Safety and immunogenicity of the intranasal H3N2 *M2*-deficient single-replication influenza vaccine alone or coadministered with an inactivated influenza vaccine (Fluzone High-Dose Quadrivalent) in adults aged 65–85 years in the USA: a multicentre, randomised, double-blind, double-dummy, phase 1b trial

Joseph Eiden, Carlos Fierro, Alexander White, Matthew Davis, Margaret Rhee, Mark Turner, Bryan Murray, Renee Herber, Roger Aitchison, David Marshall, Michael J Moser, Robert Belshe, Harry Greenberg, Kathleen Coelingh, Yoshihiro Kawaoka, Gabriele Neumann, Pamuk Bilsel

Summary

Background Older adults (aged ≥65 years) show increased susceptibility to severe disease with influenza virus infection, accounting for 70–85% of annual influenza-related fatalities in the USA. Stimulating mucosal antibodies and T cells might enhance the low vaccine effectiveness seen in older adults for currently licensed inactivated influenza vaccines, which induce mainly serum antibodies. We aimed to evaluate the safety and immunogenicity of the intranasal H3N2 *M2*-deficient single-replication (M2SR) vaccine, alone or coadministered with a licensed inactivated influenza vaccine (Fluzone High-Dose Quadrivalent; hereafter referred to as Fluzone HD), in older adults.

Methods In this multicentre, randomised, double-blind, double-dummy, phase 1b trial, individuals aged 65–85 years who were considered healthy or with stable chronic conditions with no recent (<6 months) influenza vaccinations were recruited from five clinical trial sites in the USA and randomly assigned (3:3:3:1) using a permuted block design to receive the H3N2 M2SR vaccine and Fluzone HD, the H3N2 M2SR vaccine and placebo, Fluzone HD and placebo, or placebo alone. All participants received a single intranasal spray and a single intramuscular injection, whether active or placebo, to maintain masking. The primary outcome was to assess the safety of H3N2 M2SR, administered alone or with Fluzone HD, in the safety analysis set, which included all participants who were randomly assigned and received treatment. Serum and mucosal antibodies were assessed as a secondary endpoint, and cell-mediated immunity as an exploratory endpoint, in participants in the per-protocol population, which included individuals in the safety analysis set without major protocol deviations. This trial is registered with ClinicalTrials.gov, NCT05163847.

Findings Between June 14 and Sept 15, 2022, 305 participants were enrolled and randomly assigned to receive the H3N2 M2SR vaccine plus placebo (n=89), H3N2 M2SR vaccine plus Fluzone HD (n=94), Fluzone HD plus placebo (n=92), or placebo alone (n=30). All randomly assigned participants were included in the safety analysis set. The most frequently reported local symptoms up to day 8 in groups that received M2SR were rhinorrhoea (43% [38 of 89] in the H3N2 M2SR plus placebo group and 38% [36 of 94] in the H3N2 M2SR plus Fluzone HD group), nasal congestion (51% [45 of 89] and 35% [33 of 94]), and injection-site pain (8% [seven of 89] and 49% [46 of 94]), and the most frequently reported solicited systemic symptoms were sore throat (28% [25 of 89]) for M2SR and decreased activity (26% [24 of 94]) for the M2SR plus Fluzone HD group. In the Fluzone HD plus placebo group, the most frequently reported local symptom was injection-site pain (48% [44 of 92]) and systemic symptom was muscle aches (22% [20 of 92]). The frequency of participants with any treatment-emergent adverse event related to vaccination was low across all groups (2–5%). One serious adverse event was reported, in a participant in the Fluzone HD plus placebo group. M2SR with Fluzone HD induced seroconversion (≥four-fold increase in haemagglutination inhibition antibodies from baseline to day 29) in 44 (48%) of 91 participants, compared with 28 (31%) of 90 participants who seroconverted in the Fluzone HD plus placebo group (p=0·023). M2SR with Fluzone HD also induced mucosal and cellular immune responses.

Interpretation The H3N2 M2SR vaccine coadministered with Fluzone HD in older adults was well tolerated and provided enhanced immunogenicity compared with Fluzone HD administered alone, suggesting potential for improved efficacy of influenza vaccination in this age group. Additional studies are planned to assess efficacy.

Funding US Department of Defense.

Lancet Infect Dis 2024

Published Online
July 11, 2024
https://doi.org/10.1016/S1473-3099(24)00351-7

See Online/Comment
https://doi.org/10.1016/S1473-3099(24)00403-1

FluGen, Madison, WI, USA (J Eiden MD, R Herber BS, D Marshall BS, M J Moser PhD, P Bilsel PhD); **Johnson County Clin-Trials, Lenexa, KS, USA** (C Fierro MD); **Progressive Medical Research, Port Orange, FL, USA** (A White MD); **Rochester Clinical Research, Rochester, NY, USA** (M Davis MD); **Velocity Clinical Research, Cleveland, OH, USA** (M Rhee MD); **Velocity Clinical Research, Boise, ID, USA** (M Turner MD); **Boyds Consultants, Crewe, UK** (B Murray MBBS); **North Rim Consulting, Longmont, CO, USA** (R Aitchison ScM); **Saint Louis University, St Louis, MO, USA** (Prof R Belshe MD); **Stanford University School of Medicine, Emeritus, Stanford University, Stanford, CA, USA** (Prof H Greenberg MD); **St Helena, CA, USA** (K Coelingh PhD); **Influenza Research Institute, University of Wisconsin, Madison, WI, USA** (Prof Y Kawaoka PhD, G Neumann PhD)

Correspondence to:
Dr Pamuk Bilsel, FluGen, Madison, WI 53711, USA
pbilsel@flugen.com

Research in context

Evidence before this study

Older adults (aged ≥65 years) exhibit increased susceptibility to influenza virus infection, accounting for 70–85% of annual influenza-related mortality in the USA. The effectiveness of licensed influenza vaccines in older adults, which are typically administered intramuscularly and induce mainly serum humoral immunity, is low. Strategies are needed to enhance influenza vaccine effectiveness in this group, potentially including use of intranasal vaccines, which additionally stimulate mucosal antibodies and T cells—immune effectors that are associated with protection against influenza.

We searched PubMed without language restrictions from database inception to Jan 29, 2024, with the search terms "influenza vaccine", "intranasal", "clinical trial", and "older adults", returning no results, and "live", "elderly", "influenza vaccine", and "clinical trial", returning ten results.

Six publications described clinical studies from the 1990s that suggested coadministration of live attenuated influenza vaccines and inactivated influenza vaccines might provide additional efficacy in older adults (aged ≥65 years) compared with inactivated influenza vaccines alone. However, no live attenuated influenza vaccine has been licensed or indicated for use in older adults in the USA due to absence of efficacy data. Since these studies were done, standard inactivated influenza vaccines have been replaced by enhanced influenza vaccines (given at higher doses or adjuvanted) in older adults in the USA. Live or intranasal influenza vaccines have not been assessed in older adults, alone or with inactivated influenza vaccines, in more than 20 years.

