mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials

Robert A. Feldman [a,1], Rainard Fuhr [b,1], Igor Smolenov [c], Amilcar (Mick) Ribeiro [c], Lori Panther [c], Mike Watson [c,*], Joseph J. Senn [c], Mike Smith [c], Örn Almarsson [c], Hari S. Pujar [c], Michael E. Laska [c], James Thompson [c], Tal Zaks [c], Giuseppe Ciaramella [c]

[a] Miami Research Associates, 6280 Sunset Drive, Suite 600, So. Miami, FL 33143, USA
[b] PAREXEL International GmbH Klinikum Westend, House 18, Spandauer Damm 130, 14050 Berlin, Germany
[c] Moderna, 500 Technology Square, Cambridge, MA 02139, USA

ARTICLE INFO

ABSTRACT

Background: We evaluated safety and immunogenicity of the first mRNA vaccines against potentially pandemic avian H10N8 and H7N9 influenza viruses.
Methods: Two randomized, placebo-controlled, double-blind, phase 1 clinical trials enrolled participants between December 2015 and August 2017 at single centers in Germany (H10N8) and USA (H7N9). Healthy adults (ages 18–64 years for H10N8 study; 18–49 years for H7N9 study) participated. Participants received vaccine or placebo in a 2-dose vaccination series 3 weeks apart. H10N8 intramuscular (IM) dose levels of 25, 50, 75, 100, and 400 μg and intradermal dose levels of 25 and 50 μg were evaluated. H7N9 IM 10-, 25-, and 50-μg dose levels were evaluated; 2-dose series 6 months apart was also evaluated. Primary endpoints were safety (adverse events) and tolerability. Secondary immunogenicity outcomes included humoral (hemagglutination inhibition [HAI], microneutralization [MN] assays) and cell-mediated responses (ELISPOT assay).
Results: H10N8 and H7N9 mRNA IM vaccines demonstrated favorable safety and reactogenicity profiles. No vaccine-related serious adverse event was reported. For H10N8 (N = 201), 100-μg IM dose induced HAI titers ≥ 1:40 in 100% and MN titers ≥ 1:20 in 87.0% of participants. The 25-μg intradermal dose induced HAI titers > 1:40 in 64.7% of participants compared to 34.5% of participants receiving the IM dose. For H7N9 (N = 156), IM doses of 10, 25, and 50 μg achieved HAI titers ≥ 1:40 in 36.0%, 96.3%, and 89.7% of participants, respectively. MN titers ≥ 1:20 were achieved by 100% in the 10- and 25-μg groups and 96.6% in the 50-μg group. Seroconversion rates were 78.3% (HAI) and 87.0% (MN) for H10N8 (100 μg IM) and 96.3% (HAI) and 100% (MN) in H7N9 (50 μg). Significant cell-mediated responses were not detected in either study.
Conclusions: The first mRNA vaccines against H10N8 and H7N9 influenza viruses were well tolerated and elicited robust humoral immune responses.
ClinicalTrials.gov NCT03076385 and NCT03345043.

1. Introduction

H10N8 avian influenza first breached the avian-human species barrier in 2013, and was fatal in 2 of the 3 three persons infected [1]. No additional H10N8 human infections have been reported, but the virus has a high affinity for the human receptor, and mutated strains with increased virulence are a significant concern [2]. Also in 2013, the first human H7N9 infections were reported in China, with a fatality rate of 37% [3]. Since 2013, five waves of H7N9 outbreaks have caused over 1500 documented infections

and more than 600 deaths [4]. In February 2017, the pandemic threat was further highlighted by a death due to a highly pathogenic H7N9 strain with a R292K amino acid mutation associated with neuraminidase inhibitor resistance [5].

Emerging influenza strains reinforce the urgent need for vaccine technologies with precise yet flexible antigen design that generate potent and well tolerated immune responses with rapidly scalable, high-volume manufacturing [6]. Egg-based technologies do not fulfil these requirements. During the 2009 H1N1 pandemic, 6 months elapsed from the start of the epidemic until the first vaccine doses became available, and an additional 2 months were needed to produce the tens of millions of doses required for the epidemic [6]. The vaccine itself was effective [7,8], suggesting that earlier deployment could have had greater impact. Stockpiling strategies are expensive and lack the flexibility to continuously adapt the vaccine to mutating threats [9]. For example, currently stockpiled vaccines against H7N9 are expected to offer reduced protection against the emerging "wave-five" Yangtze River Delta Lineage virus [10].

mRNA vaccines have the potential for rapid, high-volume manufacturing with the precision and flexibility of antigen design necessary to provide both timely and effective responses to emerging threats from influenza and other pathogens. They also offer the opportunity for a more flexible stockpiling approach, with the potential to store low-volume libraries of frozen plasmid and/or unformulated mRNA for many decades, which can be rapidly formulated and distributed as threat levels rise. mRNA vaccines can direct expression of virtually any membrane-bound, soluble, or polyprotein antigens, mimicking antigen expression during natural infection [11]. For influenza, mRNA vaccines could also avoid antigenic drift associated with egg-based vaccine production [12]. Additional advantages are economies in time, cost, and scale that derive from using a single development and manufacturing platform. Production of mRNA vaccines does not require pathogen growth: only identification, optimization, and mRNA expression of protective antigen(s) are required.

To assess the safety and immunogenicity of mRNA influenza vaccines, we have developed two avian influenza strains of pandemic potential [13] in our lipid nanoparticle (LNP)–formulated mRNA vaccine platform. We present safety and immunogenicity data from two phase 1, randomized, double-blind, placebo-controlled studies of H10N8 and H7N9 mRNA vaccines in healthy adults. The tolerability and immunogenicity of different dose levels and routes of administration were explored.

2. Methods

2.1. Study design and participants

Two phase 1, randomized, double-blind, placebo-controlled, dose-ranging studies evaluated mRNA H10N8 and mRNA H7N9 vaccines at single centers in Berlin, Germany (PAREXEL International) and South Miami, Florida, USA (Miami Research Associates), respectively. Eligible participants were healthy adults who provided written consent and had no prior history of adverse reactions to influenza vaccinations, diagnosis of Guillain-Barré syndrome, receipt of licensed vaccines within 2–4 weeks, receipt of H10N8 or H7N9 vaccine at any time, or history of poultry or wild bird handling.

In the H10N8 study, participants aged 18–64 years were randomized to receive two doses of vaccine or placebo 3 weeks apart at intramuscular (IM) dose levels of 25, 50, 75, 100, and 400 μg or intradermal (ID) dose levels of 25 and 50 μg. In the H7N9 study, adults aged 18–49 years received two doses of vaccine or placebo 3 weeks apart at IM dose levels of 10, 25, and 50 μg. A protocol

amendment allowed participants in the 25 and 50 μg IM dose groups to receive a booster dose at 6 months.

The H10N8 trial was approved by the Ethics Committee of the Land Berlin, State Office for Health and Social Affairs, Berlin, Germany. The H7N9 trial was approved by the Chesapeake International Review Board, Columbia, Maryland. The studies were designed in accordance with the Guidance on Clinical Evaluation of New Vaccines [14] and were conducted in compliance with the International Conference on Harmonization Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki. All participants provided written, informed consent before initiation of any study-related procedures.

