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An mRNA-based seasonal influenza vaccine in adults: Results of two phase 3 randomized clinical trials and correlate of protection analysis of hemagglutination inhibition titers

Boris Kandinov[a*], Mieke Soens[a*], Wenmei Huang[a], Conrado Llapur[b], David Ensz[c], Brandon Essink[d], Carlos Fierro[e], Jignesh Vakil[a], Alicia Pucci[a], Jia Guo[a], Sinead Rudden[a], Kristi Hall[a], Bryony Hicks[f], Kristin Schaefers[a], Honghong Zhou[a], Chong Ma[a], Lingyi Zheng[a], Andrei Avanesov[a], Yoonyoung Park[a], Evelyn Du[a], Jacqueline Miller[a], Jintanat Ananworanich[a#], and Raffael Nachbagauer[a]

[a]Development, Moderna Inc, Cambridge, MA, USA; [b]Clínica Mayo de UMCB SRL, Tucuman, Argentina; [c]Meridian Clinical Research, Sioux City, IA, USA; [d]Velocity Clinical Research, Omaha, NE, USA; [e]Johnson County Clin Trials, Lenexa, KS, USA; [f]Development, Moderna Biopharma Canada Corporation, Toronto, ON, Canada

ABSTRACT

The safety, immunogenicity, and efficacy of the original formulation of the investigational mRNA-1010 vaccine for seasonal influenza were investigated in two randomized, active-controlled, phase 3 trials in adults (NCT05415462 and NCT05566639), and the results were used to evaluate hemagglutination inhibition (HAI) titers as correlates of risk and protection against influenza-like illness. mRNA-1010 (50-µg) demonstrated an acceptable reactogenicity and safety profile among the >14,000 adult participants vaccinated in both trials. The efficacy profile of mRNA-1010 was generally reflective of immunogenicity findings, with higher immune responses against influenza A strains and lower responses against influenza B strains relative to an active comparator (licensed inactivated influenza vaccine). An analysis of HAI titers as a correlate of protection against influenza infection provided support for its use as a surrogate endpoint for mRNA-1010, similar to licensed influenza vaccines. These findings support further optimization and development of mRNA-1010 against seasonal influenza.

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Seasonal influenza; mRNA vaccine; correlate of protection; immunogenicity; safety; reactogenicity

Introduction

Despite the availability of licensed vaccines, influenza continues to cause a substantial disease burden, with approximately one billion cases worldwide each year.[1] Currently, seasonal influenza vaccines are manufactured using egg-, cell culture–, or recombinant protein–based platforms, and their vaccine effectiveness has ranged from 19% to 60% among the United States general population between 2009 and 2024.[2] Seasonal influenza vaccines based on the messenger RNA (mRNA) platform are currently under investigation and have several advantages over current vaccine approaches.[3] The mRNA platform could increase antigenic fidelity against circulating strains by potentially enabling selection of candidate vaccine viruses closer to seasonal onset,[3–5] and avoiding aberrant mutations that can occur with egg-based vaccine platforms.[3,6,7]

Seasonal influenza is caused by infection with influenza A and B viruses, which are RNA viruses consisting of eight segments encoding for viral proteins.[8,9] Among these, the hemagglutinin (HA) surface glycoprotein has a vital role in infecting host cells and is a major vaccine target.[8,9] Antibodies directed against the immunodominant globular head domain of influenza HA can prevent the virus from binding to its receptor and therefore prevent host cell infection.[10] Thus, induction of serum strain–specific antibodies against HA, via infection or vaccination, can presumably mediate protection from subsequent (re)infection.[10] Serum antibodies that bind to HA are routinely measured using the hemagglutination inhibition (HAI) assay, with the HAI titer widely recognized as a surrogate marker for protection against influenza infection and an HAI titer of 40 generally considered to provide ≥50% reduced risk of influenza illness.[9,11–13] The use of the HAI titer as a benchmark for protection has been established for clinical assessment and licensure of influenza vaccines, and thus understanding the predictiveness of this immune correlate to newer vaccine modalities such as mRNA is of utmost importance.[4,14,15]

mRNA-1010 is an investigational vaccine containing mRNAs that encode the HA proteins of seasonal influenza strains recommended by the World Health Organization (WHO).[16] The vaccine has been evaluated in multiple clinical trials, and its formulation has undergone significant optimizations and improvements over the course of investigation. Initially, an original formulation of mRNA-1010 was tested in early phase 1/2 and phase 3 clinical trials. In the phase 1/2 trial (NCT04956575), original mRNA-1010 showed an

CONTACT Raffael Nachbagauer Raffael.Nachbagauer@modernatx.com Development, Moderna Inc, 325 Binney Street, Cambridge, MA 02142, USA.
*Contributed equally.
#Employee at the time of the study.
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acceptable safety and reactogenicity profile among United States adults aged ≥18 years and elicited robust humoral and CD4+ T-cell responses.[16,17] The original mRNA-1010 formulation was then also investigated in two phase 3 trials, including a phase 3 safety and immunogenicity study in adults aged ≥18 years (P301; NCT05415462) and a phase 3 efficacy study in adults aged ≥50 years (P302; NCT05566639). The P301 trial was initiated in the 2022 Southern hemisphere (SH) season to evaluate the safety and immunogenicity of mRNA-1010. As the COVID-19 pandemic had impacted seasonal influenza virus circulation patterns, this trial aimed to establish vaccine safety and immunogenicity (primary objectives) prior to demonstrating efficacy in a subsequent confirmatory trial that would occur during a more typical influenza season. Efficacy was a secondary objective of the P301 trial, enabling a detailed immune correlate analysis on the predictiveness of vaccination-induced HAI titers for protection against infection. With the resurgence of influenza virus circulation in the 2022 SH season, the P302 trial was subsequently initiated to evaluate relative vaccine efficacy (rVE) and safety during the 2022–2023 Northern hemisphere (NH) season prior to the availability of P301 trial results. After conducting these trials, the previously described optimization of the mRNA-1010 formulation was completed, and a phase 3 trial was initiated in 2023 (P303; NCT05827978).

In this article, we report on the safety, immunogenicity, and vaccine efficacy findings on the original mRNA-1010 formulation from the P301 and P302 trials in adults aged ≥18 years or aged ≥50 years, respectively. Further, we present the immune correlate analysis based on the P301 trial to evaluate HAI titers as a surrogate endpoint for rVE of mRNA-1010 and mRNA-based influenza vaccines in general.

Methods

Clinical trial design and participants

The P301 trial is a phase 3, randomized, active-controlled, observer-blind study (NCT05415462) designed to evaluate the immunogenicity and safety of mRNA-1010 and was conducted in 53 sites across five countries in the Asia–Pacific and Latin America. Adults aged ≥18 years were randomly assigned to receive a single dose of mRNA-1010 (50 µg) or licensed quadrivalent inactivated influenza vaccine (Fluarix® Tetra, GlaxoSmithKline) prior to or during the SH 2022 influenza vaccination campaign period. Eligibility criteria and blinding details are listed in the Supplement. Random assignment to vaccine groups was stratified by influenza vaccine status in the prior 12 months (received or not received) and age group (18–49, 50–64, or ≥65 years).

The P302 trial is a phase 3, randomized, active-controlled, observer-blind study (NCT05566639) designed to evaluate the safety and rVE of mRNA-1010 and was conducted in 230 sites across 10 NH countries. Adults aged ≥50 years were randomly assigned to receive a single dose of mRNA-1010 (50 µg) or licensed quadrivalent inactivated influenza vaccine (Fluarix®/Influsplit®, GlaxoSmithKline) during the NH 2022–2023 influenza vaccination campaign period. Eligibility criteria and blinding details are listed in the Supplement. Random assignment to vaccine groups was stratified by influenza vaccine status in the prior 12 months (received or not received) and age group (50–64 or ≥65 years).

