

A Phase 2 Randomized, Double-Blind, Placebo-Controlled Trial of the Monoclonal Antibody MHAA4549A in Patients With Acute Uncomplicated Influenza A Infection

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Background. MHAA4549A, a human monoclonal antibody targeting the influenza A hemagglutinin stalk, neutralizes influenza A virus in animal and human volunteer challenge studies. We investigated the safety and tolerability, efficacy, and pharmacokinetics of MHAA4549A in outpatients with acute, uncomplicated influenza A infection.

Methods. This was a phase 2, randomized, double-blind, placebo-controlled trial of single intravenous (IV) doses of 3600 mg or 8400 mg of MHAA4549A or IV placebo in adult outpatients testing positive for influenza A. Patients were enrolled across 35 sites in 6 countries. Randomization and dosing occurred within ≤ 72 hours of symptom onset; the study duration was 14 weeks. The primary end point was the nature and frequency of adverse events (AEs). Secondary end points included median time to alleviation of all influenza symptoms, effects on nasopharyngeal viral load and duration of viral shedding, and MHAA4549A serum pharmacokinetics.

Results. Of 125 randomized patients, 124 received study treatment, with 99 confirmed positive for influenza A by central testing. The frequency of AEs between the MHAA4549A and placebo groups was similar; nausea was most common (8 patients; 6.5%). MHAA4549A serum exposure was confirmed in all MHAA4549A-treated patients and was dose-proportional. No hospitalizations or deaths occurred. Between the MHAA4549A and placebo groups, no statistically significant differences occurred in the median time to alleviation of all symptoms, nasopharyngeal viral load, or duration of viral shedding.

Conclusions. While MHAA4549A was safe and well tolerated with confirmed exposure, the antibody did not improve clinical outcomes in patients with acute uncomplicated influenza A infection.

Keywords. antiviral agents; influenza A virus; MHAA4549A; monoclonal antibody.

Seasonal influenza is a highly contagious airborne disease caused by influenza A and B viruses. Influenza can cause a range of clinical outcomes from self-limited upper respiratory tract infection to severe disease with life-threatening complications, including respiratory failure. Particularly vulnerable populations include children, the elderly, pregnant and postpartum women, people with chronic medical conditions, and those who are immunosuppressed.

The mainstay of treatment in influenza-infected patients is neuraminidase inhibitors (NAIs) that act against influenza A and B subtypes [1, 2]. If given within the first 36–48 hours of symptom onset in patients with uncomplicated

laboratory-confirmed influenza, NAIs reduce both the time to alleviation of symptoms by ~ 1 –2 days and the risk of hospitalization [3–5]. However, influenza resistance to NAIs remains a concern [6, 7], especially in immunocompromised individuals due to prolonged viral shedding [8]. Even with a seasonal influenza vaccine and NAIs, there were 37.4–42.9 million cases of influenza, 531 000–647 000 influenza-related hospitalizations, and 36 400–61 200 influenza-related deaths in the United States during the 2018–2019 influenza season [9]. Globally, seasonal influenza results in ~ 3 –5 million cases of severe illness and up to 650 000 deaths each year [10, 11]. Thus, a significant unmet need exists for developing novel antiviral agents for use with NAIs in patients with severe influenza.

MHAA4549A is a human monoclonal immunoglobulin G1 (IgG1) antibody that binds conserved epitopes in the HA stalk of human influenza A viruses and neutralizes all tested seasonal human influenza A strains [12]. MHAA4549A has 2 complementary mechanisms of action: (1) it directly neutralizes influenza virus by binding to the HA stalk, preventing HA maturation and blocking HA-mediated membrane fusion in the endosome and release of influenza particles, and (2) it binds

Received 16 August 2021; editorial decision 6 December 2021; accepted 9 December 2021; published online 10 December 2021.

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Open Forum Infectious Diseases® 2022

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HA on the surface of infected cells and recruits immune cells to lyse the virus-infected cells through antibody-dependent cellular cytotoxicity (ADCC) [13, 14]. MHAA4549A was well tolerated in 2 phase 1 studies and in a phase 2a nasal influenza challenge study [15, 16].

Preclinical studies have raised the possibility that monoclonal anti-HA stalk antibodies may promote antibody-dependent enhancement (ADE) of infection [17–19]. Specifically, non-neutralizing antiviral antibodies may facilitate virus entry into host cells, causing enhanced viral replication, shedding, and potentially more severe clinical symptoms [17, 18, 20–23]. However, the phase 2a intranasal challenge study showed none of these effects in subjects receiving MHAA4549A vs placebo [16]. To date, there is no clinical evidence of ADE with anti-HA monoclonal antibodies with similar mechanisms to MHAA4549A [24, 25].

This phase 2 study (NIGHTHAWK) assessed the safety and tolerability of MHAA4549A in otherwise healthy outpatients with naturally occurring influenza A infection. We also explored the effects of MHAA4549A on time to resolution of influenza symptoms and viral load in the upper respiratory tract.

METHODS

Study Design

This was a randomized, double-blind, placebo-controlled study to assess the safety and tolerability of single IV doses of MHAA4549A (3600 or 8400 mg) compared with placebo for treatment of acute uncomplicated seasonal influenza A infection in otherwise healthy adults in an outpatient setting. The 3600 mg MHAA4549A dose was selected based on the phase 2a challenge study, which demonstrated significant decreases in virology and symptom end points [16, 26]. The 8400 mg dose was selected to mirror the dose used in a trial in hospitalized patients [27] who are likely to have higher viral loads and longer durations of viral shedding and therefore would need higher doses of MHAA4549A [28, 29].