2.2. Vaccines

The H10N8 and H7N9 mRNA vaccines consisted of chemically modified mRNAs encoding the full-length, membrane-bound form of the hemagglutinin (HA) glycoprotein from the H10N8 influenza strain (A/Jiangxi-Donghu/346/2013) or the H7N9 influenza strain (A/Anhui/1/2013). An LNP delivery system was used as previously described [15]. The H10N8 and H7N9 vaccines were manufactured in compliance with current Good Manufacturing Processes. Each vaccine vial contained 2 mg/mL H10N8 or H7N9 mRNA and 40 mg/mL of LNP excipients formulated in isotonic 8.0% sucrose/20 mM buffer. Study vaccine was diluted with 0.9% saline and administered at a final injection volume of 200 μL. Placebo doses were 200 μL of 0.9% sodium chloride. The initial vaccine doses were selected according to the Guidance for Industry based on the preclinical animal models [13,16].

2.3. Procedures

All participants and study personnel responsible for any clinical evaluations were masked to treatment arm assignment except for 3 sentinel participants in each dose group receiving active vaccine. Vaccines were prepared and administered by unmasked study personnel with no other study involvement. A third-party biostatistician performed interim analyses. Randomization codes were generated centrally and stored at study sites with access restricted to designated personnel.

At each dose level, 3 sentinel participants receiving active vaccine were sequentially enrolled 48 h apart for safety evaluation. After review of safety data through 14 days after last sentinel vaccination, additional participants were randomized 3:1 to vaccine or placebo. The study advanced similarly for each subsequent dose level. No sentinel participants were enrolled in the H10N8 vaccine 50- and 75-μg IM dose groups as they were added after enrollment of the 100-μg dose group. IM doses were delivered in the deltoid following standard procedures; ID doses were delivered over the deltoid area. All H7N9 vaccines were administered IM in the deltoid muscle.

2.4. Safety monitoring

In both studies, physical examinations, vital signs, and clinical laboratory assessments were conducted at screening and at days 1 (prior to first vaccination), 8, 22 (prior to second vaccination), 30, and 43. Participants were observed for 60 min after vaccination and followed for 1 year after last vaccination. Safety blood testing was performed at specific timepoints through 21 days after each vaccination (eAppendix 1). Participant diary cards captured solicited local adverse events (AEs; injection site pain, tenderness, erythema, ecchymosis, and injection site swelling) and solicited systemic AEs (headache, fatigue, myalgia, arthralgia, nausea, vomiting, diarrhea, chills, loss of appetite, malaise, and fever) from the day of each vaccination through the following 6 days, and

unsolicited AEs through 21 days after each vaccination. Participants were instructed to call or return to the study site within 24 h if any AE was severe or life-threatening during the first 7 days following vaccination.

The intensity of AEs and laboratory abnormalities was graded by the investigator as mild (Grade 1), moderate (Grade 2), severe (Grade 3), or potentially life threatening (Grade 4) using the Center for Biologics Evaluation and Research toxicity grading scale [17]. AEs were determined by the investigator to be probably, possibly, or not related to study vaccine. Serious AEs (SAEs), severe AEs, medically attended AEs, events of special interest (AESI; a subset of potentially immune-mediated medical conditions that are historically associated with a vaccination), new onset of chronic illness, and AEs leading to study withdrawal were collected throughout each study. All AEs were monitored until resolution, or if the event became chronic, until a cause was identified.

For each study, an independent safety monitoring committee performed a blinded safety data review at pre-specified time points prior to proceeding to the next dose level. Rules to pause the study were in place to halt further dosing until a safety review was performed (eAppendix 1). For the H10N8 study, the study was paused for any vaccine-related anaphylactic reaction, generalized urticarial event, severe unsolicited systemic event, or any SAE. In addition, for any H10N8 cohort (with or without sentinel), the study was paused for any severe solicited AE (systemic or local), any Grade 4 vaccine-related AE, or 3 or more Grade 3 vaccine-related AEs in any one treatment arm. For the H7N9 study, the study was paused for any vaccine-related systemic hypersensitivity event, severe solicited AE (systemic or local), severe unsolicited AE, SAE, Grade 4 AE, or 3 or more severe AEs in any one treatment arm.

2.5. Immunogenicity assessments

Immunogenicity was determined by hemagglutination inhibition (HAI) using recombinant, full-length HA proteins for H10N8 (A/Jiangxi-Donghu/346/2013, Medigen) or the A/Shanghai/02/2013XPR8 virus for H7N9 and by microneutralization (MN) assays, using the A/quail/Italy/1117/1965 and the A/Shanghai/02/2013XPR8 viruses for H10N8 and H7N9, respectively, as previously described [18,19]. Testing for HAI was performed on blood samples collected at days 1, 8, 22, 30, 43, and 84, and testing for microneutralization (MN) assays was performed on blood samples collected at days 1, 22, and 43. Blood samples for HAI persistence testing were collected at approximately 6 and 12 months after the last vaccination. Peripheral blood mononuclear cells (PBMC) were collected at days 1, 6, 22, 30, 43, and 84 and were analyzed by enzyme-linked immunospot (ELISPOT).

Serum antibodies to influenza virus HA proteins (HAI assay) were measured by serial dilution of heat-inactivated sera incubated with the titer reported as the reciprocal of the highest dilution that effectively inhibited agglutination of red blood cells by a specific influenza strain. Serum neutralizing antibodies (MN assay) were measured by serial dilution of heat-inactivated sera incubated with influenza virus and transferred to plates containing Madin-Darby canine kidney (MDCK) cells, with the titer reported as the reciprocal of the highest dilution at which no cytopathic effect was observed. Influenza viruses A/quail/Italy/1117/1965 and A/Shanghai/02/2013XPR8 were used for H10N8 and H7N9 MN assays, respectively [18,19]. Cell-mediated immune response was assessed by interferon-γ ELISPOT assays of PBMC stimulated with H10N8 and N7N9 HA protein peptide libraries.

2.6. Outcomes

The primary endpoints were safety and reactogenicity as measured by frequency and severity of solicited AEs, unsolicited AEs,

and SAEs. Secondary immunogenicity endpoints were HAI (percentage of participants with HAI titers ≥ 1:40) and MN (percentage of participants with MN titers ≥ 1:20) seroprotective rates and seroconversion rates at day 43. HAI seroconversion rates were defined as baseline HAI titer < 1:10 and post-vaccination titer ≥ 1:40 or baseline titer ≥ 1:10 and ≥ 4-fold increase in post-vaccination titer. MN seroconversion rates were defined as baseline MN titer < 1:10 and post-vaccination titer ≥ 1:20 or baseline titer ≥ 1:10 and ≥ 4-fold increase in post-vaccination titer. HAI and MN antibody responses were described as the anti-log of the arithmetic mean of the log-10 transformed titers (GMTs) and geometric mean ratios (GMR, post-vaccination titer to baseline titer). Endpoints were defined according to the international guidelines for vaccine evaluation [20].

2.7. Statistical analysis

Descriptive statistics were used to summarize demographic and baseline characteristics; there was no planned formal statistical testing. Sample size was not hypothesis-driven. A sample size of 30 participants per dose level was planned in both studies; however, actual enrollment was determined by safety and reactogenicity data at each of the dose levels.

Safety and immunogenicity data were analyzed using summary statistics, and included all randomized participants who received ≥ 1 dose of vaccine or placebo. Solicited and unsolicited AEs and SAEs were reported as numbers and percentages. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA) preferred terms.

Day 43 analyses of HAI and MN GMT, GMR, seroconversion, and antibody response were conducted for participants who received both doses of vaccine and provided immunogenicity data at baseline and day 43. GMR was calculated as the ratio of GMT pre-vaccination (day 1) to GMT at day 43. The fold-increase in titer was calculated as a ratio of GMT at Day 43 (21 days after the second vaccination) to the pre-vaccination GMT on Day 1 for each participant with both Day 1 and Day 43 results. For GMT calculations, values that were reported as below the lower limit of quantitation (LLOQ) were replaced by 0.5 × LLOQ. For calculations of fold-rise, values < LLOQ were replaced by 0.5 × LLOQ for the numerator and by LLOQ for the denominator.