Both studies were conducted in accordance with the protocols, applicable laws and regulatory requirements, as well as International Council for Harmonisation Good Clinical Practice guidelines, and the consensus ethical principles derived from international guidelines, including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines. The studies were registered with ClinicalTrials.gov (NCT05415462 [P301]; NCT05566639 [P302]) and the European Union Clinical Trials Register (EudraCT: 2022-001638-12 [P302]) and approved by the relevant central and local institutional review boards and independent ethics committees (full list available upon request). Written informed consent was obtained from all participants before enrollment.

Vaccines

mRNA-1010 included mRNAs encoding HA surface glycoproteins of four influenza virus strains formulated in lipid nanoparticles. The active comparator for both trials (Fluarix®/Influsplit®) was selected due to its wide availability across geographic regions. In P301, mRNA-1010 strain selection was based on WHO recommendations for the SH 2022 cell- or recombinant-based vaccines (A/H1N1, A/Wisconsin/588/2019; A/H3N2, A/Darwin/6/2021; B/Victoria, B/Austria/1359417/2021; B/Yamagata, B/Phuket/3073/2013). In P302, mRNA-1010 strain selection was based on WHO recommendations for the NH 2022–2023 cell- or recombinant-based vaccines, which did not change from the SH 2022 season. Both mRNA-1010 and active comparator vaccines were administered intramuscularly into the deltoid muscle (preferably nondominant arm) as a single 0.5-mL injection.

Clinical trial objectives

For study P301, the co-primary objectives were the safety and reactogenicity of mRNA-1010 as well as the humoral immunogenicity of mRNA-1010 relative to that of an active comparator against vaccine-matched influenza A and B strains at Day 29. Evaluation of rVE to prevent influenza caused by any strain was a secondary objective.

For study P302, the co-primary objectives were to evaluate the safety and reactogenicity of mRNA-1010; and to evaluate the relative vaccine efficacy of mRNA-1010 as compared to an active comparator against influenza caused by influenza A or B strains. A secondary objective was to evaluate the humoral immunogenicity of mRNA-1010 relative to that of an active comparator against vaccine-matched influenza A and B strains at Day 29 in a subset of participants.

Safety assessments

Safety endpoints for both trials included solicited local and systemic adverse reactions (ARs) through 7 days after study vaccination; unsolicited adverse events (AEs) through 28 days after study vaccination; and medically attended AEs (MAAEs),

AEs of special interest (AESIs), serious AEs (SAEs), and AEs leading to study discontinuation through to the end of the study (Day 361). Participants used an electronic diary to record solicited local (injection site pain, erythema, swelling/induration, axillary swelling/tenderness) and systemic (headache, fatigue, myalgia, arthralgia, nausea/vomiting, chills, fever) ARs.

Immunogenicity assessments

Blood samples at baseline and on Day 29 were taken from all participants in P301 and from a subset of approximately 1000 participants in P302. Immunogenicity endpoints (primary for P301; secondary for P302) included HAI geometric mean titers (GMTs) at Day 29 and the proportion of participants reaching seroconversion at Day 29 as measured by HAI. Standard methods were used for the HAI assay,[16] as described in the Supplement. Seroconversion was defined as the proportion of participants with either a pre-vaccination HAI titer <1:10 and a post-vaccination titer ≥1:40, or a pre-vaccination HAI titer ≥1:10 and at least a four-fold rise in post-vaccination HAI titer.

Efficacy assessments

Key efficacy endpoints (secondary for P301; primary for P302) included first episode of the reverse transcriptase polymerase chain reaction (RT-PCR)-confirmed protocol-defined influenza-like illness (ILI) that begins at least 14 days after vaccination through Day 181 (Month 6) or end of influenza season caused by any seasonal influenza A or B strains, regardless of antigenic match to strains selected for the seasonal vaccine. In P301, protocol-defined ILI was the occurrence of ≥1 respiratory symptom concurrently with ≥1 systemic symptom, or the occurrence of ≥2 respiratory symptoms. Respiratory symptoms were sore throat, cough/rhinorrhea/nasal congestion, sputum production, wheezing, difficulty breathing; systemic symptoms were body temperature > 37.2°C (>99°F), chills, tiredness, headache, myalgia, nausea/vomiting, and diarrhea. In P302, protocol-defined ILI was the occurrence of body temperature >37.5°C (>99.5°F) accompanied by ≥1 respiratory symptom (sore throat, cough, sputum production, wheezing, difficulty breathing). Throughout the studies, participants who developed symptoms consistent with protocol-defined ILI had nasopharyngeal swabs collected for testing.

Statistical analyses

Sample size determinations are described in the Supplement. In both trials, safety (except for solicited ARs) was assessed in the safety population (all randomly assigned participants who received study vaccination); solicited ARs were assessed in all participants in the safety population who contributed any solicited AR data (solicited safety population). Participants were included in the group that corresponded to the vaccine actually received.

Immunogenicity was assessed for P301 in the per-protocol immunogenicity population, which included all randomly assigned participants who received the planned study vaccination, complied with the immunogenicity testing schedule, had no major protocol deviations that impacted key data, and excluded participants with RT-PCR–confirmed influenza between Days 1–29. The per-protocol immunogenicity subset was similarly defined for P302 in a subset of approximately 1000 participants. In the P301 trial, noninferiority tests were prespecified and were evaluated for A strains and B strains separately, each with an alpha of 0.025. Noninferiority for A strains (or B strains) was considered demonstrated if the lower bounds of the 97.5% confidence intervals (CIs) of the geometric mean ratio (GMR) exceeded 0.667 based on a noninferiority margin of 1.5 and the lower bounds of the 97.5% CIs of the seroconversion rate (SCR) difference exceeded −10% based on a noninferiority margin of 10% for both A strains (or for both B strains).

In both trials, an analysis of covariance (ANCOVA) model was performed with log-transformed HAI titers at Day 29 as the dependent variable, vaccine group as the fixed variable, log-transformed baseline HAI titers as a fixed covariate, and adjusting for stratification factors (age group and influenza vaccine status in the previous season). The geometric least squares mean (GLSM) and corresponding 95% CIs in log-transformed scale, as estimated by the model, were back-transformed to obtain an estimate of the GMT. The GMR was estimated by the ratio of the GLSM (mRNA-1010 vs comparator) and provided with corresponding two-sided 97.5% CIs (P301) or 95% CIs (P302). In both trials, the number and percentage of participants with seroconversion were determined alongside two-sided 95% CIs using the Clopper-Pearson method at Day 29. The 97.5% CI (P301) or 95% CI (P302) of the SCR difference was calculated using the Miettinen-Nurminen (score) method.

