccinated donor[10] and binds and neutralizes all human seasonal influenza A strains tested in vitro, including seasonal H1N1, H3N2, and H2N2 isolates across group 1 and group 2.[10,11] MHAA4549A binds to a conserved epitope on the influenza A hemagglutinin stalk and (1) prevents viral replication by inhibiting fusion of the viral envelope with the host endosome and (2) promotes lysis of infected host cells by natural killer cells via antibody-dependent cell-mediated cytotoxicity.[12,13] MHAA4549A demonstrates in vivo efficacy in mouse models of influenza A infection, both alone and synergizing with oseltamivir, likely because the treatments target 2 different steps in the viral life cycle (viral fusion and budding, respectively).[10]

In 2 phase 1 trials in healthy volunteers examining weight-based dosing and fixed dosing, respectively, single intravenous doses of MHAA4549A up to 10 800 mg were well tolerated and demonstrated linear pharmacokinetics (PKs), with a mean serum half-life ($t_{1/2}$) of approximately 21.5 to 23 days, exhibiting a biphasic disposition characterized by an initial rapid distribution phase followed by a slow elimination phase.[14] In healthy subjects challenged with influenza A virus, a phase 2a trial tested 3 single intravenous doses of MHAA4549A (400, 1200, and 3600 mg) and intravenous placebo on day 1.[15,16] Also beginning on day 1, a separate cohort received a standard course of oseltamivir alone along with intravenous placebo. Starting on day 7, all subjects in all cohorts then received a 5-day course of oral oseltamivir administered twice daily. The 3600-mg dose of MHAA4549A reduced viral burden, peak viral load, the duration of viral shedding, and influenza symptoms. MHAA4549A serum PKs was dose proportional, with a serum $t_{1/2}$ of approximately 23 days and a slow clearance (CL) of 152-240 mL/day. However, MHAA4549A exposure in upper respiratory tract (nasopharyngeal) samples increased approximately 10-fold in the 3600-mg group compared with the 1200-mg group, indicating that nasal PKs was not dose proportional. The lack of dose-proportionality may have been because of the saturation of neonatal Fc receptors (FcRn) that mediate antibody transcytosis from the nasal compartment to blood.[17] Patient infection status did not affect MHAA4549A serum or nasopharyngeal exposure, and MHAA4549A treatment did not affect the exposure of oseltamivir and its active metabolite, oseltamivir carboxylate.[16]

To extend these analyses, we examined the PKs of MHAA4549A in the CRANE trial (ClinicalTrials.gov: NCT02293863; EudraCT: #2014-000461-43), a phase 2b, randomized, double-blind, placebo-controlled trial testing MHAA4549A in combination with oseltamivir in patients hospitalized with severe influenza A infection.[18] The current work was part of a protocol-specified interim analysis that became final once the trial was terminated for lack of efficacy. Our objective was to further characterize MHAA4549A serum PK, as well as PKs in both the upper (nasopharyngeal) and lower (tracheal aspirate) respiratory tract and to assess the effect of MHAA4549A on the PKs of oseltamivir and its active metabolite, oseltamivir carboxylate, in these patients.

Methods

The institutional review board/ethics committee for each site approved the protocol for the CRANE trial (ClinicalTrials.gov: NCT02293863; EudraCT #2014-000461-43) before initiation of the study. All patients or their legally authorized representatives provided written informed consent. A list of the study sites is provided in the Supplemental Material. The CRANE trial was conducted in full conformity with the International Council for Harmonisation (ICH) E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki or the laws and regulations of the country in which the research was conducted, whichever afforded the greater protection to the individual. The study complied with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the European Union (EU) or European Economic Area complied with the EU Clinical Trial Directive (2001/20/EC).

Study Design and Patients

The CRANE study was a randomized, double-blind, placebo-controlled phase 2b trial of patients hospitalized with influenza A.[18] Patient eligibility in multiple global, clinical sites was determined by a diagnosis of influenza A infection by local influenza antigen test or polymerase chain reaction assay and a requirement for either supplemental oxygen to maintain oxygen saturation > 92%, as measured by pulse oximetry (SpO_2), or a requirement for positive pressure ventilation within 24 hours of hospital admission. Eligible patients also included those with confirmed or suspected bacterial pneumonia and coexisting medical conditions, except for those with a history of chronic lung disease with a documented oxygen saturation (SpO_2) < 95% off oxygen or significant immunosuppression. If patients had received 3 or more days of anti-influenza therapy, had been admitted to the hospital more than 48 hours before study treatment, or had exhibited influenza symptoms for more than 5 days before study treatment, they were excluded.