Antibody persistence analyses included all participants who received ≥ 1 dose and provided immunogenicity data at day 22, and all participants who received both doses of vaccine and provided immunogenicity data at any or all days 43, 84, or 183 (H10N8 study), and days 43, 84, or 205 (H7N9 study). HAI and MN GMTs and their associated 95% confidence intervals (CIs) were reported by study and dose level. Continuous variables were calculated as means with 95% CIs or means with standard deviations (SD). Statistical analyses were performed using SAS® version 9.1 or higher (SAS Institute Inc., Cary, North Carolina, United States).

3. Results

3.1. Participants

Participants were enrolled in the H10N8 study from December 2015 to December 2016 and in the H7N9 study from February 2016 to February 2017. There were 201 participants randomized in the H10N8 study; 145 received IM vaccination and 56 received ID vaccination (Fig. 1). In the IM dose groups, 144 participants received the first vaccination and provided immunogenicity samples at day 22, and 107 participants received both vaccinations and provided immunogenicity samples at baseline and day 43. The second vaccination in the 75-µg dose group was not initiated
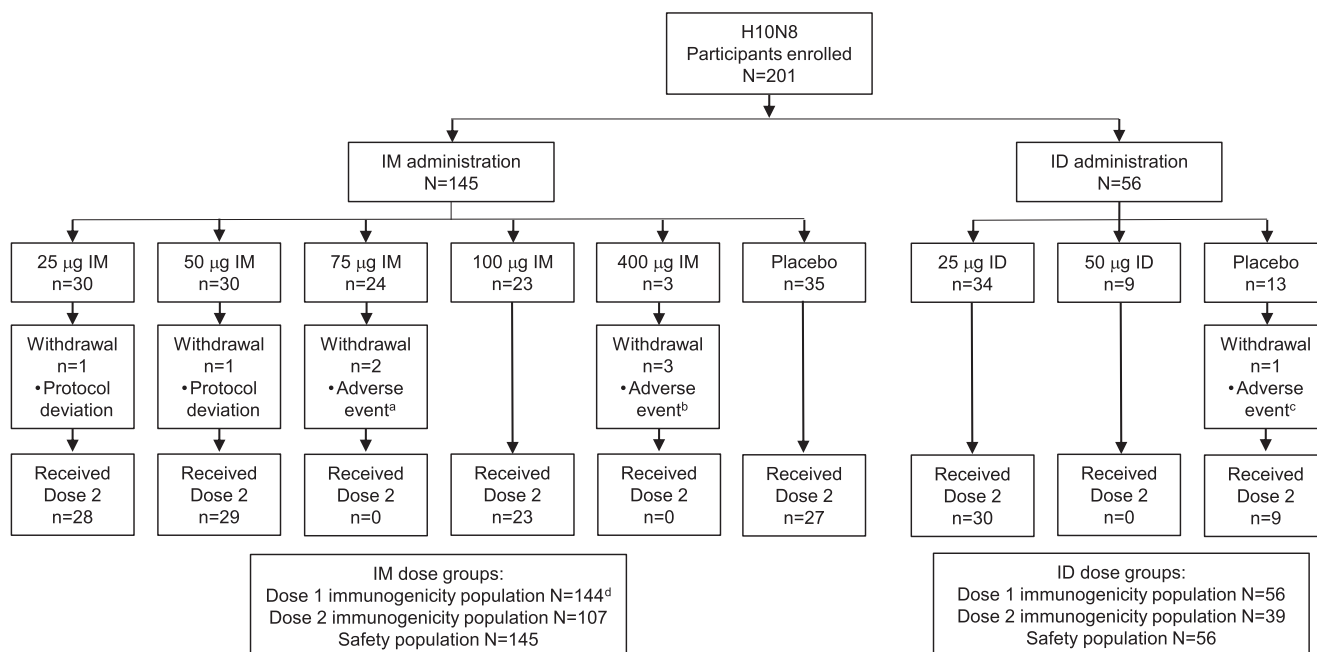
Placebo participants were pooled from all treatment arms of similar administration (IM or ID). Immunogenicity population was based on participants who provided evaluable blood samples. All enrolled participants received Dose 1. Dose 2 was administered 21 days after Dose 1. Participant withdrawals are those who withdrew by day 18. [a]Events included severe fatigue on day of vaccination (n=1) and severe erythema on day of vaccination (n=1). [b]Events included injection site erythema on day of vaccination (n=1), severe headache 2 days after vaccination (n=1), and other (n=1). [c]Event was a moderate cold 3 days after vaccination (n=1). [d]1 participant did not provide an evaluable blood sample at day 22.

Fig. 1. Patient flow for the H10N8 Study.

after finding minimal safety concerns in the previously completed 100-μg dose group. Baseline characteristics were similar across all IM dose groups (Table 1). Of the 56 participants in the ID dose groups who received the first vaccination, 39 received the second vaccination. In the 50-μg ID dose group, enrollment was halted because of local reactogenicity, and the second vaccination was not administered. Baseline characteristics for the ID dose groups are shown in eTable 1 (supplemental materials).

There were 156 participants randomized in the H7N9 study (Fig. 2). Thirty participants in the day 1 and day 21 dose groups at the 10-, 25-, and 50-μg dose levels received both vaccinations. Overall, 122 participants provided immunogenicity data at 21 days after the first dose, and 117 participants received 2 doses, provided samples at day 43, and were included in day 43 immunogenicity evaluations. Baseline characteristics were similar across all dose groups (Table 1). Ten participants in the day 1, month 6 dose groups received the first vaccination, and 3, 0, and 2 participants received the second vaccination at the 10-, 25-, and 50-μg dose levels, respectively.

3.2. Safety

3.2.1. H10N8 study

Solicited local and systemic AEs are summarized Table 2. In the IM dose groups, injection site pain after either dose was the most common solicited local AE (78.6–93.1%), followed by erythema (0–17.4%), and injection site swelling (6.7–16.7%). There were 3 Grade 3 solicited local AEs, which all occurred in the 100-μg dose group. The most common solicited systemic AEs after either IM dose were myalgia (7.8–58.6%), fatigue (26.7–47.8%), and headache (14.3–69.6%). Most solicited systemic reactions were mild to moderate in severity, of short duration (1–3 days), and resolved without intervention. The incidence of fever was higher following the second dose in the 100-μg dose group and increased with increas-

ing dose for both first and second vaccinations. In the 400-μg IM dose group, 2 sentinel participants experienced grade 3 solicited AEs (1 injection site erythema, 1 headache) within 24 h of the first vaccination, which resolved spontaneously but met study pause rules (data not shown). After safety review, further 400-μg IM vaccinations were stopped. In the 75-μg IM dose group, 2 participants experienced grade 3 solicited AEs (1 severe swelling, 1 with severe fatigue, myalgia, and injection site pain) following the first vaccination (data not shown).

Overall, 124 unsolicited AEs were reported in the IM dose groups. The most common unsolicited AEs were upper respiratory tract infection, back pain, pharyngitis, and oropharyngeal pain. Three severe unsolicited AEs (back pain, tonsillitis, ruptured ovarian cyst) and 2 SAEs (cholecystitis, ruptured ovarian cyst) were reported and deemed unrelated to vaccination. No AESIs or cases of new onset of chronic illness were reported.