The study was conducted at 35 investigational sites in 6 countries. Study duration was ~14 weeks; randomization and study drug dosing occurred ≤ 72 hours (3 days) from the onset of influenza-like illness (as determined by the investigator). Clinic visits were conducted on days 3, 5, and 7; telephone follow-ups occurred on days 2, 14, 30, and 100. The end of the study was defined as the first day when all patients had a study completion visit, early termination visit, or had otherwise been discontinued from the study.

Patient Consent

This study was conducted in full conformance with the International Council for Harmonization (ICH) E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki or the laws and regulations of the country where the

research was conducted, whichever afforded the greater protection to the individual. The study complied with ICH E2A guidelines, US Food and Drug Administration regulations, applicable local, state, and federal laws, and the EU Clinical Trial Directive (2001/20/EC). All patients provided written informed consent. Clinical trial registries: ClinicalTrials.gov: NCT02623322; EudraCT 2016-000425-40.

Participants

Enrolled participants were otherwise healthy adults, 18–65 years old, who tested positive for influenza A infection using a rapid polymerase chain reaction (PCR) test or rapid antigen test, had ≤ 72 hours (3 days) between onset of influenza-like illness and start of study treatment, and had clinical evidence of influenza infection during screening: at least 1 moderate or severe constitutional symptom (eg, headache, myalgias, fever, chills, fatigue, anorexia, nausea) and 1 moderate or severe respiratory symptom (eg, cough, sore throat, rhinorrhea). Participant exclusion criteria are described in the [Supplementary Data](#).

Outcomes

The primary end point was the nature, frequency, and severity of adverse events (AEs) and serious adverse events (SAEs), effects on laboratory values, vital signs, electrocardiogram (ECG) parameters, and other safety biomarkers in patients receiving a single IV dose of MHAA4549A or placebo.

Secondary end points included the median time to alleviation of all influenza symptoms (nasal congestion, sore throat, cough, aches, fatigue, headaches, and chills/sweats) with resolution maintained for 24 hours (without use of symptom relief medications), defined by a rating of 0 (none) or 1 (mild) for each symptom on a 4-point scale (0–3). For patients with mild symptoms, the symptom score had to be reduced by 1 point during the study. Other secondary end points included the incidence of influenza reinfection/relapse at 100 days after the end of treatment, hospital admission rate, duration of hospital stay, antibiotic usage for secondary bacterial respiratory infections, influenza reinfection rate, and the incidence of death. MHAA4549A serum pharmacokinetic (PK), nasopharyngeal viral load by quantitative PCR and TCID₅₀, and the duration of viral shedding in upper respiratory samples were also assessed.

Procedures

A single dose of MHAA4549A or placebo was diluted in 0.9% normal saline and delivered intravenously over ~120 minutes. All patients were monitored for study drug reactions during and for at least 30 minutes after study drug administration.

Patients recorded symptoms of influenza-like illness, use of symptom relief medications, and oral temperature in paper diaries twice daily: once upon waking in the morning and ~12 hours later. Patients recorded entries until day 14 or symptom resolution (symptom score of 0–1 for 24 hours without use

of symptom relief medications). Temperature was taken before administration of acetaminophen/paracetamol and/or nonsteroidal anti-inflammatory drugs. The day 1 entry was completed within 6 hours before starting study infusion.

Sample collection and assays for assessing serum and nasopharyngeal MHAA4549A pharmacokinetics and viral load are described in the [Supplementary Data](#).

Statistical Analysis

The planned sample size was ~47 patients per treatment group to obtain 120 evaluable patients (estimated dropout rate derived from country and site feasibility assessments performed by our clinical operations group and CRO = 15%). Safety analyses included all randomized patients who received the study drug. Efficacy analyses were performed on the intent-to-treat infected (ITTI) population, defined as all randomized patients with confirmed influenza A infection by a central PCR test. Day 1 samples were used when available; otherwise, the next available time point sample was used. Time to event data were analyzed using Kaplan-Meier methodology and were summarized using medians and 80% CIs. Patients who were lost to follow-up (while event-free) were censored at the time that they were last known to be event-free. MHAA4549A serum PK was summarized by estimating total serum drug exposure. Because PK samples were only collected until day 7 (PK day 6), only maximum concentration (C_{max}) and area under the serum

concentration–time curve from time 0 to day 6 (AUC_{0-6}) are reported, with estimates summarized as mean and SD. All statistical analyses were performed using SAS (version 9.2) and R (version 3.3.2).

Data Sharing

Qualified researchers may request access to individual patient-level data through the clinical study data request platform (<https://vivli.org/>). Further details on Roche's criteria for eligible studies are available here (<https://vivli.org/members/ourmembers/>). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see <https://go.gene.com/datasharing>.