Eligible patients were randomized in a ratio of 1:1:1 into 3 treatment groups (Figure 1) that received a single intravenous dose of placebo (n = 56), 3600 mg MHAA4549A (n = 55), or 8400 mg MHAA4549A

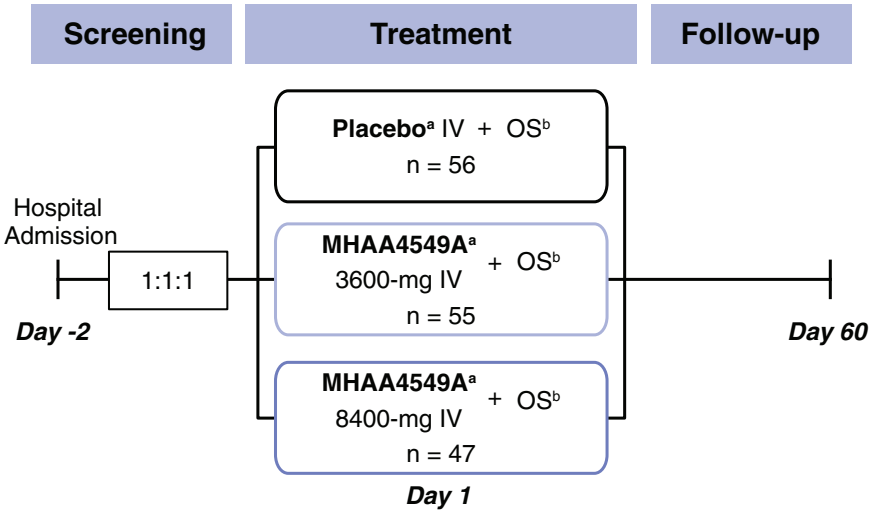

Figure 1. Study design. Patients were randomized 1:1:1 into the 3 treatment groups. [a]MHAA4549A infusions were administered on day 1 within 48 hours of hospital admission. [b]Oseltamivir was administered for ≥5 days (treatment for longer than 5 days and up to 10 days was based on investigator discretion) and no later than 12 hours after completion of MHAA4549A infusion. Patients were followed through day 60. IV, intravenous; OS, oseltamivir.

(n = 47) on day 1. The study drug was diluted in 0.9% normal saline and administered by intravenous infusion for approximately 120 minutes. Within 48 hours of hospital admission, patients began study drug infusion, and oral doses of oseltamivir (OS) of 75 or 150 mg twice daily per investigator discretion were planned for a minimum of 5 days starting on day 1 and beginning no later than 12 hours after completion of study drug administration to establish a consistent treatment duration. Some patients received protocol-allowed adjustments to lower doses of oseltamivir based on renal function per local standard-of-care practice or the local package insert. The follow-up period was defined as 60 days from the time of study drug administration.

The primary end point was the median time to the normalization of respiratory function (time to removal of supplemental oxygen support and maintenance of stable oxygen saturation ($SpO_2 \geq 95\%$). Pharmacokinetic (PK) outcomes included measurements of MHAA4549A PK parameters in serum and in nasopharyngeal and tracheal aspirate samples. In addition, we assessed the effect of MHAA4549A on oseltamivir and oseltamivir carboxylate PKs. MHAA4549A immunogenicity was also assessed.

PK and Antidrug Antibody Sample Collection and Assays

To determine MHAA4549A PKs, serum, nasopharyngeal, and tracheal aspirate samples were collected on days 1-60 from all groups to maintain blinding, but only those from MHAA4549A-treated groups were analyzed. Serum samples were collected on day 1 (30 minutes before MHAA4549A infusion and 60 minutes postdose), day 2, day 3, day 5, day 7, day 14, day 30, and day 60 (at study completion or early discontinuation), and at hospital discharge. Serum MHAA4549A concentrations were measured using a validated enzyme-linked immunosorbent assay (ELISA); lower limit of quantification (LLOQ) = 0.25 µg/mL.[14]