ID vaccination was associated with high rates of solicited AEs (eTable 2, supplemental materials), and the sponsor elected to discontinue enrollment of these cohorts.

3.2.2. H7N9 study

For H7N9, injection site pain was the most common solicited local AE after either IM dose (43.3–80.0%), followed by swelling (16.7–30.0%) (Table 2); there was no injection site erythema above Grade 1. No severe local solicited AEs were reported after first vaccination; however, 3 participants in the 50-μg dose group experienced severe injection site pain after the second vaccination. The most common solicited systemic AEs after either dose were headache (10.0–26.7%), myalgia (10.0–26.7%), and arthralgia (6.7–20.0%). Eleven of the 12 severe solicited AEs occurred in the 50-μg dose group; none required intervention or caused early termination. Except for fever in 50-μg dose group, the frequency of solicited local or systemic AEs did not increase after the second vaccination (Table 2).

Table 1
Baseline characteristics of IM administration dose groups.

	H10N8 Study (IM administration)						H7N8 Study (IM administration)			
	25 µg (n = 30)	50 µg (n = 30)	75 µg (n = 24)	100 µg (n = 23)	400 µg (n = 3)	Placebo (n = 35)	10 µg (n = 30)	25 µg (n = 30)	50 µg (n = 30)	Placebo (n = 36)
Age, mean yrs	43.1	42.8	43.3	52.5	45.3	41.4	35.3	39.3	34.6	37.7
(range)	(20–62)	(21–61)	(19–62)	(32–64)	(35–55)	(35–55)	(20–49)	(20–47)	(19–47)	(27–46)
Sex, n male (%)	17 (57)	15 (50)	10 (42)	11 (48)	2 (67)	22 (63)	18 (60)	18 (60)	15 (50)	16 (44)
Race, n white (%)	29 (97)	29 (97)	23 (96)	21 (91)	3 (100)	35 (100)	27 (90)	19 (63)	26 (87)	30 (83)
BMI, mean kg/m^2	24.3	25.5	24.6	24.9	22.3	24.7	24.9	28.8	27.3	25.5

All subjects received vaccinations at day 1 and day 21.
IM, intramuscular; BMI, body mass index.
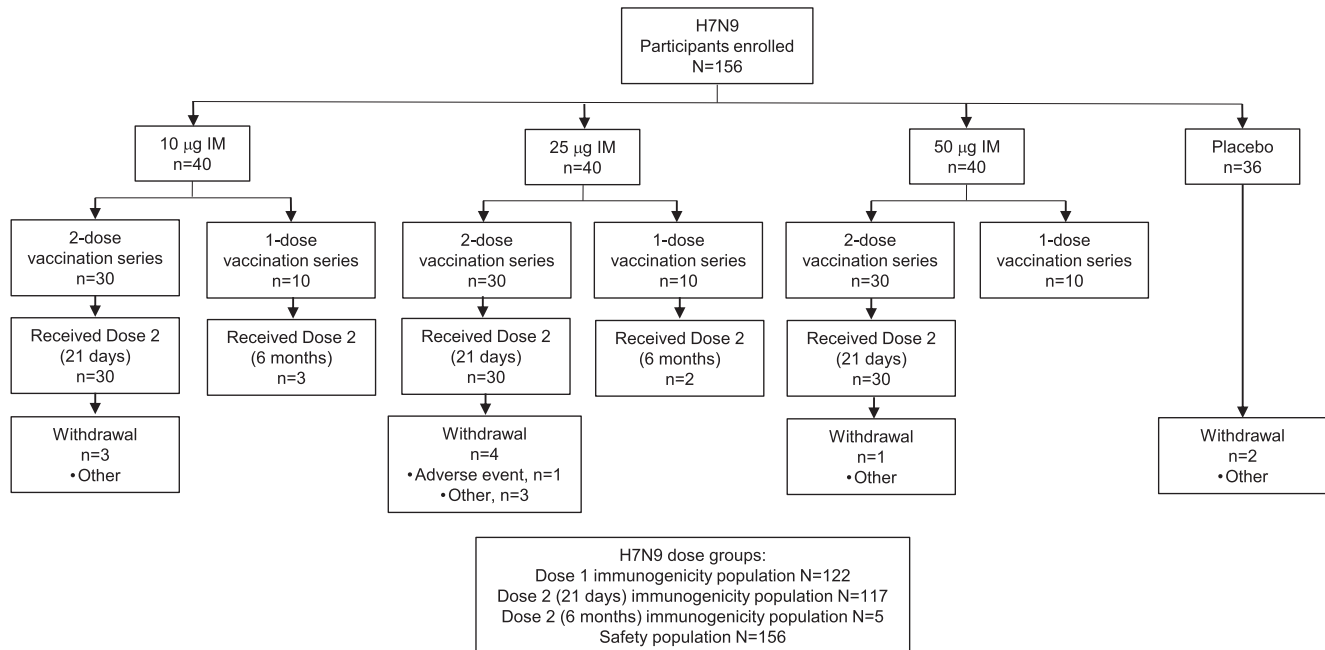
Placebo participants were pooled from all treatment arms. Immunogenicity population was based on participants who provided evaluable blood samples. All enrolled participants received Dose 1. Dose 2 was administered 21 days after Dose 1. Participant withdrawals are those who withdrew by day 18.

Fig. 2. Patient flow for the H7N9 study.

Percentages of participants who reported ≥ 1 unsolicited AE were similar across groups (53.3–73.3% vaccine; 63.9% placebo). Rates of severe unsolicited AEs were 0–20% vaccine and 8.3% placebo. The majority of possibly- and probably-related unsolicited AEs were ≥ Grade 2 laboratory abnormalities and occurred at similar rates in vaccine and placebo groups. Four severe unsolicited AEs were deemed possibly related to vaccination: 2 cases of increased alanine aminotransferase (1 50 µg, 1 placebo), 1 case of increased aspartate aminotransferase (50 µg), and 1 case of thrombocytopenia (placebo). All cases were asymptomatic and resolved without intervention. Five reported SAEs were deemed unrelated to vaccination: unintentional firearm-related death, testicular cancer, pancreatitis, facial cellulitis, and exacerbated hypertension. No AESIs or cases of new onset of chronic illness were reported.

3.3. Immunogenicity

For H10N8, HAI and MN GMT increased with increasing dose (Fig. 3A and B) and the percentage of participants with HAI titers ≥ 1:40 or MN titers ≥ 1:20 at day 43 also increased with increasing dose (Fig. 3C and D). At the 25-µg dose level, ID dosing induced higher HAI titers than IM dosing (eFigure 1, supplemental materials). In the H10N8 study, there was a discrepancy between the day 43 seroprotection rate and seroconversion rate in HAI at the 100-µg IM dose, and in MN at the 25-µg IM dose. The number of participants for each dose level was identical in the calculation of seroprotection rate and seroconversion rate. Of the 23 participants in the 100-µg dose group, 9 had baseline HAI titers < 1:10, 10 had baseline HAI titers between ≥1:10 and <1:40, and 4 had baseline HAI titers > 1:40. Of the 30 participants in the 25-µg dose group, 25 had baseline MN titers < 1:10, 1 had a baseline MN titer between ≥1:10 and <1:20, and 4 had baseline MN titers > 1:20. Six months after the second 100-µg dose, HAI GMT was 13.9 (Fig. 4A), and 22 of 23 participants (95.6%) remained seropositive (HAI titer ≥ 1:10) (data not shown).