RESULTS

Patients

The study was conducted from March 2016 until November 2017. Two hundred sixty-five patients were screened, and 124 were randomized to receive study treatment ([Supplementary Figure 1](#)). The mean age of patients (range) was 37.0 (18–65) years ([Table 1](#)). There was a higher proportion of women (57.3%), and most patients were White (61.3%). All patients had influenza A infection by local rapid antigen test (RAT) (91.9% of patients) or PCR, with ≤ 72 hours between symptom

Table 1. Patient Demographics and Baseline Characteristics (Safety-Evaluable Population)

	Placebo (n = 43)	MHAA4549A 3600 mg (n = 41)	MHAA4549A 8400 mg (n = 40)	All Patients (n = 124)
Age, y	39.3 ± 10.8	36.5 ± 12.5	35.0 ± 13.6	37.0 ± 12.4
Age group, ≥65 y	0	0	1 (2.5)	1 (0.8)
Sex, female	27 (62.8)	22 (53.7)	22 (55.0)	71 (57.3)
Race				
Asian	2 (4.7)	4 (9.8)	1 (2.5)	7 (5.6)
Black or African American	13 (30.2)	7 (17.1)	15 (37.5)	35 (28.2)
Native Hawaiian or other Pacific Islander	1 (2.3)	2 (4.9)	0	3 (2.4)
White	27 (62.8)	26 (63.4)	23 (57.5)	76 (61.3)
Multiple	0	0	1 (2.5)	1 (0.8)
Unknown	0	2 (4.9)	0	2 (1.6)
Influenza confirmation test				
Rapid antigen test	39 (90.7)	38 (92.7)	37 (92.5)	114 (91.9)
Rapid PCR test	4 (9.3)	3 (7.3)	3 (7.5)	10 (8.1)
Influenza symptom onset time category—calculated				
≤36 hours	11 (25.6)	11 (26.8)	8 (20.0)	30 (24.2)
>36 hours	32 (74.4)	30 (73.2)	32 (80.0)	94 (75.8)
Influenza subtype ^a				
H1	4/33	5/35 ^b	2/31	11/99 ^b
H3	25/33	29/35 ^b	28/31	82/99 ^b
Negative for H1 and H3	4/33	2/35	1/31	7/99

Data are mean ± SD or No. (%).

Abbreviations: ITTI, intent-to-treat infection; PCR, polymerase chain reaction.

^aITTI population; data are number/total for each cohort.

^bOne subject in the 3600 mg group tested positive for H1 and H3.

onset and study drug administration. Most patients (75.8%) were dosed >36 hours from symptom onset.

Of 124 patients who were randomized at a 1:1:1 ratio and received IV doses of placebo, 3600 mg of MHAA4549A, or 8400 mg of MHAA4549A, 121 patients (97.6%) completed the study, with 3 patients (2.4%) lost to follow-up (Supplementary Figure 1). In the placebo group, study treatment infusion was stopped in 1 patient due to an infusion-related reaction. Of the 124 safety-evaluable patients, 99 were confirmed positive for influenza A by a central PCR test (ITTI population). Eight patients had missing virology samples, and 17 patients were assumed to be false positive RAT or because there was ~24 hours between the sample collections. A majority of subjects (82 out of 99 subjects) in the ITTI population tested positive for the H3 subtype. One subject in the 3600 mg group tested positive for both the H1 and H3 subtypes, and 7 subjects tested negative for both the H1 and H3 subtypes.

Safety

Similar proportions of patients experienced AEs between the MHAA4549A and placebo groups (Table 2). Forty-one patients (33.1%) had 81 AEs. The most common AEs were nausea (6.5%) and bronchitis (4%). Bronchitis was not dose-dependent and occurred in 4 (4.9%) MHAA4549A recipients and 1 (2.5%) placebo subject with onset within 1 week of enrollment in all cases, except 1 where the diagnosis was associated with sinusitis. All bronchitis events were either mild (grade 1) or moderate (grade 2), were not serious, and were not considered related to study drug by the site investigator. Other AEs included vomiting, oropharyngeal pain, epistaxis, nasal congestion, and headache, which all occurred in <3% of patients. Two placebo-treated patients experienced 5 unrelated grade 3 AEs (nausea, diarrhea, lower respiratory tract infection, drug intolerance, and anxiety). All other events were grade ≤2. Ten

MHAA4549A-treated patients (8.1%) had treatment-related AEs (nausea and dry skin, 2 patients each; n = 1 for all other treatment-related AEs), as deemed by the site investigator, and no treatment-related AEs occurred in the placebo group. No patients were hospitalized, had influenza reinfection, or died through day 100 post-treatment (end of study). There were no clinically significant changes in laboratory parameters, ECGs, or vital signs. One patient (8400 mg MHAA4549A) had an SAE of atrial fibrillation that self-resolved without treatment and was not considered related to study drug. The only influenza-related complication was a respiratory tract infection in 1 placebo-treated patient (3.0%), who was treated with the antibiotic clarithromycin.

Efficacy

The median time to alleviation of all symptoms (total symptom score ≤1) was slightly longer in the active treatment arms (3600 mg MHAA4549A, 154 hours; 80% CI, 125–175 hours; 8400 mg MHAA4549A, 146 hours; 80% CI, 133–157 hours) compared with placebo (117 hours; 80% CI, 109–157 hours) (Figure 1), but these differences were not statistically significant. Hazard ratios (stratified analysis) for the 3600 and 8400 mg MHAA4549A arms were 0.92 (80% CI, 0.6–1.4) and 0.90 (80% CI, 0.6–1.4), respectively. In the MHAA4549A arms, a trend of increased cough duration (~1 day longer to resolve than placebo) drove the differences in resolution of the total symptom score, but these differences were not statistically significant.

The effect of MHAA4549A on influenza A nasopharyngeal viral load is shown in Figure 2. We observed no differences by PCR (Figure 2A), but with TCID₅₀, the MHAA4549A arms showed a trend toward faster viral load clearance; however, this was not statistically significant (Figure 2B). The duration of viral shedding between treatment groups did not differ (data not shown).