Added value of this study

This is the first study of an investigational intranasal influenza vaccine, M2-deficient single replication (M2SR) vaccine, in older adults (aged 65–85 years) that induces a multifaceted immune response when administered alone or concomitantly with an enhanced inactivated influenza vaccine (Fluzone High-Dose Quadrivalent; hereafter Fluzone HD). Substantially different immune responses were induced by the two types of vaccines. Intranasally administered H3N2 M2SR induced haemagglutination inhibition antibody (HAI) and neuraminidase inhibition antibody (NAI) responses, as well as local secretory IgA and cell-mediated immune responses, including granzyme B-positive cells, a correlate of protection in older adults. Intramuscular Fluzone HD elicited high serum antibodies but no mucosal or cellular immune responses. Coadministration of H3N2 M2SR with Fluzone HD significantly increased responder rates across all immune measurements and serum levels of HAI and NAI antibodies compared with either vaccine alone. Mucosal and cellular immune responses induced by coadministration were similar to the responses seen when the H3N2 M2SR vaccine was administered alone.

Implications of all the available evidence

H3N2 M2SR vaccine coadministration with Fluzone HD was well tolerated and provided enhanced immunogenicity compared with either vaccine alone, suggesting the potential for increased efficacy against influenza with this combination in older adults. Additional studies to demonstrate efficacy are planned.

Introduction

Older adults (aged ≥65 years) are highly susceptible to severe influenza disease and associated complications, partly due to immunosenescence and age-related comorbidities.[1] The US Centers for Disease Control and Prevention (CDC) estimated that 70–85% of influenza-related deaths and 50–70% of influenza-related hospitalisations during the 2010–11 and 2019–20 seasons were among older adults.[2] Similarly, 84–88% of deaths in nine influenza seasons in 2002–11 in Europe were estimated to be in individuals older than 65 years, with mortality being 35 times higher in this age group than in those younger than 65 years.[3]

Licensed influenza vaccines most commonly used in this age group are inactivated. Although annual influenza vaccination is recommended for older adults, licensed influenza vaccines often perform poorly in this population, particularly against influenza A(H3N2). In the past 12 influenza seasons in the northern hemisphere from 2010 to 2022, influenza A(H3N2) has been the dominant strain eight times, resulting in influenza seasons with increased prevalence of hospitalisations (ie, hospital admission) and death, particularly for older individuals, including those who have received recommended influenza vaccinations.[2] Vaccine effectiveness in older adults for influenza A(H3N2) was 10–42% in 2010–22.[2] For the 2022–23 influenza season, vaccine effectiveness against influenza-associated hospitalisation was 28% among older adults.[4]

To improve the immunogenicity of influenza vaccines, enhanced vaccines targeting older adults were licensed in the past 15 years and are currently in use in the USA, Europe, and other countries. Compared with standard licensed vaccines, these products contain higher antigen concentrations or an adjuvant, but the mechanism of protection (ie, generation of serum antibodies against influenza haemagglutinin) is unchanged.[5–7] Although these vaccines increase serum antibody titres, the modest improvement in efficacy seen with enhanced vaccines suggests that serum antibodies alone do not provide sufficient clinical protection against influenza. Other immune effectors are important for protection in older adults.[8–14] These include antibodies against neuraminidase,[9,10] mucosal immunity,[11,12] and $CD4^+$ and $CD8^+$ T-cell responses.[13,14] However, currently licensed intramuscular vaccines for older individuals do not induce cellular or vigorous mucosal immunity and inconsistently induce immunity to neuraminidase.[15–17]

The *M2*-deleted single-replication (M2SR) influenza vaccine is a live influenza virus in which *M2* is deleted, limiting the vaccine virus to a single round of replication after intranasal inoculation.[18] Shedding of the M2SR vaccine virus has been evaluated and has not been observed in preclinical studies or clinical trials.[19,20] Clinical studies in healthy adults (aged 18–49 years) have shown that the monovalent H3N2 M2SR vaccine is well tolerated and induces a multifaceted and protective immune response, including serum and mucosal antibodies and cellular immune responses.[19–21] The H3N2 M2SR vaccine induced durable cross-reactive serum haemagglutination inhibition (HAI) antibodies, which did not decrease for at least 6 months in younger adults (aged 18–49 years), and has shown protection against a highly drifted H3N2 challenge virus.[19,20] Moreover, the M2SR vaccine elicited serum and mucosal antibodies against a panel of H3N2-drifted viruses in addition to neuraminidase antibodies.[21] These studies show the safety of intranasally administered H3N2 M2SR vaccine and the desired broad immunogenicity that includes mucosal and cellular immune responses in next-generation influenza vaccines.

In this study, we evaluated the safety and immunogenicity of intranasally administered H3N2 M2SR vaccine, alone or concomitantly administered with an enhanced inactivated influenza vaccine (Fluzone High-Dose Quadrivalent; hereafter Fluzone HD), in older adults.

Methods

Study design and participants

This multicentre, randomised, double-blind, double-dummy, phase 1b trial was done at five clinical trial sites in the USA. It was performed in accordance with the ethical principles of Good Clinical Practice as required by the major regulatory authorities based on the Declaration of Helsinki. The institutional review board approved notices for recruitment of study participants. This study is registered with ClinicalTrials.gov, NCT05163847.

Participants were aged 65–85 years, non-smokers, healthy or with stable chronic conditions, and without known allergies or serious reactions to any vaccine. Participants had to test negative for recreational drugs and have not received an influenza vaccine in the past 6 months. Additional eligibility criteria are provided in the appendix (pp 2–3). Sex at birth was self-reported and confirmed by government issued identification. The two options for sex at birth were male and female. All participants provided written informed consent.

Randomisation and masking

Participants were randomly assigned (3:3:3:1) to receive the H3N2 M2SR vaccine and placebo (the M2SR vaccine group), the H3N2 M2SR vaccine and Fluzone HD (the combination group), Fluzone HD and placebo (the Fluzone HD group), or placebo alone (the placebo group) using a permuted block design (block size of ten). All participants received a single intranasal spray and a single intramuscular injection, whether active or placebo, to maintain masking. Intranasal H3N2 M2SR and placebo were administered in identical devices. Tape was placed over the syringe barrel so the contents were not visible.

The medical monitor (BM) and lead investigator (CF) reviewed available safety data after at least 30 participants had completed 7 days post-vaccination for an interim safety review before assigning the remaining participants. A summary of these data was provided to the independent safety review committee. The pharmacist or designee preparing the treatment and the statistician who prepared the randomisation list were unmasked to group assignment. All other investigative site staff and the participants were masked to group assignment.

Procedures

On day 1, participants received either intranasal H3N2 M2SR vaccine (FluGen, Madison, WI, USA; 10^9 50% tissue culture infectious dose) or intranasal placebo followed by intramuscular Fluzone HD (Sanofi, Swiftwater, PA, USA) or intramuscular placebo. The investigational M2SR vaccine expressed haemagglutinin and neuraminidase from A/Cambodia/e0826360/2020 (Cam2020) and the Fluzone HD vaccine was the quadrivalent 2021–22 formulation that included the Cam2020 H3N2 component in addition to the H1N1 B/Victoria and B/Yamagata components. The H3N2 M2SR vaccine was manufactured as previously described in a qualified complementing cell line[19] and delivered frozen to the sites in single-use cryovials. Fluzone HD was supplied in single-dose, pre-filled syringes and stored at 2–8°C until time of use. Placebo was sterile normal saline. A pharmacist or designee thawed the H3N2 M2SR vaccine vial contents to room temperature before administration and drew the contents into 1 mL disposable polypropylene syringes fitted with a mucosal atomisation device (MAD300; Teleflex, Morrisville, NC, USA) for intranasal delivery.

Serum and nasal swab samples for antibody response evaluation were collected on day 1 (pre-vaccination) and day 29 (28 days post-vaccination). Peripheral blood mononuclear cells for cell-mediated assessments were collected on day 1 (pre-vaccination) and day 15 (14 days post-vaccination).