For H7N9 participants dosed on days 1 and 22, post-vaccination HAI and MN GMTs were generally high across all doses (Fig. 5A and B). The rate of HAI titer ≥ 1:40 at day 43 was 96.3% in the 25-µg dose group (Fig. 5C). Across all dose levels, all but 1 participant achieved a post-vaccination MN titer ≥ 1:20 (Fig. 5D). Six months after vaccination, the HAI GMT was 13.6 (Fig. 4B), and 13 of 25 participants (52%) remained seropositive (HAI titer ≥ 1:10; data not shown).

Five participants (2 in the 25-µg dose level and 3 in the 10-µg dose level) received second doses at 6 months. HAI GMT increased from a baseline of 5 to 73 at the 10-µg dose, and 5 to 381 at the

Table 2
Solicited adverse events within 7 days after each IM vaccination on days 1 and 22.[a]

	H10N8 Study (IM administration)					H7N9 Study (IM administration)			
	25 µg	50 µg	75 µg[b]	100 µg	Placebo	10 µg	25 µg	50 µg	Placebo
Dose 1	n = 30	n = 30	n = 24	n = 23	n = 35	n = 30	n = 30	n = 30	n = 36
Injection site pain	23 (76.6) [0]	25 (83.3) [0]	21 (87.5) [4.2]	19 (82.6) [0]	2 (5.7) [0]	22 (73.3) [0]	17 (56.7) [0]	24 (80.0) [0]	5 (13.9) [0]
Erythema	1 (3.3) [0]	0	1 (3.3) [0]	3 (13.0) [0]	0	0	0	0	0
Injection site swelling	2 (6.7) [0]	5 (16.7) [0]	5 (16.7) [4.2]	3 (13.0) [0]	0	5 (16.7) [0]	5 (16.7) [0]	10 (30.0) [0]	2 (5.6) [0]
Headache	5 (16.7) [0]	12 (40.0) [0]	9 (37.5) [0]	7 (30.4) [0]	5 (14.3) [0]	5 (16.7) [0]	5 (16.7) [0]	7 (23.3) [6.7]	6 (16.7) [0]
Fatigue	8 (26.7) [0]	13 (43.3) [0]	14 (58.3) [4.2]	8 (34.8) [0]	7 (20.0) [0]	1 (3.3) [0]	4 (13.3) [0]	3 (10.0) [0]	2 (5.6) [0]
Myalgia	16 (53.3) [0]	17 (56.7) [0]	17 (70.9) [4.2]	12 (52.2) [0]	1 (2.9) [0]	3 (10.0) [0]	6 (20.0) [0]	8 (26.7) [0]	6 (16.7) [0]
Arthralgia	0	2 (6.7) [0]	4 (16.7) [0]	2 (8.7) [0]	1 (2.9) [0]	2 (6.7) [0]	3 (10.0) [0]	3 (10.0) [0]	4 (11.1) [0]
Nausea	0	1 (3.3) [0]	5 (20.8) [0]	1 (4.3) [0]	0	1 (3.3) [0]	1 (3.3) [0]	1 (3.3) [0]	1 (2.8) [0]
Fever	1 (3.3) [0]	1 (3.3) [0]	0	2 (8.7) [0]	0	0	1 (3.3) [0]	0	0
Dose 2	n = 28	n = 29	NA	n = 23	n = 27	n = 30	n = 30	n = 30	n = 36
Injection site pain	22 (78.6) [0]	27 (93.1) [0]	NA	20 (87.0) [0]	3 (11.1) [0]	14 (46.7) [0]	13 (43.3) [0]	22 (73.3) [10.0]	2 (5.6) [0]
Erythema	0	0	NA	4 (17.4) [8.7]	0	0	0	0	0
Injection site swelling	2 (7.1) [0]	4 (13.8) [0]	NA	3 (13.0) [0]	0	3 (10.0) [0]	6 (20.0) [0]	6 (20.0) [0]	1 (2.8) [0]
Headache	4 (14.3) [0]	14 (48.3) [0]	NA	16 (69.6) [0]	6 (22.2) [3.7]	3 (10.0) [0]	2 (6.7) [3.3]	8 (26.7) [6.7]	1 (2.8) [0]
Fatigue	8 (28.6) [0]	13 (44.8) [0]	NA	11 (47.8) [0]	4 (14.8) [0]	1 (3.3) [0]	3 10.0 [0]	4 (13.3) [0]	0
Myalgia	14 (50.0) [0]	17 (58.6) [0]	NA	11 (47.8) [0]	1 (3.7) [0]	3 (10.0) [0]	4 (13.3) [0]	8 (26.7) [3.3]	0
Arthralgia	0	2 (6.9) [0]	NA	7 (30.4) [0]	1 (3.7) [0]	2 (6.7) [0]	1 (3.3) [0]	6 (20.0) [3.3]	0
Nausea	1 (3.6) [0]	1 (3.4) [0]	NA	3 (13.0) [0]	0	0	0	1 (3.3) [0]	0
Fever	1 (3.6) [0]	2 (6.9) [0]	NA	4 (17.4) [0]	1 (3.7) [0]	0	0	6 (20.0) [6.7]	0

AE, adverse event; IM, intramuscular; NA, not applicable.

[a] Data represent n participants reporting any solicited AE (% of any solicited AEs) [% severe solicited AEs] in the safety population.

[b] Participants receiving 75 µg H10N8 vaccine did not receive a second dose.
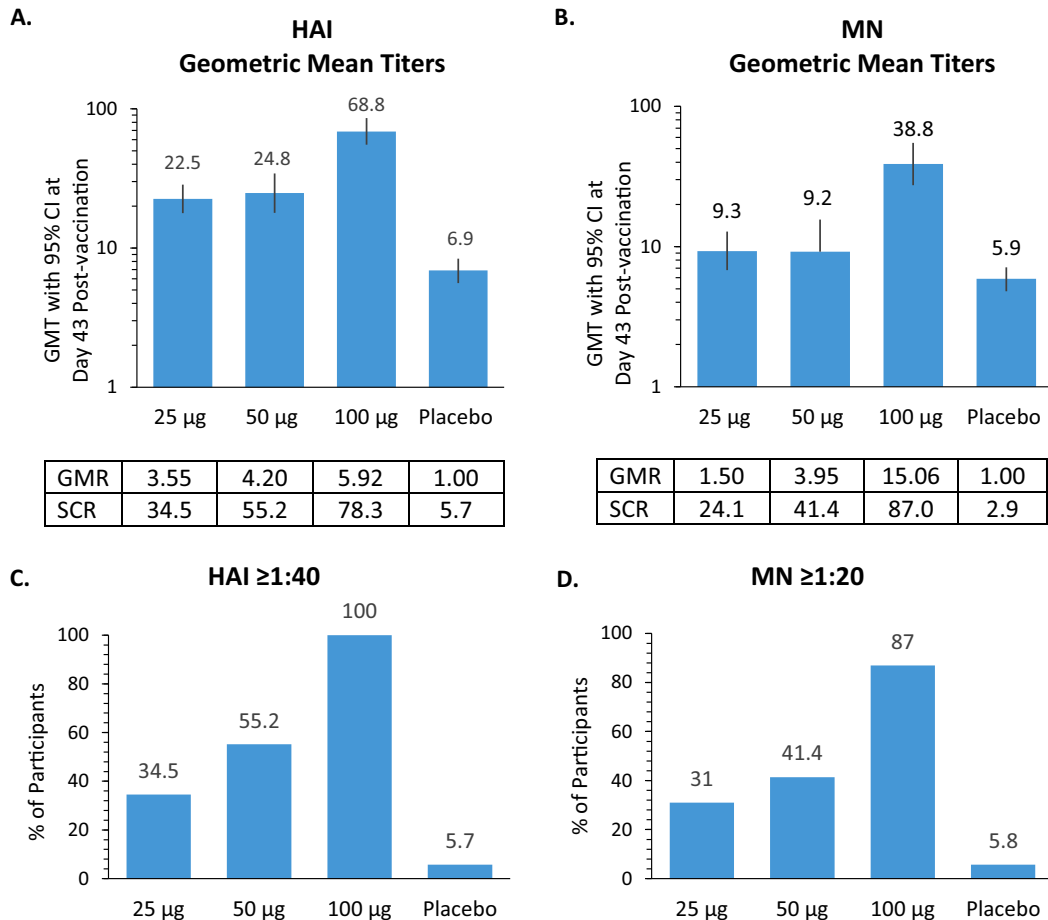