Table 2. Safety Profile; AEs in ≥2% of All Patients Are Shown

	Placebo (n = 43)	MHAA4549A 3600 mg (n = 41)	MHAA4549A 8400 mg (n = 40)	All Patients (n = 124)
Total No. of patients with ≥1 AE	13 (30.2)	16 (39.0)	12 (30.0)	41 (33.1)
Total No. of AEs	29	32	20	81
Related AE	2 (4.7)	4 (9.8)	4 (10.0)	10 (8.1)
Related AE leading to withdrawal from treatment	1 (2.3)	0	0	1 (0.8)
Total No. of patients with ≥1 SAE	0	0	1 (2.5) ^a	1 (0.8)
AEs in ≥2% of all patients				
Nausea	2 (4.7)	3 (7.3)	3 (7.5)	8 (6.5)
Bronchitis	1 (2.3)	3 (7.3)	1 (2.5)	5 (4.0)
Oropharyngeal pain	1 (2.3)	1 (2.4)	2 (5.0)	4 (3.2)
Vomiting	2 (4.7)	1 (2.4)	1 (2.5)	4 (3.2)
Epistaxis	1 (2.3)	1 (2.4)	1 (2.5)	3 (2.4)
Headache	0	2 (4.9)	1 (2.5)	3 (2.4)
Nasal congestion	0	2 (4.9)	1 (2.5)	3 (2.4)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: AE, adverse event; SAE, serious adverse event.

^aNot considered related to study drug.

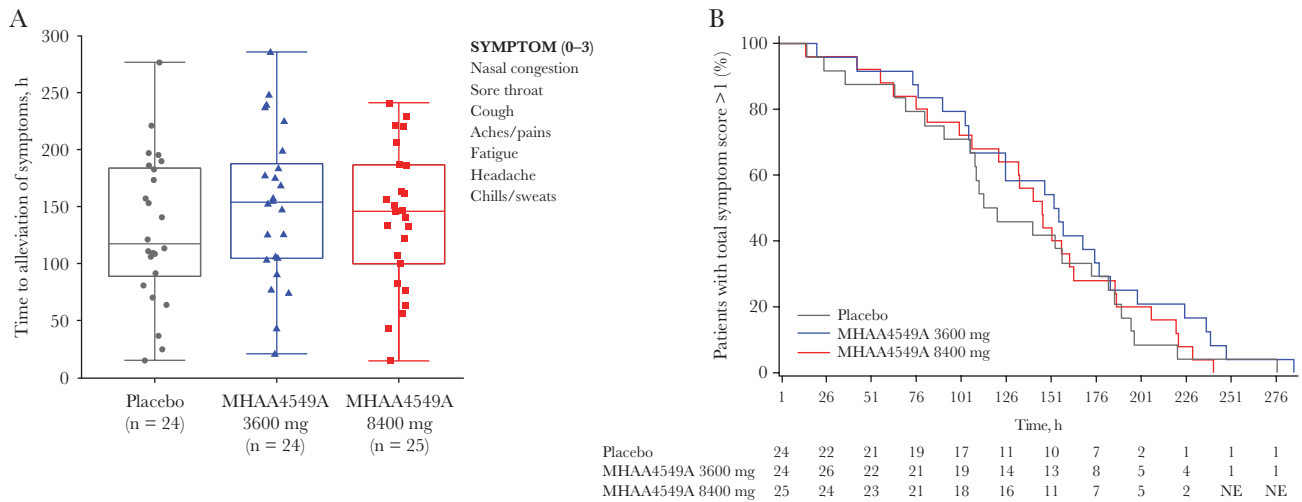


Figure 1. Median time to alleviation of total symptoms, as measured by a total symptom score ≤ 1 . A, Each symptom was scored as none (0), mild (1), moderate (2), and severe (3) and then added for a total symptom score. Horizontal bar and box boundaries indicate median and IQR, respectively; whiskers indicate 1.5 times the IQR from the upper and lower hinges of the box. B, Kaplan-Meier plot of time to alleviation of symptoms. Abbreviation: IQR, interquartile range.

Pharmacokinetics

MHAA4549A serum exposure was confirmed in all MHAA4549A-treated patients (Figure 3A) and was dose-proportional (Figure 3B). The maximum observed serum concentrations (mean \pm SD) for MHAA4549A were 1050 ± 299 $\mu\text{g/mL}$ and 2190 ± 58 $\mu\text{g/mL}$ for the 3600 and 8400 mg groups, respectively (Figure 3C).

DISCUSSION

MHAA4549A was tested as adjunctive therapy to NAIs for hospitalized patients with severe influenza A. This study evaluated single IV doses of MHAA4549A in adults with uncomplicated seasonal influenza A. MHAA4549A demonstrated acceptable safety and tolerability compared with

placebo. Similar proportions of patients experienced AEs between the MHAA4549A and placebo groups, and there were no treatment-related SAEs, cases of influenza reinfection, hospitalizations, or deaths through day 100 post-treatment. The most common AEs were nausea and dry skin. MHAA4549A serum exposure was confirmed in all MHAA4549A-treated patients and with expected dose-proportional PK.