HAI, neuraminidase inhibition (NAI), and micro-neutralisation antibody titres were measured at VisMederi (Siena, Italy), as described previously.[19] HAI and microneutralisation titres were measured for the homologous H3N2 (Cam2020egg and Cam2020cell; appendix p 14) virus and antigenically drifted H3N2 viruses (A/Hong Kong/45/2019 [HongKong 2019] and A/Darwin/09/2021 [Dar2021]). NAI antibodies to Cam2020 were measured using an enzyme-linked lectin assay directed against the influenza neuraminidase, as described in the appendix (p 4).[22] Mucosal IgA specific for Cam2020 or Dar2021 haemagglutinin and total

See Online for appendix

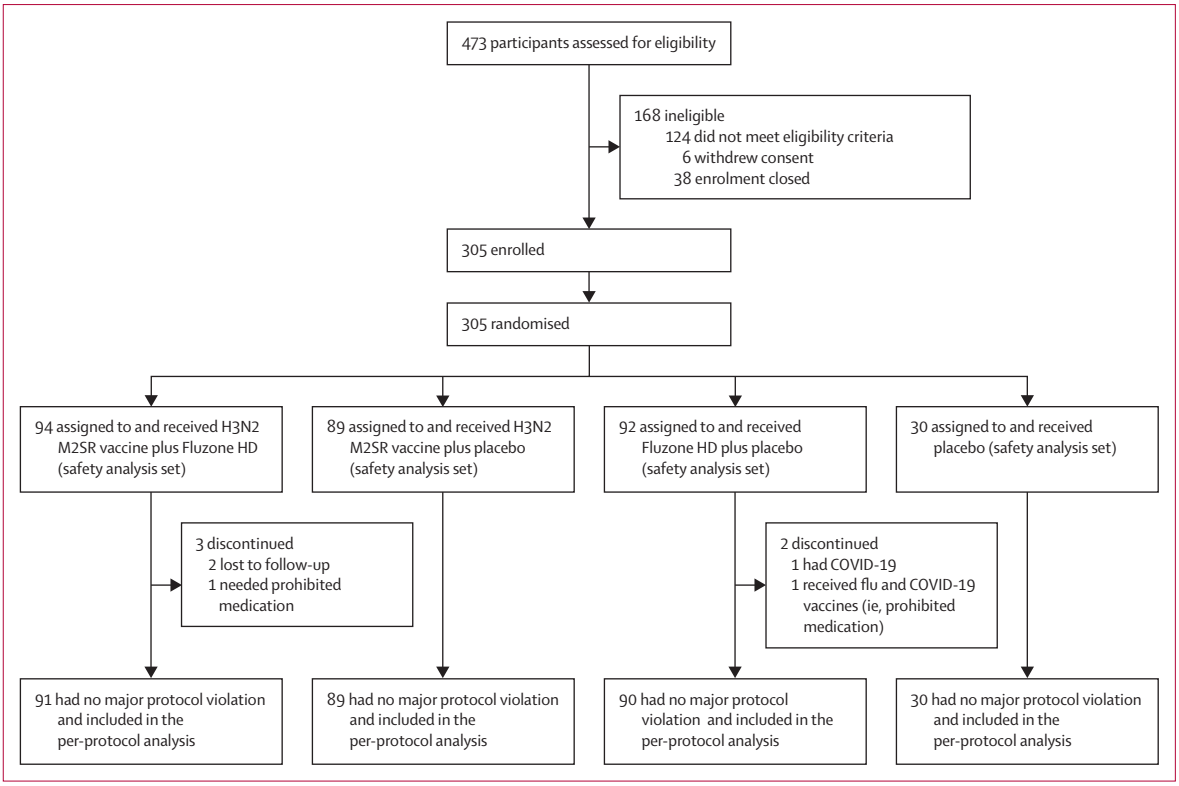

Figure 1: **Trial profile**
Fluzone HD=Fluzone High-Dose Quadrivalent. M2SR=*M2*-deficient single replication.

secretory IgA were measured using ELISA assays at FluGen (Madison, WI, USA), as described in the appendix (p 4). Double-colour fluorescent ELISpot assays detecting IFNγ and granzyme B were conducted at Cellular Technology (Shaker Heights, OH, USA).[19] Cryopreserved peripheral blood mononuclear cells (4×10^5 cells per well in triplicate) were plated onto ELISpot plates coated with human IFNγ and granzyme B capture antibodies and stimulated with a nucleoprotein peptide pool of 15-mer peptides overlapping by 11 amino acids (21st Century Biochemicals, Marlborough, MA, USA) at 1 µg/mL, media, or phytohaemagglutinin for 72 h and processed as described previously.[19] IFNγ was visualised with anti-FITC Alexa Fluor 488 and granzyme B with anti-hapten CTL-yellow using an ImmunoSpot analyser (Cellular Technology, Shaker Heights, OH, USA).

Participants were administered an intranasal dose and intramuscular dose consecutively and observed for 15 min after each. Participants were asked to record symptoms of reactogenicity in electronic diaries from first administration of vaccine or placebo until day 8. Site investigators monitored electronic diaries and recorded unsolicited adverse events and serious adverse events up to day 29. Unsolicited adverse events and serious adverse events were recorded on day 8 by telephone or on day 15 and day 29 at site visits. Medication history updates occurred at each follow-up visit. All adverse events were assessed by the investigator using the grading system from the US Food and Drug Administration vaccine toxicity criteria.

Outcomes

The primary objective was to assess the safety and tolerability of the intranasal H3N2 M2SR vaccine when given alone or concomitantly with intramuscular Fluzone HD. Primary endpoints were numbers and percentages of participants with solicited local and systemic reactions within 7 days after vaccination and unsolicited adverse events and serious adverse events during 28 days after vaccination.

Secondary endpoints were to assess the serum and mucosal antibody responses against Cam2020 (with microneutralisation and HAI assays for serum and secretory IgA ELISA for mucosal samples). Exploratory endpoints included cell-mediated immune responses; HAI and microneutralisation antibody titres against non-vaccine H3N2 strains, H1N1, and B influenza strains; and NAI titres against Cam2020 for serum and additional H3N2 strains for nasal samples. The proportions of participants who showed a two-fold or greater and a four-fold or greater increase in immune responses from baseline was assessed as an exploratory outcome for each assay.

Statistical analysis

A sample size of 90 participants per group allowed detection of an adverse event with a prevalence of 5% and approximately 99% probability. For between-group comparisons, with approximately 90 participants per group, a post-hoc power calculation showed that this study had 80% power to detect a 25% difference in the proportion responding compared with placebo (5%). Fisher's exact test compared response rates (≥two-fold or ≥four-fold titre increase) between groups. Fisher's exact test also compared safety endpoints. Log-transformed titres (exponentiated to geometric means and geometric fold rises) were analysed by pairwise comparisons of least-squares means following significant overall *F* tests from a one-way ANOVA. 95% CIs were estimated by the Clopper-Pearson method. Significance level was two-sided 0·05 with no correction for multiple testing.

Prespecified subgroup analyses of immunogenicity endpoints included baseline serostatus—ie, sero-susceptible (defined as serum HAI titres <40) versus seroprotected (defined as serum HAI titres ≥40)—and age subgroups of 65–74 years and 75–85 years.

The population for safety analyses (safety analysis set) included all participants who were randomly assigned and received treatment. All participants who were randomly assigned, received treatment, and had no major protocol violations were used for all immunogenicity analyses and were included in the per-protocol population.