Nasopharyngeal and tracheal aspirate samples were collected on day 1 (immediately before MHAA4549A infusion and oseltamivir dosing), days 2-10 (before oseltamivir dosing if oseltamivir was given), day 14, day 20, day 25, day 30, and day 60 (at study completion or early discontinuation), and at hospital discharge. Discharge day assessments were used instead of the assessments for the corresponding day except for the study completion/early termination visit. Nasopharyngeal samples were collected from both nostrils from all patients and measured MHAA4549A concentrations were normalized to urea concentrations, as described in Deng et al.[16] In addition to upper respiratory tract samples, lower respiratory tract samples were collected as tracheal aspirates in patients who were intubated; measured MHAA4549A concentrations in tracheal aspirate samples were similarly normalized to urea concentrations. In the event of extubation, collection of nasopharyngeal samples continued. Nasopharyngeal MHAA4549A concentrations were measured using a qualified ELISA (LLOQ = 320 pg/mL).[16] Tracheal aspirate MHAA4549A concentrations were measured using a qualified ELISA (LLOQ = 25.0 ng/mL).

For PK assessment of oseltamivir and oseltamivir carboxylate, only plasma samples from all groups were collected on day 1 and day 5 immediately before oseltamivir dosing. Plasma oseltamivir and oseltamivir carboxylate concentrations were quantified using a validated liquid chromatography/mass spectrometry

assay (oseltamivir LLOQ = 1 ng/mL; oseltamivir carboxylate LLOQ = 10 ng/mL).[19]

Serum samples to assess the presence of antidrug antibodies (ADAs) were collected on day 1 immediately before infusion and oseltamivir dosing, day 30, at hospital discharge, and on day 60 study completion or early discontinuation while hospitalized, and at any unscheduled visit. ADAs were detected with a validated MHAA4549A-specific bridging ELISA, using a 2-tier testing approach with screening and confirmatory assays as previously described.[14]

PK Data Analysis

PK assessments of the data available for all cohorts were performed using serum, nasopharyngeal, and tracheal aspirate concentration-time data, employing standard noncompartmental analysis (NCA) with Phoenix WinNonlin software, version 6.2 (Certara, L.P., Princeton, New Jersey). Actual sampling times were used. Predose data below the lower limit of quantification (BLQ) were set to zero. The one-third imputation rule was applied to postdose BLQ data: for a given treatment and nominal time, if more than one-third of the values were BLQ, then all concentrations for that treatment and nominal time were counted as missing. Dose-normalized concentration-time profiles were also provided for dose-proportionality assessments. The following serum PK parameters were estimated: total drug exposure defined as area under the serum concentration-time curve extrapolated to infinity ($AUC_{0\text{-inf}}$), calculated using the linear-up, log-down trapezoidal rule, clearance (CL), volume of distribution at steady state ($V_{d(ss)}$), observed maximum serum concentration (C_{max}), and terminal half-life ($t_{1/2}$). The following nasopharyngeal PK parameters were estimated: $AUC_{0\text{-inf}}$, $V_{d(ss)}$, C_{max}, time of C_{max} (T_{max}), and $t_{1/2}$. Only observed C_{max} and T_{max} were reported from the NCA for tracheal aspirate PK parameters. Statistical summaries of plasma oseltamivir and oseltamivir carboxylate concentrations on day 5 were provided.

The observed nasopharyngeal C_{max} was compared with a model-predicted nasopharyngeal C_{max} for the 3600- and 8400-mg groups. The Monte Carlo simulation (n = 1000) was conducted based on a semiquantitative population PK model, as reported by Patel et al,[20] using NONMEM software (version 7.2, ICON Development Solutions, Ellicott City, Maryland). Briefly, a 2-compartment PK model adequately described serum PKs; a third compartment was used to describe MHAA4549A serum-to-nasal equilibrium with a linear distribution from the central compartment to the nasal compartment, while using a saturable process for nasal to central distribution.

Immunogenicity analysis was performed as previously described.[14] Briefly, immunogenicity analysis included patients with at least 1 ADA sample collected at baseline and 1 postdose ADA sample. If patients were ADA-negative at baseline and developed an ADA response after study drug administration or if they were ADA-positive at baseline and had a postbaseline titer at least 4-fold greater than the baseline titer, they were considered ADA-positive. If patients were ADA-negative at baseline and had no ADA-positive postbaseline samples, they were considered ADA-negative. Patients were also considered ADA-negative if they were ADA-positive at baseline and had no postbaseline samples with titers at least 4-fold greater than their baseline titer (treatment-unaffected ADA).