In MHAA4549A-treated patients, there was a trend of cough persisting ~ 1 day longer compared with placebo-treated patients. While bronchitis was reported as one of the most common AEs (4% of patients), there were no significant differences between the treatment and placebo groups, and only 1 placebo-treated patient received an antibiotic. The cause of prolonged cough in MHAA4549A recipients is unknown; one hypothesis is that it could be due to antibody-mediated irritation

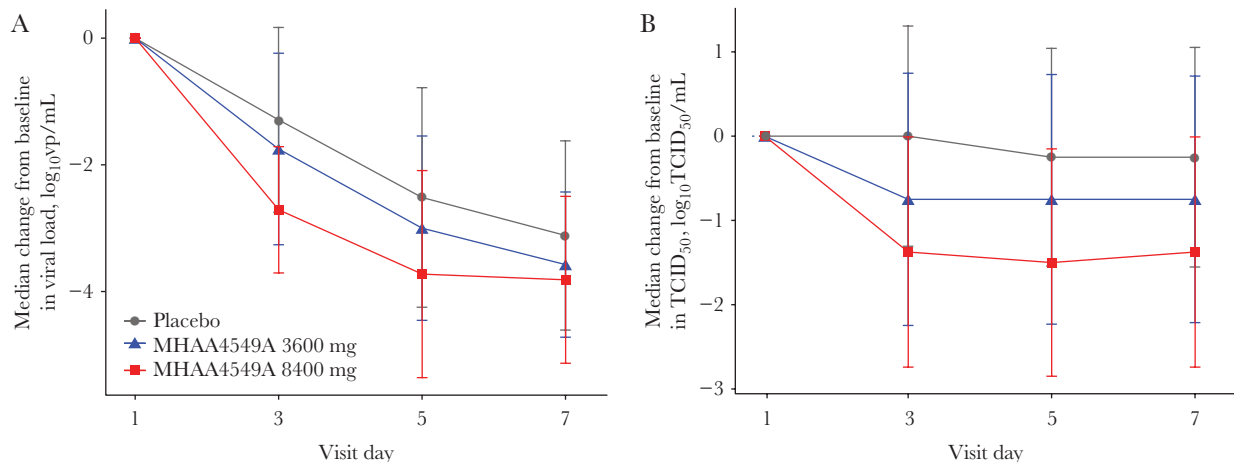


Figure 2. Median (\pm SD) change in nasopharyngeal viral load as measured by (A) qPCR and (B) TCID_{50} in the ITTI population. For (A), LLOQ = $2.18 \log_{10}[\text{vp/mL}]$. For (B), LLOQ = $0.75 \log_{10}[\text{TCID}_{50}/\text{mL}]$. Assay results below the LLOQ were imputed as the LLOQ. Abbreviations: ITTI, intent-to-treat-infected; LLOQ, lower limit of quantification; qPCR, quantitative polymerase chain reaction; TCID_{50} , 50% tissue culture infectious dose; vp, viral particles.

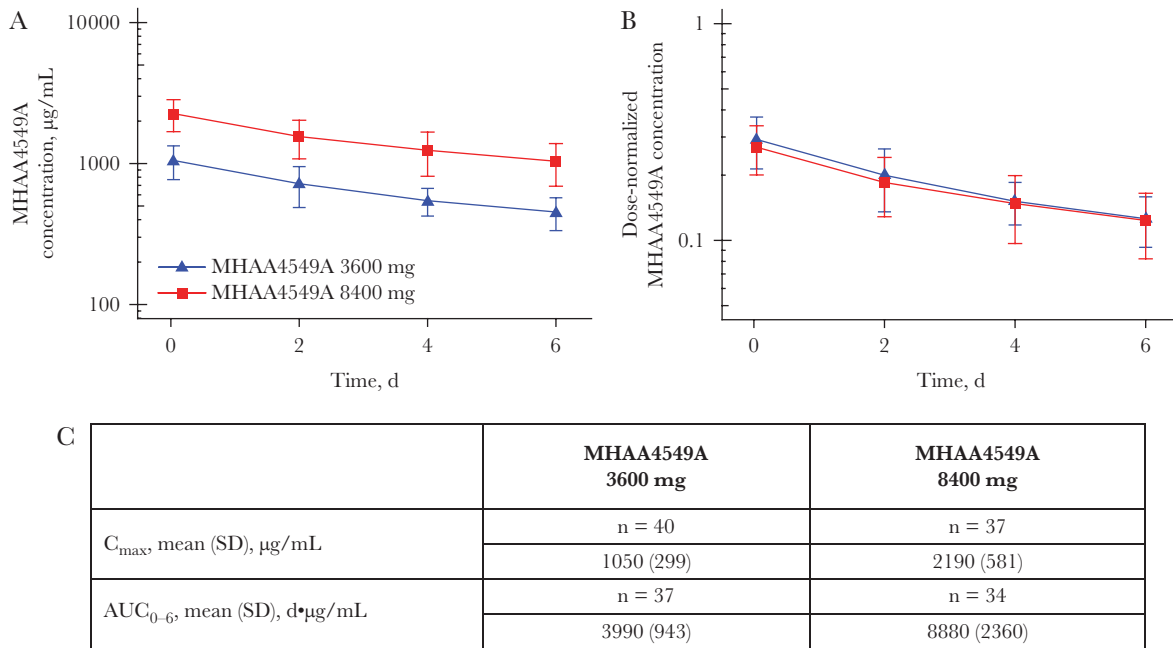


Figure 3. A, MHAA4549A serum concentration–time curve. B, MHAA4549A serum concentration normalized to dose. C, Summary of serum MHAA4549A pharmacokinetic parameters in PK-evaluable subjects.

in pleural fluid. In a phase 2 outpatient study with MEDI8852, a human IgG1 monoclonal antibody that binds the influenza A hemagglutinin stalk epitope, bronchitis occurred at a higher rate in MEDI8852 recipients than oseltamivir recipients (11.8% and 3.1%, respectively), with most events starting around day 7 and resolving by day 15 [24]. The cause of the bronchitis event is unknown; however, most of the bronchitis events occurred at a single study site. Additionally, prolonged postinfectious cough from influenza infection is common due to epithelial damage and inflammation in the airway tract. Patients with influenza infections commonly report cough lasting more than a week [30], and 11%–25% of patients with upper respiratory infections develop persistent cough (>3 weeks after acute symptoms disappear) [31–33]. Given the lack of associated influenza symptoms, lack of increase in influenza virus shedding the MHAA4549A vs placebo groups, and similar number of bronchitis cases between the MHAA4549A and placebo recipients, the finding of prolonged cough by 1 day is not considered to be consistent with ADE of influenza disease or to be clinically significant.