Efficacy was assessed in the modified intent-to-treat population (P301) and in the per-protocol population (P302). The modified intent-to-treat population (P301) included all participants who received study vaccination and who provided any follow-up for ILI beginning at least 14 days following administration of study vaccine. The per-protocol population (P302) included all participants who received study vaccination, except those who discontinued from the study prior to 14 days following study vaccination, and had no significant protocol deviations that could adversely impact efficacy. In P301, the incidence rate of the first occurrence of RT-PCR–confirmed protocol-defined ILI after vaccination was calculated as the number of participants with a case (ie, first occurrence of ILI at least 14 days after vaccination through Day 181 or end of influenza season) divided by the number of participants at risk, adjusted by person-years. The person-years were calculated as the time from randomization to the date of the first episode for participants with a case, or the time from randomization to the date of discontinuation or death or data cutoff (the later date of Day 181 or end of influenza season), whichever occurred first, for participants without a case. Relative VE was estimated as 100 × (1 – ratio of the incidence rate [mRNA-1010 vs active comparator] adjusting for person-years), and the 95% CI was computed using the exact method conditional upon the total number of cases, adjusted by person-years. To assess the primary efficacy endpoint in P302, a Cox proportional hazards regression model

was used to estimate the hazard ratio (HR), with vaccine group as a fixed effect and baseline stratification factors as strata variables; rVE was estimated using 100% × (1 − HR) along with the two-sided 95% CI and one-sided p-value for testing H_0^1: rVE ≤−10%. An interim analysis was planned around the middle of February 2023, when approximately 75% of the cases were expected to be accrued. The Lan-DeMets Pocock boundaries were pre-specified to control the overall type one error rate over the interim analysis and the final analysis. The interim analysis was conducted with 234 cases identified as of the data cutoff date of February 17, 2023, but the success criterion was not met. At the final analysis at the end of the season, the primary efficacy objective was considered met if the p-value for rejecting H_0^1: rVE ≤−10% was less than the nominal p-value (1.2%) based on the Lan-DeMets Pocock boundaries and the actual information fraction.

A correlate analysis was conducted in the P301 trial to evaluate Day 29 HAI antibody titers as a correlate of risk (CoR) and a correlate of protection (CoP) against ILI caused by each specific influenza virus strain. The study population included participants who had immunogenicity data, were in the per-protocol efficacy population, and were in the per-protocol immunogenicity population. Participants who had a strain-specific ILI event or were censored within 7 days after their Day 29 visit were excluded from CoP analysis, due to the concern that their Day 29 HAI titer may have been impacted by the potential influenza virus infection. ILI caused by influenza B was assumed to be B/Victoria based on epidemiologic data showing no circulation of B/Yamagata since 2020. To account for the potential confounding on the correlation between Day 29 HAI antibody titers and the ILI cases by baseline risk factors, the synthetic baseline risk scores were calculated based on the baseline covariates (based on a principal component analysis on seven baseline covariates: age, age group, sex, ethnicity, body mass index [BMI], BMI group, and prior influenza vaccination status). Multivariate Cox proportional hazards models were carried out for each of the influenza endpoints of ILI caused by A/H1N1, A/H3N2, and B/Victoria, respectively, adjusting for the baseline risk scores. Each of the three models incorporated vaccine group (mRNA-1010 or active comparator), Day 29 HAI titers for all four strains, and were adjusted by the baseline risk scores. Based on the fitted multivariate Cox proportional hazards models for ILI caused by each specific strain, the predictive risk of getting ILI caused by each strain was estimated (with bootstrap pointwise 95% CIs) by the assigned Day 29 A/H1N1, A/H3N2, and B/Victoria antibody titers adjusting by potential confounders in the model (non-strain-specific HAI titers and baseline risk scores).

Results

Trial populations

The P301 trial randomized 6102 participants between June 6, 2022, and August 26, 2022, to receive either original mRNA-1010 50 µg (n = 3045) or an active comparator (Fluarix® Tetra, GlaxoSmithKline) (n = 3057) (Figure 1); last participant last visit was September 4, 2023. Overall, 93.1% of participants completed the one-year study (median duration of participation was 355 days); the most common reasons for study discontinuation were participant withdrawal (3.8%) and loss to follow-up (2.2%). Among the participants who received study vaccination, the median age was 50 years and 57.8% were female, 56.9% were White, and 72.5% were Hispanic/Latino; 97.6% had not received an influenza vaccine in the past 12 months (Table 1). Participants were from Argentina (55.5%), the Philippines (22.6%), Colombia (17.1%), Australia (3.7%), and Panama (1.1%).

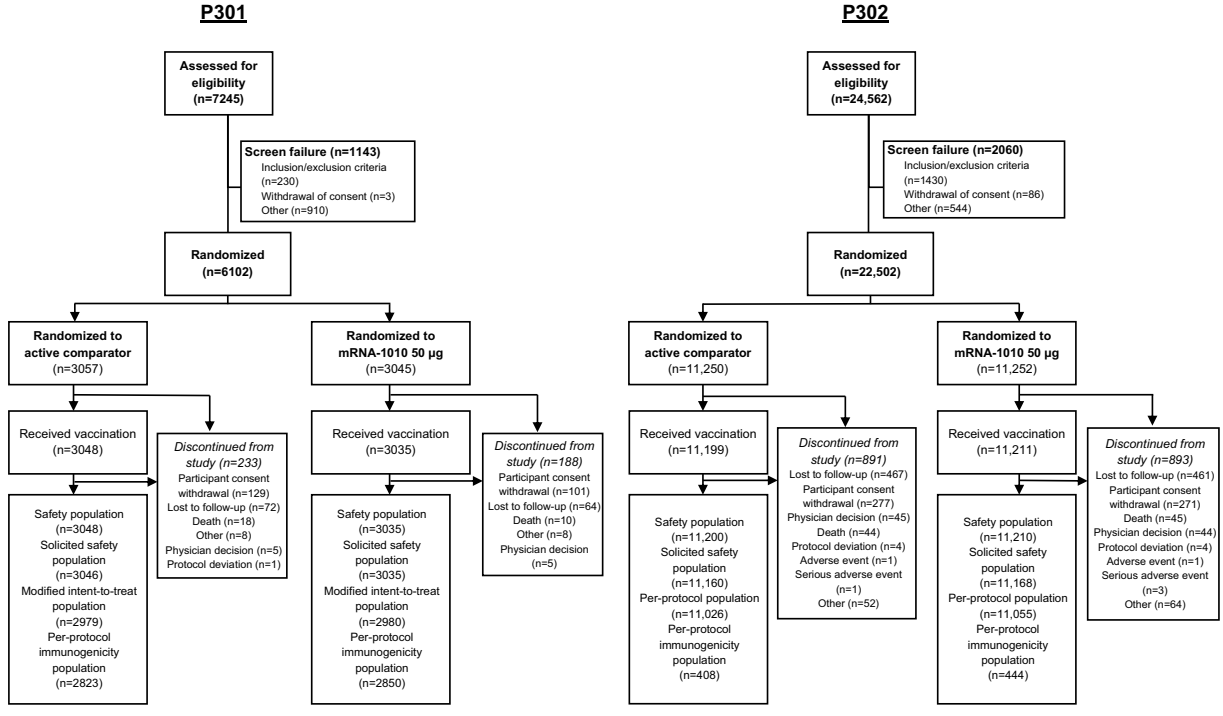

Figure 1. Participant disposition for the P301 and P302 studies. AE, adverse event.

Table 1. Baseline participant demographics/characteristics in the P301 and P302 studies (safety population).