Statistical analyses were performed using PASS 2022, GraphPad Prism 7, and SAS version 9.4.

Role of the funding source

The funder of the study had no role in the data collection, data analysis, data interpretation, or writing of the report but reviewed and approved the study protocol.

Results

Between June 14 and Sept 15, 2022, 305 participants were enrolled and randomly assigned to one of four groups (94 to the coadministration group, 89 to the M2SR vaccine group, 92 to the Fluzone HD group, and 30 to the placebo group; figure 1). All randomly assigned participants received study intervention and were included in the safety analysis set. 302 (99%) of 305 participants received vaccines or placebo, did not majorly deviate from the protocol, and were included in the per-protocol analysis (figure 1; appendix p 5).

Mean age of participants was 72·2 years (SD 5·1). 186 (61%) of 305 participants were female and 119 (39%) were male. Most participants were White (286 [94%] of 305 participants) and non-Hispanic (294 [96%] of 305; table 1). Baseline characteristics of participants were similar across treatment groups, except for the placebo group that had a higher proportion of female participants.

The H3N2 M2SR vaccine administered concomitantly with Fluzone HD or placebo was well tolerated up to day 29 (table 2). The most frequently reported solicited local symptoms up to day 8 in these groups were rhinorrhoea (43% [38 of 89] in the M2SR vaccine group and 38% [36 of 94] in the combination group), nasal congestion (51% [45 of 89] and 35% [33 of 94]), and injection-site pain (8% [seven of 89] and 49% [46 of 94]). Injection-site pain or swelling were more common in the two groups that received Fluzone HD than in the other groups (table 2; appendix p 6). Sore throat (28% [25 of 89]) in the M2SR group and decreased activity (26% [24 of 94]) in the combination group were the most frequent systemic reactions with muscle aches most common in the Fluzone HD groups (22% [20 of 92] alone and 26% [24 of 94] in combination).

Most unsolicited treatment-emergent adverse events (TEAEs) assessed by the investigators up to day 29 were mild in severity across the treatment groups (appendix p 7). The proportion of participants with at least one TEAE was 10% (nine of 89) in the M2SR vaccine group, 15% (14 of 94) in the combination group, 17% (16 of 92) in the Fluzone HD group, and 20% (six of 30) in the placebo group (table 2). The frequency of participants with any treatment-related TEAE was low across all groups (2–5%; table 2; appendix p 8). Two participants in the Fluzone HD group had a severe TEAE. One participant reported injection-site swelling of grade 3 on the same evening as the dose. The other participant's severe TEAE (grade 2 urinary tract infection) escalated to a serious adverse reaction when they were admitted to hospital for urosepsis on day 22 and was considered by the primary investigator as unrelated to M2SR vaccine (table 2). No deaths, serious adverse events, or TEAEs led to study withdrawal. No protocol-defined halting rules were met.

Mucosal secretory IgA against influenza-specific haemagglutinin in nasal swabs was measured on day 29 and normalised to total secretory IgA. Baseline mucosal secretory IgA titres were similar across the four groups

	M2SR vaccine group (n=89)	Combination group (n=94)	Fluzone HD group (n=92)	Placebo group (n=30)
Age, years	72·0 (5·2)	71·1 (5·1)	72·0 (5·0)	73·6 (5·1)
Sex				
Female	53 (60%)	52 (55%)	58 (63%)	23 (77%)
Male	36 (40%)	42 (45%)	34 (37%)	7 (23%)
Race				
Asian	1 (1%)	0	1 (1%)	0
Black or African American	4 (4%)	7 (7%)	6 (7%)	0
White	84 (94%)	87 (93%)	85 (92%)	30 (100%)
Ethnicity				
Hispanic or Latinx	1 (1%)	8 (9%)	2 (2%)	0
Not Hispanic or Latinx	88 (99%)	86 (91%)	90 (98%)	30 (100%)
BMI (kg/m²)	29·8 (6·6)	29·7 (5·9)	29·4 (5·2)	28·1 (4·8)

Data are mean (SD) or n (%). The combination group received H3N2 M2SR vaccine plus Fluzone HD. Fluzone HD=Fluzone High-Dose Quadrivalent. M2SR=*M2*-deficient single replication.

Table 1: **Demographic characteristics of participants (safety analysis set)**

	M2SR vaccine group (n=89)	Combination group (n=94)	Fluzone HD group (n=92)	Placebo group (n=30)
Electronic diary reactogenicity up to day 8				
Solicited local reactions				
Nasal congestion	45 (51%)*†‡	33 (35%)†	17 (18%)	7 (23%)
Rhinorrhoea	38 (43%)*†	36 (38%)*†	17 (18%)	4 (13%)
Injection-site pain	7 (8%)	46 (49%)*§	44 (48%)*§	3 (10%)
Injection-site swelling	0	17 (18%)§	11 (12%)§	1 (3%)
Injection-site redness	4 (4%)	13 (14%)	12 (13%)	1 (3%)
Solicited systemic reactions				
Headache	27 (30%)	20 (21%)	18 (20%)	6 (20%)
Sore throat	25 (28%)*	16 (17%)	15 (16%)	1 (3%)
Cough	13 (15%)	15 (16%)	8 (9%)	1 (3%)
Muscle aches	13 (15%)	24 (26%)	20 (22%)	4 (13%)
Decreased activity	17 (19%)	24 (26%)*	15 (16%)	2 (7%)
Decreased appetite	9 (10%)	15 (16%)	9 (10%)	1 (3%)
Chills	4 (4%)	9 (10%)	9 (10%)	1 (3%)
Fever	0	1 (1%)	3 (3%)	0
Unsolicited adverse events up to day 29				
Participants with at least one TEAE	9 (10%)	14 (15%)	16 (17%)	6 (20%)
Participants with at least one related TEAE	2 (2%)	5 (5%)	2 (2%)	1 (3%)
Participants with severe TEAEs	0	0	2 (2%)	0
Participants with related severe TEAEs	0	0	0	0
Participants with possibly life-threatening TEAEs	0	0	0	0
Participants with related possibly life-threatening TEAEs	0	0	0	0
Serious adverse events up to day 29				
Participants with serious adverse events	0	0	1 (1%)	0
Participants with treatment-related serious adverse events	0	0	0	0
Participants who died	0	0	0	0

Data are n (%). The combination group received H3N2 M2SR vaccine plus Fluzone HD. Exact p values for comparisons between groups are shown in the appendix (p 6). Fluzone HD=Fluzone High-Dose Quadrivalent. M2SR=*M2*-deficient single replication. TEAE=treatment-emergent adverse event. *p<0·05 versus placebo-only group. †p<0·05 versus Fluzone HD plus placebo group. ‡p<0·05 versus M2SR vaccine plus Fluzone HD group. §p<0·05 versus M2SR vaccine plus placebo group.

Table 2: **Summary of adverse events up to day 29**

(figure 2A). The geometric mean fold rise at day 29 was 2·69 (95% CI 2·20–3·30) in the M2SR vaccine group, 1·93 (1·67–2·23) in the combination group, 1·13 (0·98–1·30) in the Fluzone HD group, and 0·99 (0·91–1·08) in the placebo group (figure 2B). 43 (48%) of 89 participants in the M2SR vaccine group showed a two-fold or greater rise from baseline in normalised secretory IgA titres compared with none (0%) of 30 participants in the placebo group and nine (10%) of 89 participants in the Fluzone HD group (both p<0·0001; figure 2C). One participant in the Fluzone HD group did not have a secretory IgA titre result. A significantly higher proportion of participants (33 [36%] of 91; p<0·0001) in the combination group showed a two-fold or greater rise from baseline in secretory IgA titres compared with the placebo group and the Fluzone HD group (both p<0·0001; figure 2C). Secretory IgA titres significantly increased independent of baseline serostatus (figure 2D–I). In participants aged 75–85 years, the proportion of participants with a two-fold or higher increase from baseline in mucosal secretory IgA was higher in the M2SR group than in the other groups and higher in the M2SR group than in the Fluzone HD group for participants who were serosusceptible at baseline (appendix p 20). Additionally, there was a higher proportion of responders in the M2SR group in people aged 75–85 years.