Results

Patient Disposition

One hundred sixty-six patients were randomized into this study (Figure S1). Patients who were 65 years or older comprised 42.4% (n = 67) of the population, 92% (n = 146) had at least one comorbidity, and 50% (n = 79) had confirmed or suspected bacterial pneumonia at baseline.[18] Fifty-six patients received placebo plus oseltamivir (placebo + OS), with 9 patients discontinuing the study. In the 3600-mg MHAA4549A + OS group, 55 received study drug and 13 discontinued the study. In the 8400-mg MHAA4549A + OS group, 47 received study drug and 9 discontinued the study. Among these patients, 22 received 150 mg oral oseltamivir (placebo, n = 10; 3600 mg MHAA4549A + OS, n = 9; 8400 mg MHAA4549A + OS, n = 3), and because of renal impairment, some patients received either 10 mg oseltamivir (placebo, n = 1) or 30 mg oseltamivir (placebo, n = 5; 3600 mg MHAA4549A + OS, n = 4; 8400 mg MHAA4549A+OS, n = 5). The remaining patients received 75 mg oseltamivir. Because the results of this interim analysis demonstrated a lack of MHAA4549A efficacy, the trial was terminated.

MHAA4549A PKs

Serum MHAA4549A concentrations in patients with severe influenza were dose-proportional and biphasic (Figure 2A and Figure S2), demonstrating an initial rapid distribution phase followed by a slow elimination phase. We confirmed serum exposure in all patients receiving MHAA4549A. The mean clearance (CL) across the 2 treatment groups ranged from 288 to 350 mL/day, with a mean elimination half-life ($t_{1/2}$) ranging from 17.8 to 19.0 days (Table 1).

Because serum PKs of MHAA4549A does not necessarily reflect the conditions within the respiratory tract, we first determined MHAA4549A concentrations in the upper respiratory tract by measuring MHAA4549A in nasopharyngeal swab samples. Nasopharyngeal MHAA4549A concentrations were non-dose-proportional, with the mean maximum

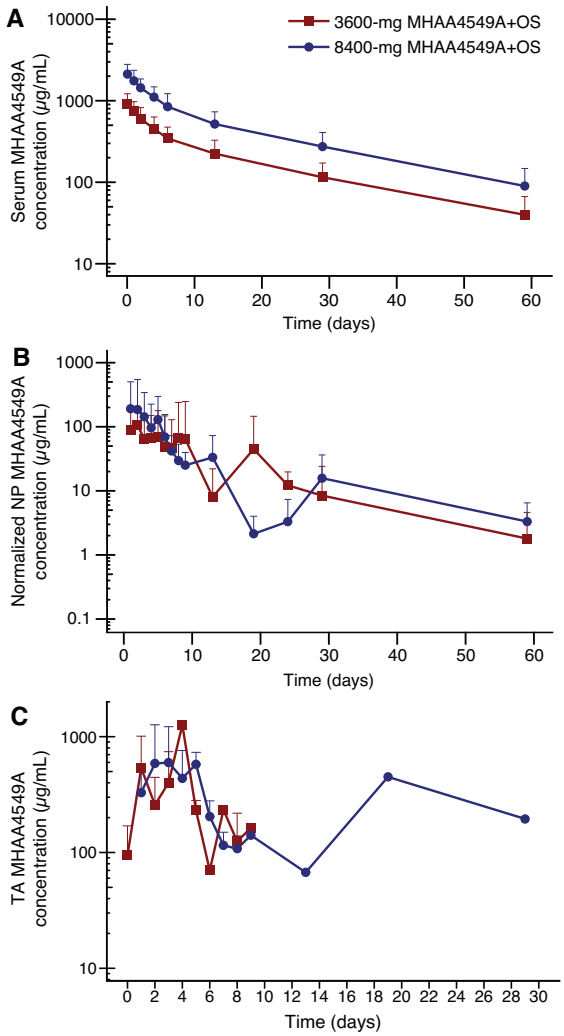

Figure 2. MHAA4549A (A) serum, (B) nasopharyngeal, and (C) tracheal aspirate concentration-time profiles. Data are mean + SD. Nasopharyngeal and tracheal aspirate concentrations were normalized to urea concentrations as previously described.[14] NP, nasopharyngeal; SD, standard deviation; TA, tracheal aspirate..

observed concentration (C_{max}) ranging from 132 to 281 μg/mL (Table 2, Figure 2B). The mean T_{max} ranged from 2.72 to 4.70 days, and the mean $t_{1/2}$ ranged from 14.0 to 24.1 days.