	P301 Study		P302 Study	
	Active comparator (*N* = 3048)	mRNA-1010 50 µg (*N* = 3035)	Active comparator (*N* = 11,200)	mRNA-1010 50 µg (*N* = 11,210)
Age, y				
Mean ± SD	48.0 ± 16.5	48.0 ± 16.4	63.8 ± 8.4	63.9 ± 8.4
Median (range)	50 (18–93)	50 (18–98)	64 (50–96)	64 (50–99)
Sex, n (%)				
Male	1313 (43.1)	1256 (41.4)	4941 (44.1)	4975 (44.4)
Female	1735 (56.9)	1779 (58.6)	6259 (55.9)	6235 (55.6)
Race, n (%)				
White	1738 (57.0)	1725 (56.8)	8750 (78.1)	8820 (78.7)
Black/African American	18 (0.6)	15 (0.5)	1976 (17.6)	1941 (17.3)
Asian	706 (23.2)	707 (23.3)	280 (2.5)	252 (2.2)
American Indian or Alaska Native	319 (10.5)	304 (10.0)	55 (0.5)	57 (0.5)
Native Hawaiian or other Pacific Islander	3 (<0.1)	10 (0.3)	15 (0.1)	11 (<0.1)
Multiracial	216 (7.1)	220 (7.2)	37 (0.3)	47 (0.4)
Other/not reported/unknown/missing	48 (1.6)	54 (1.8)	87 (0.8)	82 (0.7)
Ethnicity, n (%)				
Hispanic or Latino	2213 (72.6)	2200 (72.5)	1828 (16.3)	1854 (16.5)
Not Hispanic or Latino	812 (26.6)	818 (27.0)	9248 (82.6)	9236 (82.4)
Not reported	6 (0.2)	1 (<0.1)	116 (1.0)	113 (1.0)
Unknown	17 (0.6)	16 (0.5)	8 (<0.1)	7 (<0.1)
BMI, kg/m^2				
Mean ± SD	27.2 ± 5.5	27.5 ± 5.7	30.0 ± 6.6	29.9 ± 6.5
Received influenza vaccine in the last season, n (%)				
Yes	70 (2.3)	74 (2.4)	4787 (42.7)	4802 (42.8)
No	2978 (97.7)	2961 (97.6)	6413 (57.3)	6408 (57.2)

BMI, body mass index; SD, standard deviation.

The P302 trial randomized 22,502 participants between September 14, 2022, and December 22, 2022, to receive either original mRNA-1010 50 µg (*n* = 11,252) or active comparator (Fluarix®/Influsplit®) (*n* = 11,250) (Figure 1); last participant last visit was January 2, 2024. Overall, 92.1% of participants completed the one-year study (median duration of participation was 354 days); the most common reasons for study discontinuation were loss to follow-up (4.1%) and participant withdrawal (2.4%). Among participants who received study vaccination, the median age was 64 years and 55.8% were female, 78.4% were White, and 82.5% were not Hispanic/Latino; 57.2% had not received an influenza vaccine in the previous season (Table 1). Participants were from the United States (79.2%), Bulgaria (6.1%), Canada (5.2%), Germany (3.5%), Estonia (2.5%), Poland (1.8%), Spain (0.6%), Taiwan (0.6%), the United Kingdom (0.5%), and Denmark (<0.1%).

Safety

Solicited adverse reactions

Safety and reactogenicity were primary objectives in both the P301 and P302 trials. In both studies, the overall rates of any solicited local and systemic ARs within 7 days of study vaccination were higher with original mRNA-1010 than active comparator and rates tended to decrease with increased age for both vaccination groups (Figure 2a,b). Most local and systemic solicited ARs were grade one or two in severity. Injection site pain was the most common local AR, and myalgia, headache, fatigue, and arthralgia were the most common solicited ARs in both studies (Figures S1 and S2).

Unsolicited adverse events

In the P301 trial, unsolicited AEs within 28 days after vaccination were reported for 800 participants (26.4%) receiving original mRNA-1010 and 749 participants (24.6%) receiving active comparator; few were considered vaccine-related by the investigator (0.7% and 1.0%, respectively) (Table 2). At the end of the study, median duration of safety follow-up was 355 (interquartile range [IQR], 350–364) days. During the study period, MAAEs considered vaccine-related by the investigator were reported in 14 participants (0.5%) in the original mRNA-1010 group and 20 participants (0.7%) in the active comparator group. In the original mRNA-1010 group, one participant had a SAE that was considered vaccine-related by the investigator (acute coronary syndrome [onset Day 3, resolved Day 6]); no SAEs were considered vaccine-related in the active comparator group. AESIs were reported in nine (0.3%) and 13 (0.4%) participants in the original mRNA-1010 and active comparator groups, respectively. No fatal AEs or AEs leading to study discontinuation were considered vaccine-related in either group.

In the P302 trial, unsolicited AEs within 28 days after vaccination were reported for 1351 participants (12.1%) receiving original mRNA-1010 and 1495 participants (13.3%) receiving active comparator; few were considered vaccine-related by the investigator (0.8% and 0.7%, respectively) (Table 2). At the end of the study, median duration of safety follow-up was 354 (IQR, 349–362) days. During the study period, MAAEs assessed as vaccine-related by the investigator were reported in 13 participants (0.1%) and 17 participants (0.2%) in the mRNA-1010 and active comparator groups, respectively. Two participants had SAEs that were considered vaccine-related by the investigator (pulmonary embolism [onset Day 9, resolved Day 17] and