The geometric mean fold rise in mucosal secretory IgA against Dar2021 haemagglutinin was significantly higher in the M2SR vaccine groups than Fluzone HD alone or placebo. More participants in the M2SR vaccine group (33 [37%] of 89) and combination group (30 [33%] of 91) had a two-fold or greater rise from baseline in Dar2021-specific secretory IgA than in the Fluzone HD group (11 [12%] of 90; appendix p 9).

Detailed HAI and microneutralisation responses against Cam2020 are presented in the appendix (pp 15–16). HAI titres measured in serum samples on day 1 and day 29 showed that significant responses to matched H3N2 Cam2020 influenza virus were induced in all vaccine groups (figure 3A). A two-fold or greater rise in HAI titres occurred in 26 (29%) of 89 participants in the M2SR vaccine group compared with one (3%) of 30 participants in the placebo group (p=0·0022; figure 3A). Vaccination with the combination of H3N2 M2SR vaccine plus Fluzone HD was the most immunogenic of all groups as measured by HAI titres: 74 (81%) of 91 participants in the combination group had a two-fold or greater increase in HAI titre, and 44 (48%) had a four-fold or greater increase, compared with 58 (64%) of 90 participants who had a two-fold or greater rise (p=0·012) and 28 (31%) who had a four-fold or greater rise (p=0·023) in the Fluzone HD group (figure 3A).

For participants classified as serosusceptible at baseline, a greater proportion had a four-fold or greater rise in HAI titre in the combination group than in the Fluzone HD group (33 [75%] of 44 participants *vs* 17 [46%] of 37 participants; p=0·011; figure 3B). The geometric mean titre at day 29 in participants who were serosusceptible was 91·4 (95% CI 62·7–133·2) for the combination group and 55·0 (36·5–83·0) for the Fluzone HD group (p=0·033; figure 3E). The geometric mean fold rise was 6·48 (95% CI 4·37–9·62) in the combination group and 4·14 (2·88–5·95) in the Fluzone HD group (p=0·044; figure 3H).

In participants who were seroprotected at baseline, the two-fold or greater seroconversion rate was significantly higher in the combination group than in the Fluzone HD group (34 [72%] of 47 participants *vs* 26 [49%] of 53 participants; p=0·024; figure 3C). There were no significant differences between the combination and Fluzone HD groups in geometric mean titres and geometric mean fold rises on day 29.

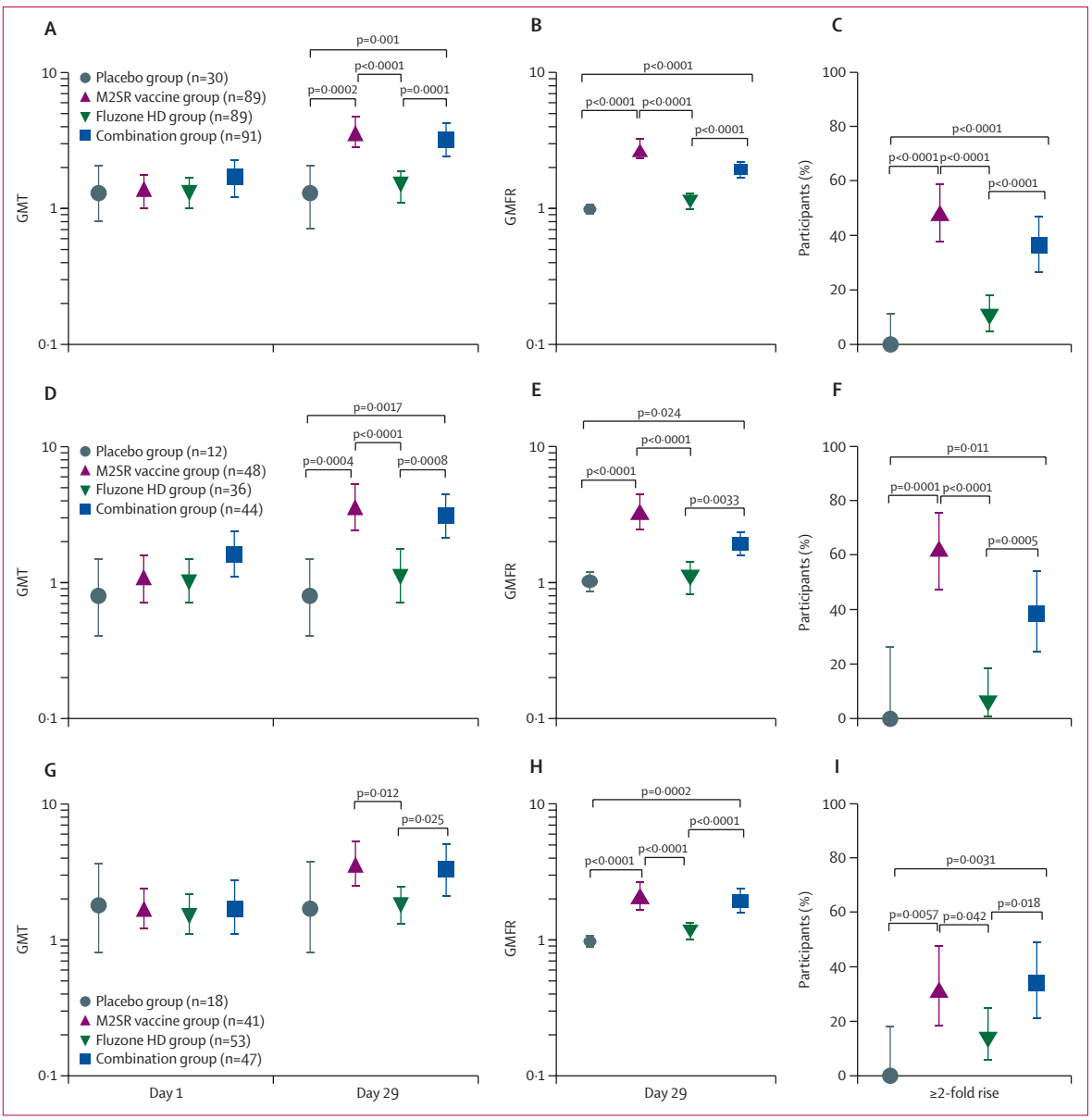

Figure 2: **Mucosal secretory IgA Cam2020 haemagglutinin ELISA titres in nasal swabs after vaccination**
(A) GMTs of secretory IgA normalised to total secretory IgA on day 1 and day 29. (B) GMFRs of secretory IgA normalised to total secretory IgA from baseline to day 29. (C) Proportions of participants with two-fold or higher rises in secretory IgA titres from baseline to day 29. (D) GMTs of secretory IgA normalised to total secretory IgA on day 1 and day 29 in participants serosusceptible to Cam2020 on day 1. (E) GMFRs of secretory IgA normalised to total secretory IgA from baseline to day 29 in participants serosusceptible to Cam2020 on day 1. (F) Proportions of participants with two-fold or higher rises in secretory IgA titres from baseline to day 29 in participants serosusceptible to Cam2020 on day 1. (G) GMTs of secretory IgA normalised to total secretory IgA on day 1 and day 29 in participants seroprotected against Cam2020 on day 1. (H) GMFRs of secretory IgA normalised to total secretory IgA from baseline to day 29 in participants seroprotected against Cam2020 on day 1. (I) Proportions of participants with two-fold or higher increases in secretory IgA titres from baseline to day 29 in participants seroprotected against Cam2020 on day 1. Error bars are 95% CIs. The combination group received H3N2 M2SR vaccine plus Fluzone HD. H3N2 strain is Cam2020. Fluzone HD=Fluzone High-Dose Quadrivalent. GMFR=geometric mean fold rise. GMT=geometric mean titre. M2SR=*M2*-deficient single replication.