We also examined MHAA4549A concentrations in tracheal aspirates to investigate MHAA4549A PKs in the lower respiratory tract. Tracheal aspirate samples were collected from patients who were intubated (n = 8 for the 3600-mg group; n = 7 for the 8400-mg group). MHAA4549A concentrations in tracheal aspirates had high intersubject variability (Figure 2C), with a C_{max} (mean ± SD) of 542 ± 528 and 581 ± 427 μg/mL for the 3600- and 8400-mg groups, respectively. T_{max} (mean ± SD) was 3.13 ± 2.70 and 3.57 ± 1.51 days for the 3600- and 8400-mg groups, respectively.

Oseltamivir PKs

To investigate the effect of MHAA4549A on the PKs of both oseltamivir and oseltamivir carboxylate in patients with severe influenza A, we analyzed oseltamivir and oseltamivir carboxylate concentrations only in plasma samples from both placebo- and MHAA4549A-treated patients. Because of the small number of samples from patients who received 150 mg oseltamivir, we only report results from patients who had received 75 mg oseltamivir twice daily. After at least 5 days of dosing with oseltamivir, oseltamivir concentrations on day 5 ranged from BLQ to 47.5 ng/mL and BLQ to 22.3 ng/mL (Figure 3A), and oseltamivir carboxylate plasma concentrations on day 5 ranged from 82.5 to 3040 ng/mL and from BLQ to 2570 ng/mL for the 3600- and 8400-mg groups, respectively (Figure 3B). Oseltamivir and oseltamivir carboxylate concentrations in the MHAA4549A-treated groups were comparable to the placebo groups, indicating that MHAA4549A did not affect oseltamivir PKs (Figure 3).

Immunogenicity

Two of 158 patients tested positive for ADAs at baseline, resulting in an ADA prevalence of 1.3%. Of the 127 postbaseline evaluable patients in the study, 1 patient in the 3600-mg MHAA4549A + OS group had treatment-unaffected ADA. This patient had an MHAA4549A PK profile that was comparable to other patients who were ADA-negative (data not shown).

Table 1. Summary of Serum MHAA4549A PK Parameters by Treatment (PK-Evaluable Subjects)

	3600 mg MHAA4549A + OS		8400 mg MHAA4549A + OS	
MHAA4549A PK parameters	n	Mean (SD)	n	Mean (SD)
Terminal $t_{1/2}$ (days)	38	19.0 (4.91)	31	17.8 (3.88)
C_{max} (μg/mL)	55	916 (294)	46	2220 (556)
$AUC_{0\text{-inf}}$ (μg·day/mL)	38	11 400 (4530)	31	26 700 (9810)
CL_{obs} (mL/day)	38	288 (158)	31	350 (130)
$V_{d(ss)\_obs}$ (mL)	38	6410 (3170)	31	7450 (2270)

OS, oseltamivir; SD, standard deviation; terminal $t_{1/2}$, elimination half-life; C_{max}, maximum observed serum concentration; $AUC_{0\text{-inf}}$, area under the concentration-time curve from time 0 to infinity; CL_{obs}, observed clearance; $V_{d(ss)\_obs}$, observed steady-state volume of distribution.
PK parameters were not estimated for some patients because of an insufficient number of samples collected.

Table 2. Summary of Nasopharyngeal MHAA4549A PK Parameters by Treatment (PK-Evaluable Subjects)

	3600 mg MHAA4549A + OS		8400 mg MHAA4549A + OS	
MHAA4549A PK parameters	n	Mean (SD)	n	Mean (SD)
Terminal $t_{1/2}$ (days)	26	14.0 (8.18)	22	24.1 (29.1)
C_{max} (μg/mL)	53	132 (148)	45	281 (399)
T_{max} (days)	53	4.70 (6.26)	45	2.72 (2.19)
$AUC_{0\text{-}inf}$ (μg· day/mL)	26	570 (444)	22	1850 (1600)

OS, oseltamivir; SD, standard deviation; terminal $t_{1/2}$, elimination half-life; C_{max}, maximum observed serum concentration; T_{max}, time of C_{max}; $AUC_{0\text{-}inf}$, area under the concentration-time curve from time 0 to infinity.
PK parameters were not estimated for some patients because of an insufficient number of samples collected.