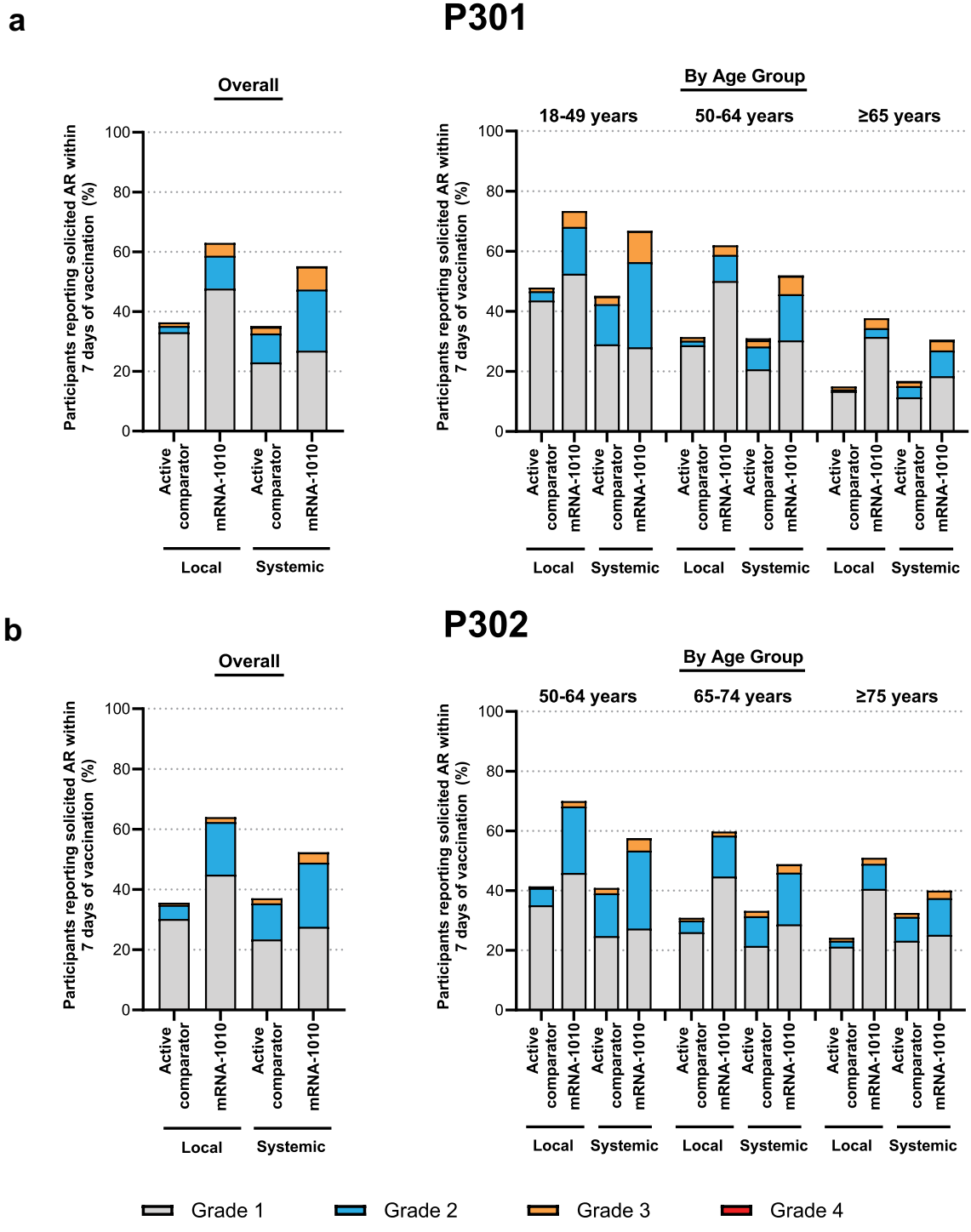

Figure 2. Participants with local and systemic adverse reactions within seven days after vaccination in the P301 study (a) and the P302 study (b) by grade, vaccine, and age group (solicited safety population). The solicited safety population consisted of all participants who received study vaccination and contributed any solicited AR data; in P301 this included 3035 mRNA-1010 recipients and 3046 comparator recipients, and in P302, this included 11,168 mRNA-1010 recipients and 11,160 comparator recipients. AR, adverse reaction.

angioedema [onset Day 5, resolved Day 5]), in the original mRNA-1010 group; no SAEs were considered vaccine-related in the active comparator group. AESIs were reported in 10 (<0.1%) and 13 (0.1%) participants in the original mRNA-1010 and active comparator groups, respectively; none was considered vaccine-related by the investigator. No fatal AEs or AEs leading to study discontinuation were considered vaccine-related in either group.

In both studies, the SAEs were heterogeneously distributed across organ systems without identified patterns or trends (Tables S1 and S2).

Immunogenicity

Humoral immunogenicity of the original mRNA-1010 relative to an active comparator against vaccine-matched influenza

Table 2. Summary of unsolicited adverse events (safety population).

	P301 study		P302 study	
	Active comparator (*N* = 3048)	mRNA-1010 50 µg (*N* = 3035)	Active comparator (*N* = 11,200)	mRNA-1010 50 µg (*N* = 11,210)
All unsolicited AEs, n (%)				
Within 28 days	749 (24.6)	800 (26.4)	1495 (13.3)	1351 (12.1)
Severe AEs	10 (0.3)	10 (0.3)	40 (0.4)	51 (0.5)
SAEs	15 (0.5)	13 (0.4)	52 (0.5)	63 (0.6)
Fatal	0	1 (<0.1)	5 (<0.1)	3 (<0.1)
MAAEs	454 (14.9)	479 (15.8)	642 (5.7)	578 (5.2)
Leading to study discontinuation	0	1 (<0.1)	5 (<0.1)	4 (<0.1)
AESI	4 (0.1)	1 (<0.1)	0	1 (<0.1)
Through to end of study[a]				
SAEs	131 (4.3)	132 (4.3)	495 (4.4)	518 (4.6)
Fatal	18 (0.6)	10 (0.3)	44 (0.4)	45 (0.4)
MAAEs	1481 (48.6)	1422 (46.9)	3166 (28.3)	3126 (27.9)
Leading to study discontinuation	17 (0.6)	10 (0.3)	47 (0.4)	49 (0.4)
AESI	13 (0.4)	9 (0.3)	13 (0.1)	10 (<0.1)
Related unsolicited AEs, n (%)				
Within 28 days	29 (1.0)	22 (0.7)	79 (0.7)	90 (0.8)
Severe	0	0	0	4 (<0.1)
SAEs	0	1 (<0.1)	0	2 (<0.1)
Fatal	0	0	0	0
MAAEs	17 (0.6)	13 (0.4)	16 (0.1)	12 (0.1)
Leading to study discontinuation	0	0	0	0
AESI	1 (<0.1)	1 (<0.1)	0	0
Through to end of study[a]				
SAEs	0	1 (<0.1)	0	2 (<0.1)
Fatal	0	0	0	0
MAAEs	20 (0.7)	14 (0.5)	17 (0.2)	13 (0.1)
Leading to study discontinuation	0	0	0	0
AESI	1 (<0.1)	2 (<0.1)	0	0

Data are n (%) of participants. The safety population consisted of all randomly assigned participants who received study vaccination. AEs were classified as not related (no reasonable possibility) or related (reasonable possibility) to the study vaccine by the investigator.
[a]Median duration of safety follow-up was 355 (IQR, 350–364) days in P301 and 354 (IQR, 349–362) days in P302.
AE, adverse event; AESI, adverse event of special interest; MAAE, medically attended adverse event; SAE, serious adverse event.

A and B strains at Day 29 was a primary objective in the P301 trial and was assessed in a small subset of participants in the P302 trial (~3.8% of total population and all were located in North America; Table S3) as a secondary objective. In both trials, a single dose of original mRNA-1010 50 µg elicited high HAI titers against vaccine-matched influenza A (H1N1 and H3N2) and B (Victoria and Yamagata) strains at Day 29 (Figure 3 and Figure S3; Tables S4, S5). In the P301 trial, the primary immunogenicity objective based on prespecified non-inferiority threshold (original mRNA-1010 versus active comparator) at Day 29 was met for each influenza A but not influenza B strains; the lower bound of the 97.5% CI for the GMR at Day 29 exceeded 0.667 and the lower bound of the 97.5% CI for SCR difference was greater than −10% for A/H1N1 and A/H3N2 but not for B/Victoria or B/Yamagata (Figure 3). Additionally, the lower bound of the 97.5% CI for the GMR exceeded one for A/H3N2 but not for A/H1N1. The lower bound of the 97.5% CI for the SCR difference exceeded 0% for both A/H1N1 and A/H3N2. Immunogenicity findings for the P302 trial are described in the Supplement.

Efficacy

Efficacy was assessed as secondary (P301) and primary (P302) objectives, through demonstration of rVE to prevent the first episode of RT-PCR-confirmed protocol-defined ILI starting 14 days after vaccination to Day 181 or end of influenza season, whichever occurred later.

In the P301 trial, which was not powered to detect any efficacy differences between vaccine groups, a total of 118 cases of protocol-defined ILI caused by any influenza A or B strain were observed during the study. The rVE against protocol-defined ILI caused by influenza A was 17.2% (−45.7, 53.3) and −66.0% (−187.9, 2.3) against influenza B, in line with the immunogenicity results that showed higher responses against influenza A strains.