MHAA4549A displayed PK properties consistent with previous observations [15, 16, 26], indicating that therapeutic serum concentrations were reached. Despite expected exposure to study drug, MHAA4549A did not significantly improve the time to resolution of signs and symptoms of influenza compared with placebo. Similarly, MHAA4549A did not reduce the time to clearance of viral load over placebo. The absence of increased viral loads, persistent viral shedding, and prolonged clinical symptoms in the MHAA4549A arm vs the placebo arm suggests

a lack of ADE of infection or influenza disease. We also saw no evidence of viral resistance to MHAA4549A, based on the resolution of viral shedding and clinical symptoms, although 3 MHAA4549A-resistant viruses that either abolished or reduced HA binding have been previously isolated in vitro [13].

It is unclear why MHAA4549A did not demonstrate clinical activity in patients with uncomplicated influenza infection, though a number of factors can be considered. First, unlike neuraminidase inhibitors, there are limited data to determine the appropriate time-to-treatment window with monoclonal antibodies, and patients may have received MHAA4549A too late in the course of disease. While we saw no differences among patients based on time to treatment (\leq or $>$ 36 hours from symptom onset; data not shown), most patients (76%) were dosed $>$ 36 hours from symptom onset due to operational constraints associated with diagnostic testing and delivery of an IV therapy. Accordingly, we could not fully assess efficacy trends regarding timing of therapy with respect to symptom onset.

Previous influenza outpatient studies with the polymerase inhibitors baloxivir, marboxil, and pimodivir [34, 35] that demonstrated efficacy required a 48-hour window from symptom onset for enrollment, allowing for treatment within 24–48 hours with respect to symptom onset in the majority of patients. In studies with the anti-HA stalk antibodies MEDI8852 and VIS410 [24, 25], most patients were treated within 24–48 hours of symptom onset. The median time to treatment from symptom onset in the VIS410 study was 42 hours, with favorable effects on symptom resolution seen early (~day 2) and decreasing by day 7 in the trial (end points were not presented

based on time to treatment). MEDI8852 did not demonstrate a treatment benefit even in those who received drug within 48 hours. Based on the data from these influenza trials, a 72-hour treatment window after symptom onset may be too long for influenza therapy to reverse signs and symptoms of clinical influenza, especially in otherwise healthy patients with mild disease where the viral load typically peaks on the day of symptom onset or within 1–2 days [30] as seen in this study.

Second, the use of monoclonal antibodies for the treatment of influenza is challenging from a drug exposure perspective. There are many uncertainties surrounding the PK and distribution of a systemically administered antibody at the respiratory epithelial surface. Failure to achieve adequate and potent concentrations at the site of action early in the course of disease progression may contribute to lack of efficacy [36]. In an influenza challenge study in healthy volunteers, MHAA4549A showed dose-proportional serum PK with a long terminal half-life and slow clearance, though nasopharyngeal swab PKs were not dose-proportional [26]. MHAA4549A reached concentrations exceeding influenza concentrations in tracheal aspirates in hospitalized patients with severe influenza infection with the same doses used in this study [26, 27, 37].

Other anti-HA stalk antibodies with the same mechanism of action as MHAA4549A have shown signs of preliminary efficacy. In patients with acute influenza, MEDI8852 was comparable to oseltamivir for time to resolution of symptoms, time to reduction of viral load, and duration of viral shedding [24]. In a trial of outpatients with uncomplicated influenza, VIS410 demonstrated a tendency toward faster symptom resolution by day 3 and reduced median nasopharyngeal viral load vs placebo [25]. Notably, the study used a 32-question FLUPRO patient-reported outcome assessment, which may have been more sensitive than the scale used in this study. VIS410 evaluated in combination with oseltamivir vs oseltamivir alone in hospitalized adults with influenza A requiring oxygen showed improvements in time to normal oxygenation, cessation of virus shedding, and time to complete clinical response in subset analyses of patients presenting within 72 hours of symptom onset [38]. In a more recent study, a single dose of VIS410 reduced the median viral load in healthy volunteers inoculated with H1N1 [39].

A number of promising antibody candidates have emerged as potential therapeutic agents in the prevention and treatment of COVID-19 infection in the outpatient setting. Antibodies have demonstrated efficacy in COVID-19 trials, possibly due to the longer window of ~5–7 days between onset of symptoms and progression to severe illness requiring hospitalization [36, 40, 41]. In contrast, antibody-based therapeutics have not demonstrated widespread efficacy in hospitalized patients with COVID-19. Further, the emergence of resistant variants significantly threatens the efficacy of nAb-based therapeutics [42].

Similar to other influenza studies, this trial faced significant enrollment challenges relating to study design, operational

constraints, and the seasonal variability of influenza [43, 44]. In particular, patients were required to have PCR- or RAT-confirmed influenza and be dosed within 72 hours of symptom onset. From an operational standpoint, this was challenging and slowed trial enrollment. During the trial, patients were required to receive a 120-minute IV infusion in an outpatient setting followed by postinfusion monitoring for 30 minutes. This was a less attractive option to patients as compared with oral oseltamivir, which was readily available during the trial period and was prohibited in this study.