H3N2 M2SR vaccine plus Fluzone HD significantly increased immune responses in the subset of participants aged 75–85 years compared with Fluzone HD alone: 11 (42%) of 26 participants in the combination group had a four-fold or greater increase in HAI titres against the Cam2020 vaccine antigen (p=0·008 against placebo) compared with six (21%) of 28 participants in the Fluzone HD group (appendix p 17). The difference between these groups in proportion of participants with four-fold or higher responses was even greater in participants aged 75–85 years who were serosusceptible at baseline (67% [ten of 15] in the combination group *vs* 25% [three of 12] in the Fluzone HD group (appendix p 17). In participants aged 75–85 years who were

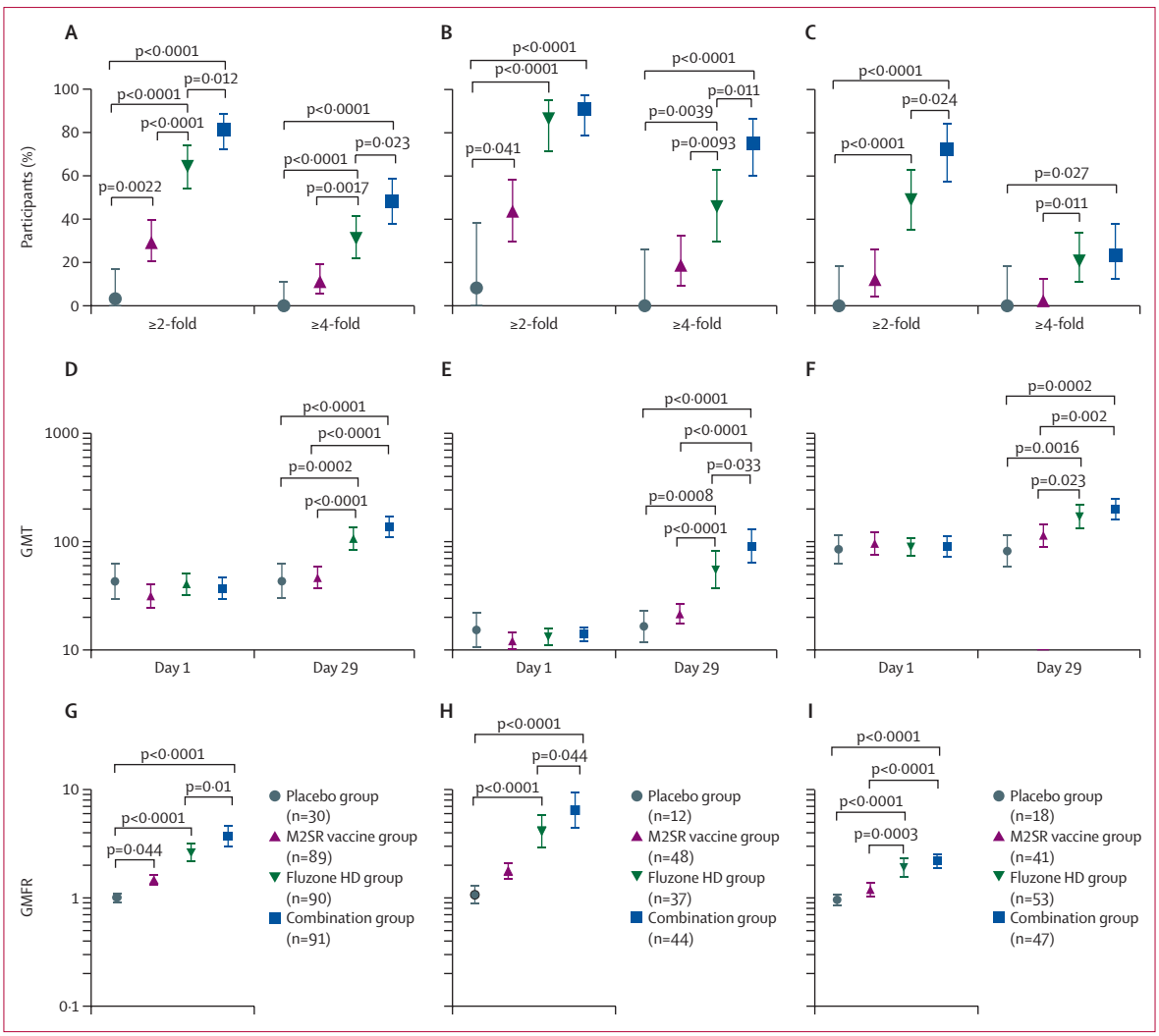

Figure 3: **Serum HAI antibody titres after vaccination**

Participants with two-fold or greater and four-fold or greater rises in HAI antibody titres against Cam2020 from baseline to day 29 in all participants (A), participants serosusceptible to Cam2020 on day 1 (B), and participants with seroprotective Cam2020 HAI titres on day 1 (C). Geometric mean HAI titres against Cam2020 on day 1 (baseline) and day 29 in all participants (D), participants serosusceptible to Cam2020 on day 1 (E), and participants with seroprotective Cam2020 HAI titres on day 1. GMFRs in HAI titres from baseline to day 29 for all participants (G), participants serosusceptible to Cam2020 at baseline (H), and participants with seroprotective Cam2020 HAI titres at baseline (I). Error bars are 95% CIs. The combination group received H3N2 M2SR vaccine plus Fluzone HD. Fluzone HD=Fluzone High-Dose Quadrivalent. GMFR=geometric mean fold rise. GMT=geometric mean titre. HAI=haemagglutination inhibition. M2SR=*M2*-deficient single replication.

seroprotected at baseline, two-fold or higher seroconversion rates were seen in 36% of participants (four of 11) in the combination group versus 56% of participants (nine of 16) in the Fluzone HD group (appendix p 17). Findings for the drifted H3N2 strains (HongKong 2019 and Dar2021), and epidemic wild-type Cam2020 virus, were also higher in the combination group than in the Fluzone HD group in participants aged 75–85 years, especially those who were serosusceptible at baseline (appendix p 19). HAI titres assessed for the H1N1 and B/Victoria components of the 2022–23 Fluzone HD vaccine showed that the effect of the Cam2020 M2SR vaccine was specific to the H3N2 component (appendix p 10).

Broader immune responses were seen in serum microneutralisation titres in the combination group than in the Fluzone HD group. Microneutralisation titres against the two antigenically drifted H3N2 influenza strains, HongKong 2019 and Dar2021, showed higher response frequencies in the combination group than in the Fluzone HD group (appendix pp 13–14). Additionally, in the M2SR vaccine group, proportions of responders in microneutralisation and HAI titres were higher against the cell-produced Cam2020 virus (epidemic virus-like) than against the egg-produced Cam2020 virus, whereas this difference was not observed in the groups that received Fluzone HD (appendix pp 13–16).