At the time of the six-month analysis, the P302 trial had not accrued the target number of cases planned to demonstrate noninferiority of the rVE of original mRNA-1010 versus the active comparator. Additional enrollment or case accrual was not pursued in the P302 trial as optimization of the mRNA-1010 formulation was planned; therefore, failure to meet non-inferiority was anticipated given the low number of cases. A total of 282 cases of RT-PCR-confirmed protocol-defined ILI caused by any influenza A or B strain were detected (77% of target number [365]), with 140 cases (1.3%) in the mRNA-1010 group and 142 cases (1.3%) in the active comparator group. The resulting overall rVE to prevent protocol-defined ILI based on the hazard ratio was 1.7% (95% CI, −24.1, 22.2; one-sided p-value = .17), which did not meet the prespecified criteria for noninferiority (Table S6). The rVE against protocol-defined ILI caused by any influenza A was 7.2% (95% CI,

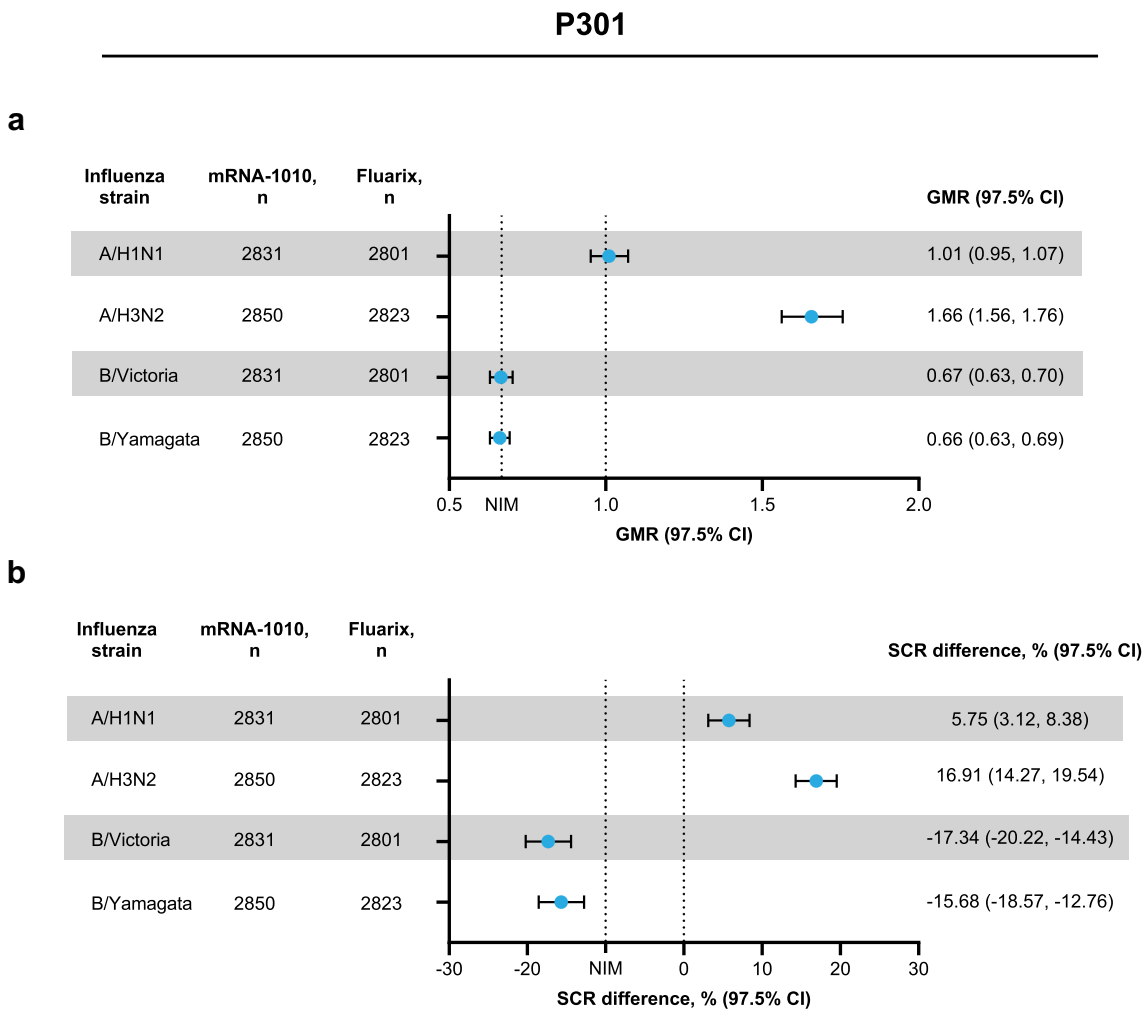

Figure 3. Geometric mean titer ratio (a) and seroconversion rate difference (b) of anti-hemagglutinin antibodies against influenza strains A/H1N1, A/H3N2, B/Victoria, and B/Yamagata for mRNA-1010 vs active comparator at Day 29 in the P301 study (per-protocol immunogenicity population). The log-transformed antibody levels were analyzed using an ANCOVA model with vaccination group as the fixed variable, log-transformed baseline HAI titers as a fixed covariate, adjusting for the randomization stratification factors: age group (18–49 years, 50–64 years, and ≥65 years) and flu vaccine status. For GMR, the dashed lines indicate the prespecified NI threshold for the lower bound 97.5% CI (>0.667) and a GMR of one indicating equivalence of mRNA-1010 to the active comparator. For SCR difference, the dashed lines indicate the prespecified NI threshold for the lower bound 97.5% CI (>−10%) and an SCR difference of 0 indicating equivalence of mRNA-1010 to the active comparator. ANCOVA, analysis of covariance; GMR, geometric mean titer ratio; HAI, hemagglutination inhibition; LLOQ, lower limit of quantification; NI, noninferiority; NIM, noninferiority margin; SCR, seroconversion rate; ULOQ, upper limit of quantification.

−18.8, 27.5) and any influenza B was −62.7% (−244.6, 23.1), again demonstrating a higher rVE point estimate against influenza A strains. rVE by influenza A subtype was 0.3% (95% CI − 45.4, 31.6) against A/H1N1 and 16.7% (−18.9, 41.5) against A/H3N2.

Correlates of protection

A correlate analysis was conducted in the P301 trial to assess Day 29 HAI titers as CoR and CoP for influenza strain-specific ILI endpoints, and hence as surrogate endpoints for VE. The P301 trial was used for this analysis because all participants had immunogenicity assessments at baseline and Day 29; by contrast, only a subset of participants in the P302 trial had immunogenicity assessments at these timepoints and few ILI cases were observed in the subset. Overall, in the P301 trial, 2808 original mRNA-1010 recipients and 2767 active comparator recipients were eligible for inclusion in the CoP analysis (had immunogenicity data and were included in both the per-protocol immunogenicity and per-protocol efficacy populations). Among that subset, two original mRNA-1010 recipients and five active comparator recipients who had early ILI events or discontinued within 7 days after the Day 29 timepoint were excluded from the CoP analysis.

From 7 days after the Day 29 visit through Day 181 or the end of influenza season (whichever occurred later), there were a total of 103 cases of protocol-defined ILI caused by influenza A and B strains. By influenza strain, 17 cases were caused by A/H1N1 (eight in the original mRNA-1010 group and nine in the comparator group), 26 cases were caused by A/H3N2 (11 in the original mRNA-1010 group and 15 in the comparator group) and 60 cases caused by B/Victoria (39 in the original mRNA-1010 group and 21 in the comparator group). Based on a multivariable-antibody Cox proportional hazards model adjusted by participant baseline risk scores (age, age group, sex, ethnicity, BMI, BMI group, and prior influenza vaccination status; see Methods), the strain-specific Day 29 HAI titers was statistically significant in reducing strain-specific ILI endpoints

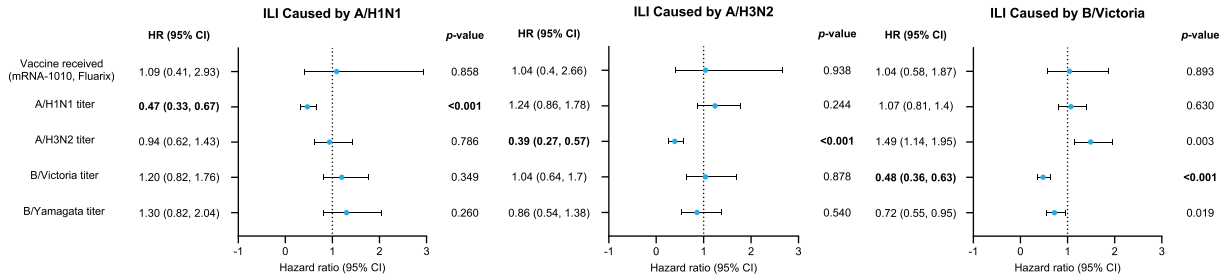

Figure 4. Multivariate analysis of Day 29 HAI titers for predicting influenza infections in the P301 study. The multivariable Cox regression model included vaccine group, Day 29 HAI titers against all strains, and baseline risk scores. ILI caused by influenza B was assumed to be B/Victoria based on epidemiologic data showing no circulation of B/Yamagata. HAI, hemagglutination inhibition; HR, hazard ratio; ILI, influenza-like illness.