Fig. 3. H10N8 vaccine HAI and MN results at 3 weeks (day 43) after the second IM vaccination at day 21. (A) HAI GMTs, (B) MN GMTs, (C) HAI seroprotective rates (titer ≥ 1:40), and (D) MN seroconversion rates (titer ≥ 1:20) are shown. Error bars represent 95% confidence intervals. HAI, hemagglutination inhibition; MN, microneutralization; GMT, geometric mean titer, GMR, geometric mean ratio (day 43 post-vaccination titer/day 1 pre-vaccination titer); SCR, seroconversion rate (% of participants who achieved seroconversion).
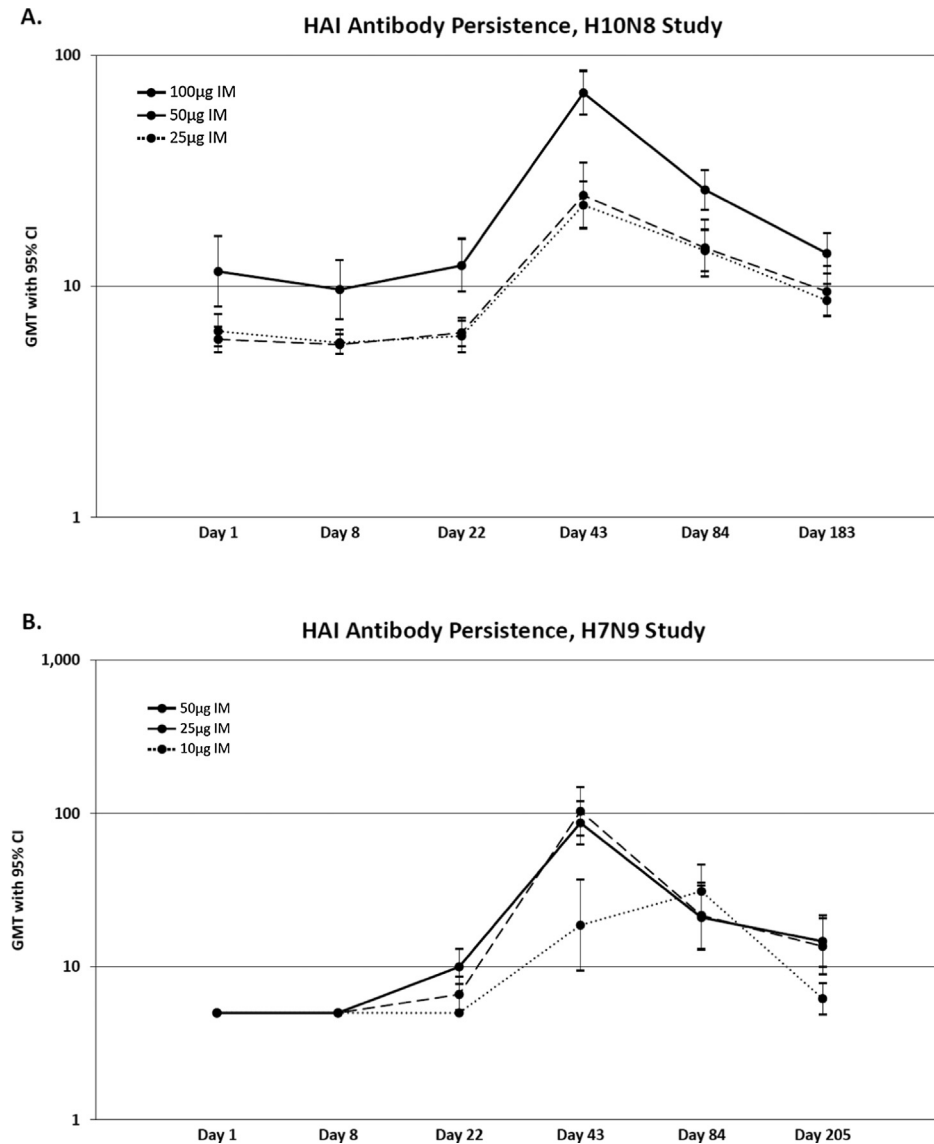
Fig. 4. H10N8 and H7N9 HAI antibody persistence up to 6 months after vaccine doses administered at day1 and day 22. HAI GMT for (A) H10N8 100 μg and (B) H7N9 25 μg dose groups are shown through day 183 (H10N8) or day 205 (H7N9). HAI, hemagglutination inhibition; GMT, geometric mean titers.

25-μg dose. MN GMT increased from 9 to 453 and 7 to 1280 at the 10- and 25-μg dose levels, respectively (eTable 2, supplemental materials).

Significant HA-specific cell-mediated responses were not detected by interferon-γ ELISPOT in either study (data not shown).

4. Discussion

These findings demonstrate the ability of mRNA vaccines to elicit robust humoral immune responses in healthy adults against H10N8 and H7N9 influenza viruses without adjuvantation [21]. Our studies demonstrate proof-of-concept that LNP-formulated mRNA provides an effective vaccine platform.

Low immune responses observed with unadjuvanted vaccines and low HAI titers seen with natural infection suggest that the HA protein of H7N9 is poorly immunogenic [21,22]. Other H7N9 vaccine candidates have required adjuvantation to elicit acceptable seroconversion and seroprotection rates [23]. Without adjuvant, HAI GMTs and seroconversion rates for these candidates were low (40–47% seroconversion, GMTs 24.1–32.8) [23]. The highest seroconversion rates (96% HAI, 93% MN) were reported with an

AS03-adjuvanted vaccine [24] and were comparable to our H7N9 mRNA vaccine seroconversion rates of 36.0–89.7%, and HAI GMTs of 18.7–87.0 (although intrinsic variability in HAI assays precludes direct comparisons). The HA protein, particularly H7N9 HA, is not predicted to be a robust T-cell antigen [21], perhaps explaining the lack of significant HA-specific cell-mediated responses in our studies.

In addition, our H7N9 mRNA vaccine showed HAI titers that were detectable and persistent 6 months post-vaccination, suggesting the development of memory B-cell responses. A rapid and high anamnestic-like immune response was observed in participants with undetectable HAI titers 43 days after the first 10-μg dose, suggesting robust antibody maturation [25]. Although based on results from only 5 participants, post-vaccination titers at 6 months exceeded the level of immunity observed after 2 doses 3 weeks apart at the 10- and 25-μg dose levels, suggesting that a day 1, month 6 immunization schedule in pandemic settings could confer sufficient protective immunity.