End point selection is critical to the effective evaluation of novel therapeutic agents, and there were no standardized outcomes across influenza trials at the time of this trial. While symptom duration has been used for registrational trials of acute uncomplicated influenza [45], these studies are subject to reporting bias, and it is challenging to quantify differences between patients. Virologic end points are not considered appropriate measures of efficacy for influenza therapeutics as there is no established relationship between viral reduction and clinical symptoms. Similar to end points used in this study, trials evaluating viral polymerase inhibitors and anti-HA stalk antibodies have used end points focusing on the time to symptom resolution and measures of antiviral activity, including reductions in viral load or duration of viral shedding.

In conclusion, this study demonstrated that MHAA4549A was well tolerated with acceptable PK and likely reached efficacious exposures at the site of activity, but it did not improve clinical outcomes in patients with acute uncomplicated influenza A infection. Even though the sponsor has decided to discontinue the clinical development of MHAA4549A for reasons unrelated to safety, this study has contributed to our understanding of anti-HA stalk monoclonal antibody therapy in patients with influenza A and supported the feasibility of this targeting approach in future programs.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

The authors wish to thank all participating patients and their families as well as all the contributing staff at the attending sites. Editing and writing assistance was provided by Genentech, Inc.

Financial support. This work was supported by Genentech, Inc.

Potential conflicts of interest. J.J.L., P.K., A.N., J.M.M., R.D., J.G., T.C., E.M.N., J.A.T., and M.C.P. were employees of Genentech, Inc. a member of the Roche group, and own/owned Roche stock and/or options at the time this study was performed. Genentech, Inc., provided research funding to the institutions of S.D., D.V., and J.P.H.G. for the conduct of this study. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Trial registration. ClinicalTrials.gov #NCT02623322; EudraCT #2016-000425-40.