(Figure 4), indicating that Day 29 HAI titers against each respective strain were CoRs for ILI caused by each respective strain. Hazard ratios against the A/H1N1-specific ILI endpoint per a standard deviation (SD) increase in Day 29 HAI titers were 0.47 (95% CI, 0.33, 0.67; $p < .001$), 0.39 (95% CI, 0.27, 0.57; $p < .001$) against A/H3N2, and 0.48 (95% CI, 0.36, 0.63; $p < .001$) against B/Victoria. After accounting for the Day 29 HAI titers in each model, the hazard ratio of vaccine (mRNA-1010 vs active comparator) was close to one and statistically insignificant to any of the three strain-specific ILI endpoints, indicating that Day 29 HAI titer is adequate to predict the risk of ILI regardless of vaccine platform. The prediction model also showed that the risk decreased as the levels of Day 29 HAI titers increased (Figure 5) for both mRNA-1010 and active comparator, supporting that Day 29 HAI titers were likely a CoP biomarker against ILI.

Discussion

The clinical evaluation of the mRNA-1010 seasonal influenza vaccine has consisted of multiple clinical trials that led to significant optimizations and improvements to the original vaccine formulation over the course of investigation. In this article, we describe phase 3 findings on the original formulation of the investigational mRNA-1010 vaccine from two trials: the P301 trial intended to establish vaccine safety and immunogenicity as well as the confirmatory P302 efficacy trial.

While primary immunogenicity (P301) endpoints based on noninferiority to an active comparator at Day 29 were met for influenza A strains, these endpoints were not met for influenza B strains. The primary noninferiority objective for efficacy (P302) against influenza caused by any A or B strain was not met. Lower immune responses against influenza B strains have been previously observed for other seasonal influenza vaccines, which could potentially be attributed to immune exposure history, HAI assay limitations, and inherent immunogenic properties of influenza B antigens.[18] These preliminary findings, wherein strain-specific efficacy results generally mirrored the observed immunogenicity profile of mRNA-1010 versus a comparator (higher for influenza A and lower for influenza B), have led to optimizing the mRNA-1010 formulation to improve immune responses against B strains.[19] In a phase 3 trial (P303) among US adults aged ≥18 years, the optimized mRNA-1010 formulation induced strong immune responses against all vaccine-matched influenza A and B strains and met prespecified noninferiority success criteria for all eight coprimary immunogenicity endpoints versus a licensed standard-dose (18–64 years) or high-dose (≥65 years) vaccine.[19] Further, mRNA-1010 immunogenicity was superior to standard-dose and high-dose vaccine comparators for all four vaccine-included influenza strains.[19] The findings from these 3 trials thus support continued investigation of mRNA-1010 and exemplify the possibility of mRNA vaccine technology against seasonal influenza, which could potentially enable influenza strain selection closer to seasonal onset.[3–5,20,21]

There is an established precedent for using HA-based immune correlates for clinical assessment and licensure of influenza vaccines. The presence of serum HAI antibodies after vaccination is considered an important protective component, with the HAI antibody response a potential surrogate marker of activity that reasonably predicts clinical benefit. The availability of immunogenicity and efficacy data from P301 participants enabled a detailed analysis of the predictiveness of HAI titers on the likelihood of protection against influenza infection. Our analysis supports the Day 29 HAI titer as a surrogate endpoint for mRNA platform-based influenza vaccines, similar to licensed standard-dose, egg-based vaccines. Of note, the HAI assay has been previously recognized for certain limitations, including interassay variability and sensitivity differences across age groups.[12] However, HAI titers have also been previously shown to generally correlate with alternative assessments for humoral immune responses after influenza vaccination, such as functional neutralizing antibody titers measured by microneutralization assay.[22–26] Therefore, while protection against influenza is likely to be complex and multifactorial, these findings support that strain-specific HAI titers at Day 29 predict risk of infection independent of vaccine type and may provide a surrogate measure of mRNA-based vaccine protection. Accordingly, optimization of mRNA-1010 (evaluated in the P303 trial) elicited higher HAI titers at Day 29 than active comparators,[19] suggesting that this updated formulation may reduce the risk of influenza compared with current vaccine approaches.

Overall, the original mRNA-1010 formulation demonstrated an acceptable reactogenicity and safety profile among the >14,000 adult participants in both trials. Within each study, the rates of any unsolicited AEs of any cause during the 28 days after vaccination were similar for the original mRNA-1010 versus the active comparator vaccine. During long-term safety follow-up, no fatal AEs or AEs leading to study discontinuation were considered related to the original mRNA-1010 vaccine. Three participants had SAEs that were classified by the investigator as having a reasonable possibility of a relationship with the mRNA-