To our knowledge, no other H10N8 vaccine has been evaluated; therefore, no immunological benchmark for vaccine response exists. High seroconversion rates observed in our study are consis-
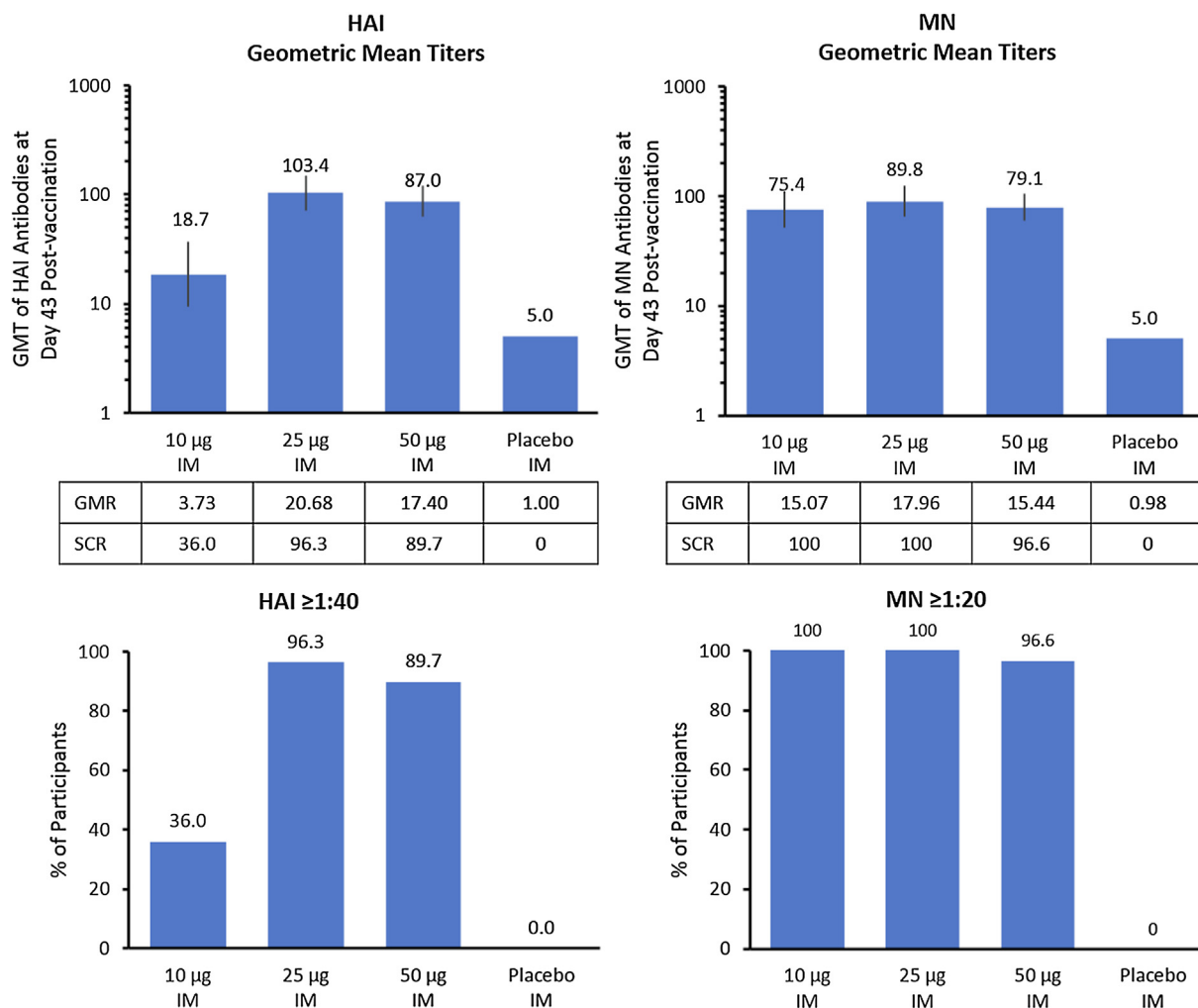
Error bars represent 95% CI. GMR = geometric mean ratio (day 43 post-vaccination/day 1 pre-vaccination);
SCR = seroconversion rate (% of participants who achieved seroconversion)

Fig. 5. H7N9 HAI and MN results at 3 weeks (day 43) after the second IM vaccination at day 21. (A) HAI GMTs, (B) MN GMTs, (C) HAI seroprotective rates (titer ≥ 1:40), and (D) MN seroconversion rates (titer ≥ 1:20) are shown. Error bars represent 95% confidence intervals. HAI, hemagglutination inhibition; MN, microneutralization; GMT, geometric mean titer, GMR, geometric mean ratio (day 43 post-vaccination titer/day 1 pre-vaccination titer); SCR, seroconversion rate (% of participants who achieved seroconversion).

tent with a similarly immunogenic vaccine to H7N9, albeit requiring a higher dose. Overall, for doses up to 100 μg, safety and reactogenicity profiles for our H10N8 and H7N9 vaccines were comparable to licensed adjuvanted and unadjuvanted influenza vaccines [26–30]. The nature, severity, frequency, and patterns of AEs were consistent with those seen with other vaccinations [28–30].

A limitation in the H10N8 MN assay was the lack of availability of a live H10N8 strain; therefore, a surrogate quail virus (A/quail/1117/1965) with 91% homology for the HA protein was used for MN assays. This may have contributed to differences in dose levels required to elicit ~100% seroconversions. Although HAI and MN titers correlated with levels expected to provide protection with seasonal influenza vaccines, it is unknown if these titers are protective [23,31–34]. Though HAI and MN parameters are current standards for vaccine response, these tests may underestimate immunogenicity [35], and may not accurately estimate protective immunity for pandemic influenza strains [14,36]. However, based on these tests, our mRNA vaccines elicited some of the highest seroprotective and seroconversion rates observed for influenza vaccines.

Both influenza strains A/H7N9 and A/H10N8 are serious potential threats to public health, which emphasizes the need for effective, rapidly deployable vaccines. Recent mechanistic studies with the mRNA vaccine platform [37,38] confirm translatability from preclinical studies, and safety data from a non-LNP-formulated vaccine [39] provide further support for this new class of vaccines. These phase 1 studies demonstrate both safety and robust immune responses to mRNA vaccines against H10N8 and H7N9 influenza viruses, and support the potential of mRNA to deliver a vaccine platform with precision, speed, adaptability, and scalability.

Author disclosures

R. Feldman has nothing to disclose. R Fuhr is an employee of PAREXEL International, a contract research organization that was sponsored to conduct this study. I. Smolenov, A. Ribeiro, L. Panther, M. Watson, J.J. Senn, M. Smith, Ö. Almarsson, H.S. Pujar, M.E. Laska, J. Thompson, T. Zaks, and G. Ciaramella are employees of Moderna Therapeutics. G. Ciaramella also has a patent 9872900 licensed to Modera Therapeutics.

Author contributions

G. Ciaramella led the projects. G. Ciaramella and T. Zaks designed the studies. R.A. Feldman and R. Fuhr were the principal investigators for the H7N9 and the H10N8 studies, respectively, and contributed equally to this work. A. Ribeiro and L. Panther were responsible for the clinical operations of the studies. I. Smolenov, M. Watson, and T. Zaks had overall supervision for the clinical and safe conduct of the studies. Ö. Almarsson, M. Smith, J.S. Pujar, and M.E. Laska were responsible for mRNA process and formulation development. J.J. Senn conducted GLP toxicology studies. J. Thompson and M.E. Laska were responsible for process development for the vaccine manufacturing. I. Smolenov and G. Ciaramella reviewed and analyzed the data and wrote the manuscript. All authors reviewed and approved the final version of this manuscript for publication.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.04.074.