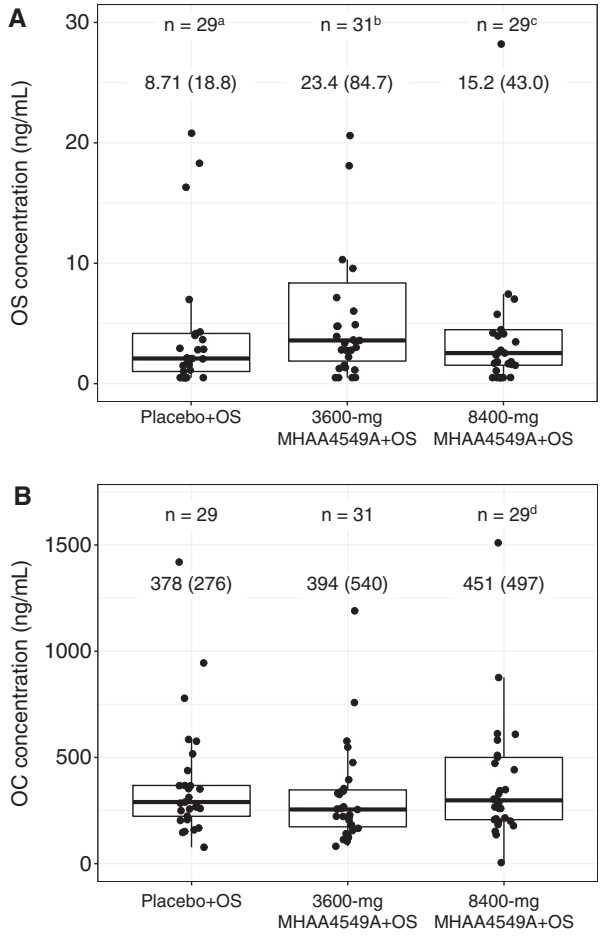

Figure 3. Plasma concentrations of (A) oseltamivir and (B) oseltamivir carboxylate in patients who received 75 mg oseltamivir twice daily and had day 5 samples. All groups received oral oseltamivir. Because of limited data, day 5 plasma concentrations of oseltamivir and oseltamivir carboxylate were not summarized for individual patients who received 150 mg oseltamivir twice daily. The number of BLQ values included in the analysis were (A) [a]n = 7, [b]n = 4, [c]n = 6, and (B) [d]n = 1; the 1/3 imputation rule was applied to postdose BLQ data for PK analysis. The number of patients and the mean (SD) values for each cohort are indicated above each box plot; median and 25th and 75th percentiles are represented by the middle line and boundaries of each box; the upper whisker extends from hinge to highest value within 1.5 times the IQR of hinge; the lower whisker extends from hinge to lowest value within 1.5 times the IQR of hinge. BLQ, below the limit of quantification; IQR, interquartile range; OS, oseltamivir; OC, oseltamivir carboxylate; SD standard deviation.

Discussion

The current study investigated the PKs of the anti-influenza A antibody MHAA4549A in serum and in the upper and lower respiratory tracts in patients hospitalized with influenza A infection. Single intravenous doses of 3600 and 8400 mg were selected to assess the safety, efficacy, and PKs of MHAA4549A and to provide data for further clinical development. Our analysis demonstrated MHAA4549A exposure in all these sites, and furthermore, we found that oseltamivir and oseltamivir carboxylate exposure in MHAA4549A treatment groups was comparable to the placebo group, confirming our prior observations from the phase 2a challenge study that MHAA4549A does not affect oseltamivir PKs.[15,16] Although 2 patients tested positive for ADAs at baseline, we did not observe MHAA4549A-induced or MHAA4549A-enhanced ADAs in MHAA4549A-treated patients, but it is possible that a high concentration of MHAA4549A in a given ADA sample may have prevented detection of ADAs in the assay. The vast majority of the patients in the study, however, had at least 1 sample tested for ADAs in which the measured drug concentration was not expected to prevent ADA detection.

The selection of dose in this study was based on nonclinical efficacy data from in vivo mouse influenza A infection models that identified the target serum concentration (C_{max} = 345 μg/mL).[21] PKs in clinical studies and the relationship between the PKs, pharmacodynamics, and efficacy observed in the phase 2a challenge study in influenza A also contributed to dose selection.[14-16,21] However, the predictive translatability of data from preclinical studies and the phase 2a challenge model is still poorly understood for a monoclonal antibody and created challenges for dose selection in the MHAA4549A phase 2b trial. The 3600-mg dose was based on the phase 2a challenge study, which demonstrated both a significant decrease in viral shedding in the upper respiratory tract and a decrease in the AUC of symptom scores in patients who received the 3600-mg dose of MHAA4549A compared with patients who received placebo.[15] In that study, an