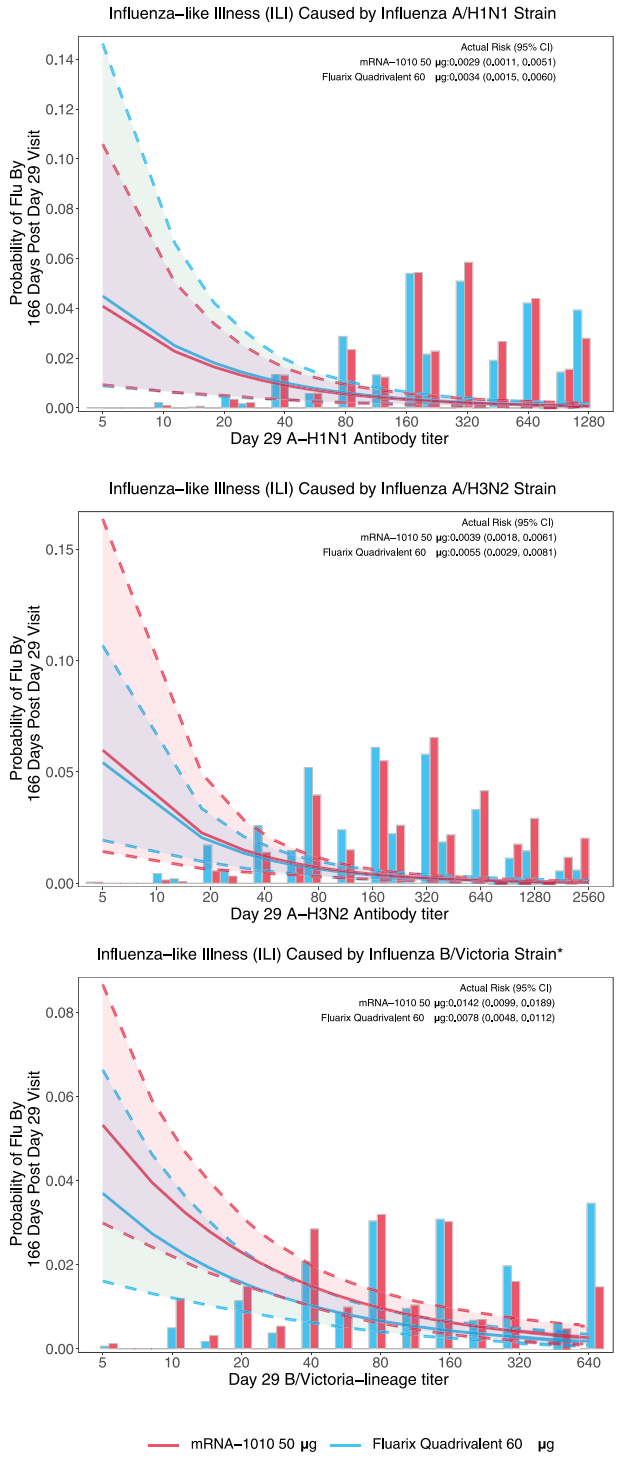

Figure 5. Influenza infection risk by Day 29 HAI titer for each strain in the P301 study. The histograms display the distribution of Day 29 HAI titers. The probability of having ILI (solid line) was estimated individually for vaccine recipients; and the CI (dotted line) was determined through bootstrap resampling. *ILI caused by influenza B was assumed to be B/Victoria based on epidemiologic data showing no circulation of B/Yamagata. HAI, hemagglutination inhibition; ILI, influenza-like illness.

1010 vaccine. However, as these cases were heavily confounded by the participants' preexisting risk factors and comorbidities, which provided more plausible explanations for the events, the sponsor assessed the events as not related to mRNA-1010. Further, upon review of all adverse event data, acute coronary syndrome, angioedema, and pulmonary embolism did not occur with greater frequency among participants who received mRNA-1010 vaccine than among participants who received the licensed comparator.

Strengths include the randomized, active comparator-controlled study designs, administration of original mRNA-1010 to >14,000 adults of varying age and ethnicity, and that the P301 trial design, which included the collection of both immunogenicity and efficacy data for all participants, allowed for a CoP analysis. Limitations include that the study populations for the P301 and P302 studies differed; 97.6% of P301 participants had not received influenza vaccine in the previous season versus 57.2% of P302 participants. Although the study population in the P301 study may have impacted the immunogenicity analyses, it does not limit the HAI conclusions. While these two studies did not examine immune responses to vaccine-heterologous strains, previous findings from the phase 1/2 trial suggest that cross-reactivity would be maintained with mRNA-1010.[17] Limitations of the P302 study include the underpowering of efficacy analyses and that secondary immunogenicity analyses were conducted in a subset of participants. The CoP analysis was not defined in the statistical analysis plan and therefore was conducted post hoc, although the analysis is largely consistent with the approach used in the phase 3 COVE study (mRNA-1273; Moderna, Inc.).[27]

In conclusion, these two phase 3 trials on the original formulation of the investigational mRNA-1010 seasonal influenza vaccine have provided key insights into the mRNA vaccine platform for this important respiratory pathogen. In both trials, the original formulation of mRNA-1010 showed an acceptable safety profile in adult participants. The efficacy profile of original mRNA-1010 was consistent with immunogenicity findings, which demonstrated higher immune responses against influenza A strains and lower responses against influenza B strains relative to an active comparator. Further, immune correlate analyses from the P301 trial support D29 HAI titers as CoR and likely as CoP for influenza illness. Accordingly, HAI titers are likely predictive of VE of mRNA-1010 or rVE to licensed influenza vaccines. Further, the results of the correlates analysis are presumed supportive of mRNA-based influenza vaccines in general, including both the original and optimized mRNA-1010 formulations. These findings support the continued investigation of mRNA-1010 against seasonal influenza, with the optimized formulation having been shown to elicit superior immune responses to standard-dose and high-dose vaccines in the P303 trial[19]; a phase 3 efficacy trial (P304 [NCT06602024]) of the optimized vaccine is ongoing.

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Author contributions

CRediT: **Boris Kandinov:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – review & editing; **Mieke Soens:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – review & editing; **Wenmei Huang:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – review & editing; **Conrado Llapur:** Formal analysis, Investigation, Visualization, Writing – review & editing; **David Ensz:** Conceptualization, Investigation, Visualization, Writing – review & editing; **Brandon Essink:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – review & editing; **Carlos Fierro:** Investigation, Visualization, Writing – review & editing; **Jignesh Vakil:** Formal analysis, Visualization, Writing – review & editing; **Alicia Pucci:** Conceptualization, Formal analysis, Visualization, Writing – review & editing; **Jia Guo:** Conceptualization, Formal analysis, Investigation, Visualization, Writing – review & editing; **Sinead Rudden:** Conceptualization, Investigation, Visualization, Writing – review & editing; **Kristi Hall:** Conceptualization, Investigation, Visualization, Writing – review & editing; **Bryony Hicks:** Conceptualization, Investigation, Visualization, Writing – review & editing; **Kristin Schaefers:** Conceptualization, Investigation, Visualization, Writing – review & editing; **Honghong Zhou:** Formal analysis, Visualization, Writing – review & editing; **Chong Ma:** Formal analysis, Visualization, Writing – review & editing; **Lingyi Zheng:** Formal analysis, Visualization, Writing – review & editing; **Andrei Avanesov:** Formal analysis, Investigation, Visualization, Writing – review & editing; **Yoonyoung Park:** Conceptualization, Formal analysis, Visualization, Writing – review & editing; **Evelyn Du:** Conceptualization, Formal analysis, Visualization, Writing – review & editing; **Jacqueline Miller:** Conceptualization, Formal analysis, Visualization, Writing – review & editing; **Jintanat Ananworanich:** Conceptualization, Formal analysis, Visualization, Writing – review & editing; **Raffael Nachbagauer:** Conceptualization, Formal analysis, Visualization, Writing – review & editing.

Data availability statement

Access to participant-level data presented in this article and supporting clinical documents with external researchers who provide methodologically sound scientific proposals will be available upon reasonable request for products or indications that have been approved by regulators in the relevant markets and subject to review from 24 months after study completion. Such requests can be made to Moderna Inc., 325 Binney Street, Cambridge, MA 02142. A materials transfer and/or data access agreement with the sponsor will be required for accessing shared data. All other relevant data are presented in the paper.

Disclosure statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
B.K., M.S., W.H., J.V., A.P., J.G., S.R., K.H., B.H., K.S., H.Z., C.M., L.Z., A.A., Y.P., E.D., J.M., and R.N. are employees of Moderna, Inc., and may hold stock/stock options in the company.
J.A. is a former employee of Moderna, Inc.
C.A., D.E., B.E., and C.F. have no conflict of interest to declare.

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Notes on contributor

Raffael Nachbagauer is a Vice President overseeing Platform and Technology Integration within Moderna Inc., where he is responsible for strategic and timely implementation of new technology emerging from platform science and technical development within clinical development. Prior to taking on this role, Dr Nachbagauer oversaw the seasonal and pandemic influenza vaccine development portfolio. Under his leadership, Moderna Inc., has initiated multiple phase 1 to 3 clinical studies spanning across first- and next-generation influenza and influenza/COVID-19 combination vaccines. Prior to joining Moderna Inc., Dr Nachbagauer was an Assistant Professor at the Icahn School of Medicine at Mount Sinai, where his research focused on the immune responses to virus infections and vaccinations, as well as the development of novel influenza vaccines.

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