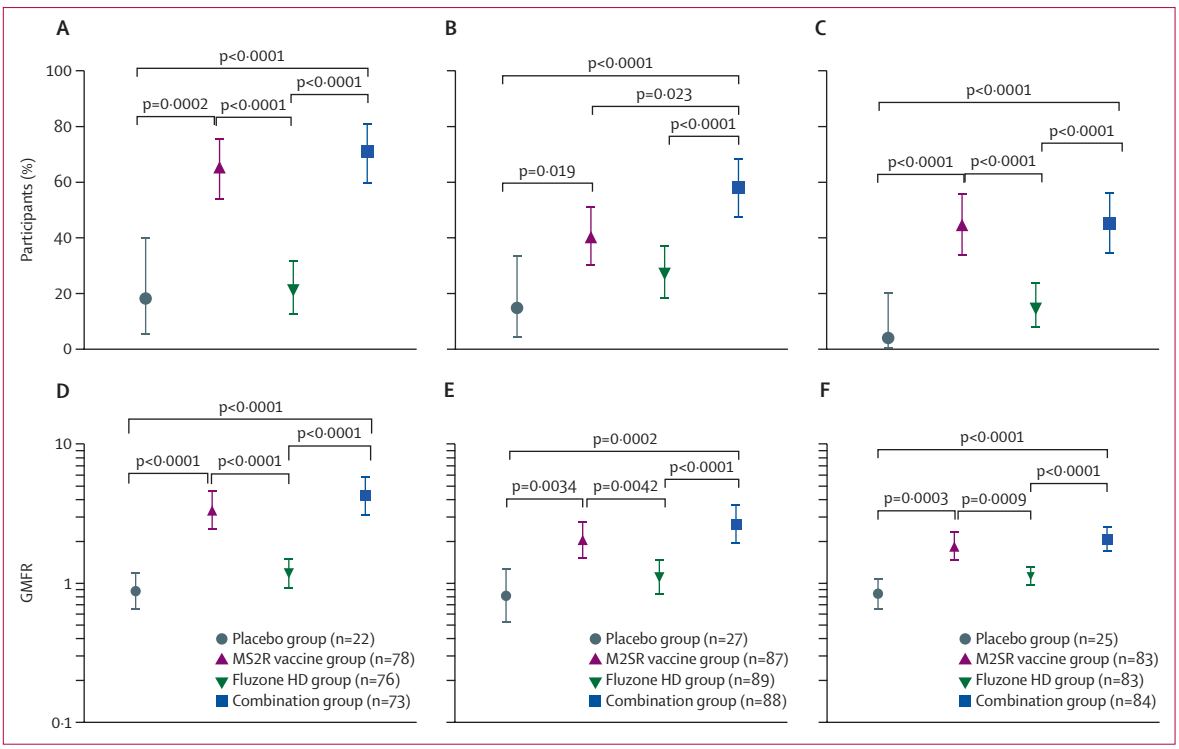

Figure 4: **Cell-mediated immune responses after vaccination**
Proportions of participants with two-fold or higher rises in IFNγ-producing cells (A), granzyme B-producing cells (B), and dual IFNγ-producing and granzyme B-producing cells (C) from baseline to day 15, as measured by ELISpot assays. GMFRs in spot-forming cells per 10^6 peripheral blood mononuclear cells from baseline to day 15 for IFNγ (D), granzyme B (E), and double-positive IFNγ and granzyme B (F). Peripheral blood mononuclear cells were stimulated with an influenza nucleoprotein peptide pool. Error bars are 95% CIs. The combination group received H3N2 M2SR vaccine plus Fluzone HD. Fluzone HD=Fluzone High-Dose Quadrivalent. GMFR=geometric mean fold rise. M2SR=*M2*-deficient single replication.

Serum antibodies against Cam2020 influenza virus neuraminidase were also elicited, with the highest proportions of responders seen in the combination group. Proportions of participants with two-fold or higher or four-fold or higher increases in NAI titres from baseline were 74% (67 of 91) and 26% (24 of 91), respectively, in the combination group, 26% (23 of 89) and 9% (eight of 89), respectively, in the M2SR vaccine group, 44% (40 of 90) and 10% (nine of 90), respectively, in the Fluzone HD group, and 7% (two of 30) and none, respectively, in the placebo group (appendix pp 11–12).

107 (36%) of 301 participants had NAI titres of 40 or more at baseline (appendix pp 11–12). After vaccination, 59 (65%) of 91 participants in the combination group had an NAI titre of 40 or more compared with ten (33%) of 30 participants in the placebo group, 47 (52%) of 90 participants in the Fluzone HD group, and 43 (48%) of 89 participants in the M2SR vaccine group (appendix pp 11–12). The two-fold or greater increase in NAI titre was significantly higher in the combination group than in the Fluzone HD group for serosusceptible participants (33 [75%] of 44 *vs* 13 [35%] of 37; p=0·0006) and seroprotected participants (34 [72%] of 47 *vs* 27 [51%] of 53; p<0·040; appendix pp 11–12).

In individuals aged 75–85 years, proportions with two-fold or higher or four-fold or higher increases in Cam2020-specific NAI titres were significantly higher in the combination group than in the Fluzone HD group (appendix p 18). Furthermore, among those who were serosusceptible at baseline, 11 (73%) of 15 participants in the combination group and three (25%) of 12 participants in the Fluzone HD group had a two-fold or greater rise in Cam2020 NAI titre from baseline (appendix p 18). In seroprotected participants, two-fold or higher responses from baseline in NAI titres were seen in nine (82%) of 11 participants in the combination group compared with seven (44%) of 16 participants in the Fluzone HD group (appendix p 18).

Post-vaccination cell-mediated immune responses assessed by IFNγ plus granzyme B ELISpot assay showed a significant increase from baseline in influenza nucleoprotein-reactive cells in the M2SR vaccine group (figure 4). Pre-vaccination baselines were similar in all groups. At day 15, proportions of participants with two-fold or greater rises from baseline in granzyme B-producing cells (35 [40%] of 87 *vs* 24 [27%] of 89) were higher in the M2SR vaccine group than in the Fluzone HD group whereas IFNγ-producing cells (51 [65%] of 78 participants *vs* 16 [21%] of 76 participants) and dual

IFNγ-producing and granzyme B-producing cells were significantly higher in the M2SR group than in the Fluzone HD group (37 [45%] of 83 *vs* 12 [15%] of 83; p<0·0001; figure 4A–C). Similarly, in the combination group, the proportions with two-fold or greater rises from baseline in IFNγ-producing cells (52 [71%] of 73), granzyme B-producing cells (51 [58%] of 88), and dual IFNγ-producing and granzyme B-producing cells (38 [45%] of 84) were significantly higher than in the Fluzone HD group (all p<0·0001; figure 4A–C). At day 15, the geometric mean fold rise from baseline was 3·4 (95% CI 2·42–4·64) in the M2SR vaccine group and 4·3 (3·08–5·94) in the combination group for IFNγ, 2·04 (1·51–2·77) and 2·65 (1·91–3·67), respectively, for granzyme B, and 1·83 (1·44–2·34) and 2·07 (1·67–2·57), respectively, for IFNγ-producing cells and granzyme B-producing cells. Lower fold-changes were seen in the other groups (figure 4D–F).