References

[1] Pascua PN, Choi YK. Zoonotic infections with avian influenza A viruses and vaccine preparedness: a game of "mix and match". Clin Exp Vaccine Res 2014;3(2):140–8.
[2] Vachieri SG, Xiong X, Collins PJ, Walker PA, Martin SR, Haire LF, et al. Receptor binding by H10 influenza viruses. Nature 2014;511(7510):475–7.
[3] Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, et al. Human infection with a novel avian-origin influenza A (H7N9) virus. N Engl J Med 2013;368(20):1888–97.
[4] World Health Organization. Human cases of influenza at the human-animal interface, January 2015-April 2017. Wkly Epidemiol Rec 2017;92(33):460–75.
[5] Ke C, Mok CKP, Zhu W, Zhou H, He J, Guan W, et al. Human infection with highly pathogenic avian influenza A(H7N9) virus. China. Emerg Infect Dis 2017;23(8):1332–40.
[6] Fineberg HV. Pandemic preparedness and response–lessons from the H1N1 influenza of 2009. N Engl J Med 2014;370(14):1335–42.
[7] Borse RH, Shrestha SS, Fiore AE, Atkins CY, Singleton JA, Furlow C, et al. Effects of vaccine program against pandemic influenza A(H1N1) virus, United States, 2009–2010. Emerg Infect Dis 2013;19(3):439–48.
[8] Larson RC, Teytelman A. Modeling the effects of H1N1 influenza vaccine distribution in the United States. Value Health 2012;15(1):158–66.
[9] Yen C, Hyde TB, Costa AJ, Fernandez K, Tam JS, Hugonnet S, et al. The development of global vaccine stockpiles. Lancet Infect Dis 2015;15(3):340–7.
[10] World Health Organization. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness. Wkly Epidemiol Rec 2014;89(11):105–15.
[11] Cohen J. Why is the flu vaccine so mediocre? Science 2017;357(6357):1222–3.
[12] Wu NC, Zost SJ. A structural explanation for the low effectiveness of the seasonal influenza H3N2 vaccine. PLoS Pathog 2017;13(10):e1006682.
[13] Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. Mol Ther 2017;25(6):1316–27.
[14] US Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry. Clinical data needed to support the licensure of seasonal inactivated influenza vaccines. (https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091990.pdf).
[15] Richner JM, Himansu S, Dowd KA, Butler SL, Salazar V, Fox JM, et al. Modified mRNA vaccines protect against Zika Virus infection. Cell 2017;168(6). 1114-25.e10.
[16] US Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. (http://www.fda.gov/downloads/drugs/guidances/ucm078932.pdf).
[17] US Food and Drug Administration, Center for Biologics Evaluation and Research. Guidance for Industry. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. (https://wayback.archive-it.org/7993/20180124164056/https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-bio-gen/documents/document/ucm091977.pdf).
[18] Webster R, Cox N, Stohr K. World Health Organization Manual on Animal Influenza Diagnosis and Surveillance. (http://www.who.int/csr/resources/publications/influenza/whocdscsrncs20025rev.pdf).
[19] Kallen KJ, Heidenreich R, Schnee M, Petsch B, Schlake T, Thess A, et al. A novel, disruptive vaccination technology: self-adjuvanted RNActive® vaccines. Hum Vaccin Immunother 2013;9(10):2263–76.
[20] The European Agency for the Evaluation of Medicinal Products. Note for guidance on harmonisation of requirements for influenza vaccines. (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003945.pdf).
[21] De Groot AS, Ardito M, Terry F, Levitz L, Ross T, Moise L, et al. Low immunogenicity predicted for emerging avian-origin H7N9: implication for influenza vaccine design. Hum Vaccin Immunother 2013;9(5):950–6.
[22] Guo L, Zhang X, Ren L, Yu X, Chen L, Zhou H, et al. Human antibody responses to avian influenza A(H7N9) virus, 2013. Emerg Infect Dis 2014;20(2):192–200.
[23] Hu Z, Jiao X, Liu X. Antibody immunity induced by H7N9 avian influenza vaccines: evaluation criteria, affecting factors, and implications for rational vaccine design. Front Microbiol 2017;8(1898).
[24] Jackson LA, Campbell JD, Frey SE, Edwards KM, Keitel WA, Kotloff KL, et al. Effect of varying doses of a monovalent H7N9 influenza vaccine with and without AS03 and MF59 adjuvants on immune response: a randomized clinical trial. JAMA 2015;314(3):237–46.
[25] Ledgerwood JE, Wei CJ, Hu Z, Gordon IJ, Enama ME, Hendel CS, et al. DNA priming and influenza vaccine immunogenicity: two phase 1 open label randomised clinical trials. Lancet Infect Dis 2011;11(12):916–24.
[26] Della Cioppa G, Nicolay U, Lindert K, Leroux-Roels G, Clement F, Castellino F, et al. A dose-ranging study in older adults to compare the safety and immunogenicity profiles of MF59®-adjuvanted and non-adjuvanted seasonal influenza vaccines following intradermal and intramuscular administration. Hum Vaccin Immunother 2014;10(6):1701–10.
[27] Greenberg ME, Lai MH, Hartel GF, Wichems CH, Gittleson C, Bennet J, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. N Engl J Med 2009;361(25):2405–13.
[28] Moa AM, Chughtai AA, Muscatello DJ, Turner RM, MacIntyre CR. Immunogenicity and safety of inactivated quadrivalent influenza vaccine in adults: a systematic review and meta-analysis of randomised controlled trials. Vaccine 2016;34(35):4092–102.
[29] Reisinger KS, Baxter R, Block SL, Shah J, Bedell L, Dull PM. Quadrivalent meningococcal vaccination of adults: phase III comparison of an investigational conjugate vaccine, MenACWY-CRM, with the licensed vaccine, Menactra. Clin Vaccine Immunol 2009;16(12):1810–5.
[30] Roman F, Vaman T, Kafeja F, Hanon E, Van Damme P. AS03(A)-Adjuvanted influenza A (H1N1) 2009 vaccine for adults up to 85 years of age. Clin Infect Dis 2010;51(6):668–77.
[31] Coudeville L, Bailleux F, Riche B, Megas F, Andre P, Ecochard R. Relationship between haemagglutination-inhibiting antibody titres and clinical protection against influenza: development and application of a bayesian random-effects model. BMC Med Res Methodol 2010;10(18).
[32] Cox RJ. Correlates of protection to influenza virus, where do we go from here? Hum Vaccin Immunother 2013;9(2):405–8.
[33] Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. Virus Res 2004;103(1–2):133–8.
[34] Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg (Lond) 1972;70(4):767–77.
[35] Kamal RP, Blanchfield K, Belser JA, Music N, Tzeng WP, Holiday C, et al. Inactivated H7 influenza virus vaccines protect mice despite inducing only low levels of neutralizing antibodies. J Virol 2017;91(20).
[36] Weir JP, Gruber MF. An overview of the regulation of influenza vaccines in the United States. Influenza Other Respir Viruses 2016;10(5):354–60.
[37] Lindgren G, Ols S, Liang F, Thompson EA, Lin A, Hellgren F, et al. Induction of robust B cell responses after influenza mRNA vaccination is accompanied by circulating hemagglutinin-specific ICOS+ PD-1+ CXCR3+ T follicular helper cells. Front Immunol 2017;8(1539).
[38] Liang F, Lindgren G, Lin A, Thompson EA, Ols S, Rohss J, et al. Efficient targeting and activation of antigen-presenting cells in vivo after modified mRNA vaccine administration in Rhesus Macaques. Mol Ther 2017;25(12):2635–47.
[39] Alberer M, Gnad-Vogt U, Hong HS, Mehr KT, Backert L, Finak G, et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet 2017;390(10101):1511–20.