exploratory exposure-response analysis of patients receiving 3600 mg MHAA4549A demonstrated that subjects with nasopharyngeal MHAA4549A C_{max} greater than the median C_{max} had shorter times to resolution of viral shedding compared with the placebo group (median, 75.8 versus 113.7 hours), whereas subjects with MHAA4549A C_{max} less than the median C_{max} had times to resolution of viral shedding similar to the placebo group (median, 112.1 versus 113.7 hours). Thus, higher doses of MHAA4549A were expected to be more efficacious. We selected the 8400-mg dose because patients hospitalized with influenza infection likely have high viral loads and longer durations of viral shedding and would therefore require increased doses of MHAA4549A. Simulations from a semiquantitative PK model developed from the phase 2a challenge study suggested that 8400 mg was the minimum dose that would show a separation of nasopharyngeal exposure from a dose of 3600 mg.[16] Both the 3600- and 8400-mg doses of MHAA4549A were expected to be safe based on the previous phase 1 and phase 2a clinical studies[14,15]; in the current work, adverse events were generally balanced across treatment groups,[18] supporting the previous results. Furthermore, the current doses of 3600 and 8600 mg surpassed the serum target concentration derived from the preclinical study.[21]

MHAA4549A + OS treatment in the CRANE trial did not meet the primary end point of reducing the time to normalization of respiratory function compared with placebo + OS, nor were there significant differences between the 2 MHAA4549A doses. MHAA4549A + OS also showed no statistically significant improvement over placebo + OS in various secondary end points, such as the time to ICU discharge, time to hospital discharge, and 30-day mortality. In addition, MHAA4549A did not reduce the duration of viral shedding, the viral AUC, or the peak viral load in nasopharyngeal samples beyond the antiviral activity of oseltamivir.[18] This lack of significant differences held true regardless of whether virology measures were compared $\leq$ 48 versus $>$ 48 hours from the time of symptom onset. Attempts were made to collect daily tracheal aspirate samples in intubated patients for assessment of infectious titers. The data were severely limited because of sampling and technical challenges; thus, we are unable to make any conclusive statements on the activity of MHAA4549A. For inflammatory cytokines in the serum and other compartments, we did not see any differences between treatment groups (J. M. McBride, manuscript in preparation).

Several factors may explain the discrepancy in efficacy between the phase 2a data healthy volunteer challenge study and this trial. For instance, naturally occurring viral strains are more virulent than the inoculation strain, and the route of inoculation may not reflect routes of natural exposure. Older patients with severe influenza also often have comorbid conditions and a lesser ability to fend off infection compared with healthy younger people. Although the phase 2a study controlled the interval from infection to treatment at 24-36 hours to directly compare efficacy to oseltamivir within the 48-hour window,[15] the phase 2b study aimed to assess MHAA4549A efficacy both within and beyond this 48-hour time frame. In this study, MHAA4549A may have been administered too late after symptom onset, even though the treatment window was similar to that used for neuraminidase inhibitor treatment[22] and the phase 3 zanamivir study.[23] However, we observed no clear differences in efficacy or virology between patients who received MHAA4549A earlier versus later than 48 hours after symptom onset[18] (J. M. McBride et al, manuscript in preparation).

Despite the lack of efficacy, MHAA4549A serum and nasopharyngeal exposures were confirmed in all MHAA4549A-treated patients. MHAA4549A has a shorter serum $t_{1/2}$ (17.8-19.0 days) and a faster serum CL ($\sim$288-350 mL/day) in patients hospitalized with influenza A compared with healthy subjects in the phase 1 trial and subjects challenged with influenza virus ($t_{1/2}$, 21.9-24.6 days; CL, $\sim$152-240 mL/day).[14-16] These results could be because of the faster intrinsic catabolism of IgG in patients with severe influenza infection, a hypothesis supported by studies suggesting that disease status can affect monoclonal antibody PKs. For example, trastuzumab, bevacizumab, and pertuzumab have lower exposure and faster CL in patients with gastric cancer;[24-26] similarly, in patients with psoriasis, diabetic comorbidity led to a 28.7% higher CL/F of ustekinumab.[27] Of note, phase 2b patients exhibited higher variability in CL compared with healthy subjects in the phase 1 and phase 2a studies. This higher variability could be because of the lesser homogeneity of this patient population compared with healthy subjects. Population PK modeling with covariate analysis with disease as a covariate on CL could be used to further understand this difference. MHAA4549A nasopharyngeal exposure was less than 10% of serum concentrations, and the observed MHAA4549A nasopharyngeal C_{max} agrees with the simulated nasopharyngeal C_{max} from a semiquantitative PK model developed from the phase 2a challenge study (Figure S3).