References

1. Uyeki TM, Bernstein HH, Bradley JS, et al. Clinical practice guidelines by the Infectious Diseases Society of America: 2018 update on diagnosis, treatment, chemoprophylaxis, and institutional outbreak management of seasonal influenza. *Clin Infect Dis* **2019**; 68:e1–47.
2. Kamali A, Holodniy M. Influenza treatment and prophylaxis with neuraminidase inhibitors: a review. *Infect Drug Resist* **2013**; 6:187–98.
3. Monto AS. Effectiveness of the neuraminidase inhibitors: the supporting evidence increases. *J Infect Dis* **2019**; 221:340–2.
4. Dobson J, Whitley RJ, Pocock S, et al. Oseltamivir treatment for influenza in adults: a meta-analysis of randomised controlled trials. *Lancet* **2015**; 385:1729–37.
5. Venkatesan S, Myles PR, Leonardi-Bee J, et al. Impact of outpatient neuraminidase inhibitor treatment in patients infected with influenza A(H1N1)pdm09 at high risk of hospitalization: an individual participant data metaanalysis. *Clin Infect Dis* **2017**; 64:1328–34.
6. Li TC, Chan MC, Lee N. Clinical implications of antiviral resistance in influenza. *Viruses* **2015**; 7:4929–44.
7. Hurt AC, Besselaar TG, Daniels RS, et al. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2014–2015. *Antiviral Res* **2016**; 132:178–85.
8. Abed Y, Boivin G. A review of clinical influenza A and B infections with reduced susceptibility to both oseltamivir and zanamivir. *Open Forum Infect Dis* **2017**; 4:XXX–XX.
9. Centers for Disease Control and Prevention. Influenza (flu). Available at: <https://www.cdc.gov/flu/index.htm>. Accessed 20 August 2019.
10. World Health Organization. Influenza (seasonal) fact sheet. **2018**. Available at: [https://www.who.int/news-room/fact-sheets/detail/influenza-\(seasonal\)](https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal)). Accessed 31 December 2021.
11. Iuliano AD, Roguski KM, Chang HH, et al. Estimates of global seasonal influenza-associated respiratory mortality: a modelling study. *Lancet* **2018**; 391:1285–300.
12. Nakamura G, Ning C, Park S, et al. An in vivo human-plasmablast enrichment technique allows rapid identification of therapeutic influenza A antibodies. *Cell Host Microbe* **2013**; 14:93–103.
13. Chai N, Swem LR, Reichelt M, et al. Two escape mechanisms of influenza A virus to a broadly neutralizing stalk-binding antibody. *PLoS Pathog* **2016**; 12:e1005702.
14. Vandervyn HA, Jegaskanda S, Wheatley AK, et al. Antibody-dependent cellular cytotoxicity and influenza virus. *Curr Opin Virol* **2017**; 22:89–96.
15. Lim JJ, Deng R, Derby MA, et al. Two phase 1, randomized, double-blind, placebo-controlled, single-ascending-dose studies to investigate the safety, tolerability, and pharmacokinetics of an anti-influenza A virus monoclonal antibody, MHAA4549A, in healthy volunteers. *Antimicrob Agents Chemother* **2016**; 60:5437–44.
16. McBride JM, Lim JJ, Burgess T, et al. Phase 2 randomized trial of the safety and efficacy of MHAA4549A, a broadly neutralizing monoclonal antibody, in a human influenza A virus challenge model. *Antimicrob Agents Chemother* **2017**; 61:e01154–17.
17. Khurana S, Loving CL, Manischewitz J, et al. Vaccine-induced anti-HA2 antibodies promote virus fusion and enhance influenza virus respiratory disease. *Sci Transl Med* **2013**; 5:200ra114.
18. Chan-Hui P-Y, Swiderek KM. Immunological considerations for developing antibody therapeutics for Influenza A. *Hum Vaccin Immunother* **2016**; 12:474–7.
19. Ramakrishnan B, Viswanathan K, Tharakaraman K, et al. A structural and mathematical modeling analysis of the likelihood of antibody dependent enhancement in influenza. *Trends Microbiol* **2016**; 24:933–43.
20. Takada A, Kawaoka Y. Antibody-dependent enhancement of viral infection: molecular mechanisms and in vivo implications. *Rev Med Virol* **2003**; 13:387–98.
21. Whitehead SS, Blaney JE, Durbin AP, et al. Prospects for a dengue virus vaccine. *Nat Rev Microbiol* **2007**; 5:518–28.
22. Dejnirattisai W, Jumnainsong A, Onsririsakul N, et al. Cross reacting antibodies enhance dengue virus infection in humans. *Science* **2010**; 328:745–8.
23. Crowe JE. Universal flu vaccines: primum non nocere. *Sci Transl Med* **2013**; 5:200fs34.
24. Ali SO, Takas T, Nyborg A, et al. Evaluation of MEDI8852, an anti-influenza A monoclonal antibody, in treating acute uncomplicated influenza. *Antimicrob Agents Chemother* **2018**; 62:e00694–18.
25. Hershberger E, Sloan S, Narayan K, et al. Safety and efficacy of monoclonal antibody VIS410 in adults with uncomplicated influenza A infection: results from a randomized, double-blind, phase-2, placebo-controlled study. *EbioMedicine* **2019**; 40:574–82.
26. Deng R, Lee AP, Maia M, et al. Pharmacokinetics of MHAA4549A, an anti-influenza A monoclonal antibody, in healthy subjects challenged with influenza A virus in a phase IIa randomized trial. *Clin Pharmacokinet* **2018**; 57:367–77.
27. Lim JJ, Nilsson AC, Silverman M, et al. A phase 2 randomized, double-blind, placebo-controlled trial of MHAA4549A, a monoclonal antibody, plus oseltamivir in patients hospitalized with severe influenza A virus infection. *Antimicrob Agents Chemother* **2020**; 64:e00352–20.
28. Lee N, Chan PK, Hui DS, et al. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. *J Infect Dis* **2009**; 200:492–500.
29. Lee N, Chan PK, Wong CK, et al. Viral clearance and inflammatory response patterns in adults hospitalized for pandemic 2009 influenza A (H1N1) virus pneumonia. *Antivir Ther* **2011**; 16:237–47.
30. Ip DKM, Lau LLH, Chan KH, et al. The dynamic relationship between clinical symptomatology and viral shedding in naturally acquired seasonal and pandemic influenza virus infections. *Clin Infect Dis* **2016**; 62:431–7.
31. Lin L, Yang ZF, Zhan YQ, et al. The duration of cough in patients with H1N1 influenza. *Clin Respir J* **2017**; 11:733–8.
32. Poe RH, Harder RV, Israel RH, et al. Chronic persistent cough. Experience in diagnosis and outcome using an anatomic diagnostic protocol. *Chest* **1989**; 95:723–8.
33. Irwin RS, Madison JM. The diagnosis and treatment of cough. *N Engl J Med* **2000**; 343:1715–21.
34. Hayden FG, Sugaya N, Hirotsu N, et al. Baloxavir marboxil for uncomplicated influenza in adults and adolescents. *N Engl J Med* **2018**; 379:913–23.
35. Finberg RW, Lanno R, Anderson D, et al. Phase 2b study of pimodivir (JNJ-63623872) as monotherapy or in combination with oseltamivir for treatment of acute uncomplicated seasonal influenza A: TOPAZ trial. *J Infect Dis* **2019**; 219:1026–34.
36. Cruz-Teran C, Tiruthani K, McSweeney M, et al. Challenges and opportunities for antiviral monoclonal antibodies as COVID-19 therapy. *Adv Drug Deliv Rev* **2021**; 169:100–17.
37. Deng R, She G, Maia M, et al. Pharmacokinetics of the monoclonal antibody MHAA4549A administered in combination with oseltamivir in patients hospitalized with severe influenza A infection. *J Clin Pharmacol* **2020**; 60:1509–18.
38. Oldach D, Narayan K, Schaefer K, et al. A global randomised, double-blind, placebo-controlled study evaluating safety and efficacy of VIS410 in combination with oseltamivir versus oseltamivir alone in hospitalized adults with influenza A requiring oxygen. Paper presented at: Options X for the Control of Influenza; 28 August–1 September 2019; Suntec, Singapore.
39. Sloan SE, Szretter KJ, Sundaresh B, et al. Clinical and virological responses to a broad-spectrum human monoclonal antibody in an influenza virus challenge study. *Antiviral Res* **2020**; 184:104763.
40. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* **2020**; 323:1061–9.
41. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet* **2020**; 395:1054–62.
42. Su J, Lu H. Opportunities and challenges to the use of neutralizing monoclonal antibody therapies for COVID-19. *Biosci Trends* **2021**; 15:205–10.
43. de Jong MD, Ison MG, Monto AS, et al. Evaluation of intravenous peramivir for treatment of influenza in hospitalized patients. *Clin Infect Dis* **2014**; 59:e172–85.
44. Corti D, Cameroni E, Guarino B, et al. Tackling influenza with broadly neutralizing antibodies. *Curr Opin Virol* **2017**; 24:60–9.
45. US Food and Drug Administration. Guidance for industry. Influenza: developing drugs for treatment and/or prophylaxis. **2011**. Available at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/influenza-developing-drugs-treatment-and-or-prophylaxis>. Accessed 7 November 2019.