There is limited literature on drug concentrations in the lower respiratory tract, especially for monoclonal antibodies. One of the few examples is KB001, a PEGylated, recombinant anti-*Pseudomonas aeruginosa* PcrV antibody fragment (Fab′), which was rapidly (as early as day 1) and consistently detected for up

to 28 days in pulmonary secretions obtained from infected, antibody-treated cystic fibrosis patients, with endotracheal aspirate/serum concentration ratios of 8% to 9%.[28] Although removal of intubation and limited sampling restricted the number of patient samples, our study is the first to report monoclonal antibody concentrations in tracheal aspirates. We found that the between-subject variability for MHAA4549A concentrations in tracheal aspirate samples was large, likely because of inherent technical challenges in the uniformity of sample collection, as well as the wide heterogeneity of tracheal aspirate samples. In particular, the volume and visually observed physical properties of tracheal aspirate samples varied considerably, leading to challenges in obtaining a fully homogenized sample, which was needed for placement in immunoassay plates in the PK ELISA procedure. Physiologically based PK (PBPK) or mini-PBPK modeling could provide another approach to providing additional insight into monoclonal antibody distribution at the site of action, including the respiratory tract.

Serum drug exposure does not necessarily reflect exposure at the site of action. To identify the optimal target exposure for eliciting a desired pharmacological response, it is critical to determine the amount and time course of drug exposure at the site of action. Although using nasopharyngeal samples for determining MHAA4549A concentration in the upper respiratory tract provided key information for dose selection, the lower respiratory tract is an important reservoir of influenza virus that drives severe clinical infection. While the sample size was limited (n = 15 subjects with tracheal aspirate samples), our tracheal aspirate exposure data confirmed that MHAA4549A reached the lower respiratory tract with a median ratio of tracheal aspirate concentration to serum concentration of 0.4. In these patients, MHAA4549A mean C_{max} was 2- to 4-fold higher in tracheal aspirate samples than in nasopharyngeal samples. We estimated that the ratio of MHAA4549A to viral load in tracheal aspirate was approximately 150, while assuming a tracheal aspirate viral load of 10 $\log_{10}$ viral particles (vp)/mL (based on viral load measurements in hospitalized patients[29]), with 1500 binding sites per viral particle (L. Swem, personal communication). These data suggest that MHAA4549A concentrations in the lower respiratory tract well exceeded the virus concentration and that the lack of efficacy was unlikely because of inadequate dosing.

Conclusions

In this study, treatment with MHAA4549A in combination with oseltamivir did not provide clinical benefit over oseltamivir in patients hospitalized with severe influenza A infection. However, we detected MHAA4549A in both the upper and lower respiratory tracts after intravenous infusion, reducing the concern that efficacy failure was from lack of drug exposure at the desired site of activity. We also confirmed that MHAA4549A treatment did not affect the PKs of oseltamivir or oseltamivir carboxylate. More work is needed to further understand the mechanisms underlying the faster serum CL of MHAA4549A in this patient population.

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Conflicts of Interest

All authors were full-time employees of Genentech, Inc., a member of the Roche group, at the time this work was performed and owned Roche stock and/or options during the time of their employment. Aide Castro is currently an employee of Calico Life Sciences, LLC. William D. Hanley is currently an employee of Seattle Genetics and no longer holds Roche stock and/or options.

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Data Availability

Qualified researchers may request access to individual patient-level data through the Vivli Center for Global Clinical Research Data (https://vivli.org/). Further details on Roche's criteria for eligible studies are available at https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Roche.aspx. For further details on Roche's data-sharing policy and the requesting of access to related clinical study documents, see https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm.

Author Contributions

Study conceptualization: R.D., J.J.L., M.C.P., J.M.M., E.M.N., J.A.T. Formal analysis: R.D., G.S., M.M., J.M.M., P.K. Funding acquisition: E.M.N., J.A.T. Investigation: R.D., M.M., J.J.L., M.C.P., P.K., P.H., A.C., E.M.N., J.A.T. Methodology: R.D., G.S., M.M., J.J.L., M.C.P., J.M.M., P.K., P.H., A.C., E.M.N., J.A.T., W.D.H. Project administration: P.H., A.C., E.M.N. Resources: E.M.N., J.A.T., W.D.H.

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