In participants aged 75–85 years, proportions with two-fold or greater rises in IFNγ-producing and granzyme B-producing cells were higher in the M2SR and combination groups than in the Fluzone HD group. Participants in the M2SR and combination groups who were serosusceptible at baseline also showed higher rises. No difference was seen in those seroprotected at baseline (appendix p 21).

Discussion

This phase 1b trial shows that the investigational intranasal H3N2 M2SR vaccine is well tolerated and immunogenic in older adults, whether administered alone or concomitantly with intramuscular Fluzone HD. Striking differences in immune responses were induced by the two types of vaccines, with intranasally administered H3N2 M2SR inducing superior local secretory IgA and cell-mediated responses and Fluzone HD eliciting high serum antibody titres. Additionally, the H3N2 M2SR vaccine augmented serum antibodies in participants given both vaccines concomitantly. Inactivated influenza vaccines have shown only modest success against influenza, partly due to the dependence on matching future antigenic strains with inactivated antigen and their limited ability to stimulate other immune compartments, including secretory IgA and cellular responses. Coadministration of intranasal H3N2 M2SR vaccine with intramuscular Fluzone HD in this study resulted in a multifaceted immune response in older adults, with a higher proportion of responders across all immune measurements compared with either vaccine alone. These broad immune responses support the potential for safe coadministration of the M2SR vaccine with Fluzone HD to enhance protection against influenza infection in this vulnerable age group.

The M2SR vaccine has the immune response characteristics of the wild-type influenza virus when administered to the nasal mucosa, a key site for the vaccine virus to stimulate a full complement of immune responses, including mucosal secretory IgA and cellular immunity, to influenza. Mucosal secretory IgA has been shown to protect against influenza virus infection and enhance cross-reactivity against drifted strains.[11,12] Experimental infection of participants with influenza virus has shown that mucosal secretory IgA contributes to protection against influenza infection and reduces virus shedding.[11,12] Licensed intramuscular influenza vaccines do not induce substantial mucosal responses.[15,23]

Cellular immunity has also been associated with protection against influenza disease in older adults and might be a better predictor of protection than serum antibody responses.[8] However, T-cell responses induced by parenteral investigational T-cell-based vaccines in recent efficacy studies (eg, NCT03450915) have not shown protective benefit against influenza.[24] By contrast, the presence of T-cell immunity, presumably due to natural infection or live attenuated influenza vaccination, is associated with protection against influenza.[13,14] T-cell responses induced by intranasal M2SR vaccine either alone or with Fluzone HD were not seen with Fluzone HD alone, which is in line with previous studies showing that intramuscular enhanced influenza vaccines generate limited T-cell responses.[25,26] The M2SR vaccine encodes for all the internal proteins of influenza that are the main targets of T-cell responses. In studies in adults, ex-vivo stimulation of peripheral blood mononuclear cells with matrix or nucleoprotein peptide pools increased T-cell responses.[19–21] In a mouse heterosubtypic challenge model, the M2SR vaccine elicited $IFN\gamma^+$ $CD8^+$ T cells with effector memory phenotype similar to those observed to be protective in humans.[14,18] Additionally, like natural influenza infection and unlike parenteral vaccines, the M2SR vaccine can establish resident memory T cells in the human respiratory tract.

As expected, H3N2 M2SR vaccine also induced antibodies to neuraminidase since all antigens except M2 are expressed within the airway by the delivery of vaccine virus RNA to the mucosa. In addition to pronounced seroconversion rates in participants aged 65–75 years who were serosusceptible at baseline against the vaccine haemagglutinin, those with existing seroprotection against the vaccine antigen still displayed significant seroconversion of HAI and NAI titres (similar to participants who were serosusceptible) after coadministration of the H3N2 M2SR vaccine plus Fluzone HD compared with Fluzone HD alone. Currently there are no licensed intranasal influenza vaccines for older adults in the USA, although older adults can induce mucosal responses as seen in this study and previous studies.[27–29]

Coadministration of live attenuated influenza vaccine or Russian-backbone live attenuated influenza vaccine and standard inactivated influenza vaccines to older adults in previous studies have suggested that the approach might provide increased efficacy against influenza, presumably partly due to the addition of

mucosal immunity.[27–29] Although use of inactivated influenza vaccines has been substantially replaced by use of enhanced influenza vaccines, efficacy remains low in older adults. Our results suggest that H3N2 M2SR vaccine coadministered with Fluzone HD might reduce the risk of influenza disease in this age group.

This study has limitations. Coadministration of a monovalent H3N2 M2SR vaccine with quadrivalent Fluzone was evaluated in this study. The impact of an M2SR vaccine encoding influenza A(H1N1) or influenza B/Victoria on immune responses in older adults remains to be seen in future trials. Similarly, although three licensed inactivated influenza vaccine products (Fluzone HD, FluBlok, and FluAd) are recommended by the CDC for adults older than 65 years, we tested only Fluzone HD. Lastly, we did not evaluate the effect of the H3N2 M2SR vaccine on the durability of the immune responses in this age group.

In summary, coadministration of the intranasal H3N2 M2SR vaccine with Fluzone HD shows enhanced and broad serum, mucosal, and cellular immune responses in older adults. The data suggest that coadministration of the H3N2 M2SR vaccine with Fluzone HD has the potential to enhance the efficacy provided by Fluzone HD alone. Additional studies are needed to evaluate its clinical efficacy.

Contributors

JE, PB, and RH designed the trial. CF, AW, MD, MR, and MT were study investigators. RH and BM provided clinical operational support. RA provided statistical analysis, output listings, and tables. JE, RA, RH, and PB verified the data. PB drafted the manuscript. JE, RB, HG, KC, YK, and GN supported development of trial design and provided scientific expertise on data analysis. DM and MJM contributed to data collection. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

JE is a consultant for FluGen. RB, HG, and KC serve on a clinical advisory board for FluGen. YK and GN are founders of FluGen. RH, DM, MJM, and PB are employees of FluGen. All other authors declare no competing interests.

Data sharing

The study protocol and the statistical analysis plan will be made available on request to the corresponding author (pbilsel@flugen.com). De-identified data (tables and listings) will be available for reasonable requests after contacting the corresponding author and citing the intended use. Approved requestors will be required to sign a data access agreement. Requests should be sent to the corresponding author within 36 months of publication.

Acknowledgments

This work was supported by The Assistant Secretary of Defense for Health Affairs endorsed by the Department of Defense, in the amount of US$11 439 843 through the Peer Reviewed Medical Research Program under award number W81XWH-21–1-0563. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by The Assistant Secretary of Defense for Health Affairs endorsed by the Department of Defense.

References

1 McElhaney JE, Verschoor CP, Andrew MK, Haynes L, Kuchel GA, Pawelec G. The immune response to influenza in older humans: beyond immune senescence. *Immun Ageing* 2020; **17:** 10.

2 US Centers for Disease Control and Prevention. Influenza (flu). 2024. https://www.cdc.gov/flu/ (accessed Jan 23, 2024).

4 Lewis NM, Zhu Y, Peltan ID, et al. Vaccine effectiveness against influenza A—associated hospitalization, organ failure, and death: United States, 2022–2023. *Clin Infect Dis* 2023; **78:** 1056–64.

3 Paget J, Danielle Iuliano A, Taylor RJ, Simonsen L, Viboud C, Spreeuwenberg P. Estimates of mortality associated with seasonal influenza for the European Union from the GLaMOR project. *Vaccine* 2022; **40:** 1361–69.

5 Ng TWY, Cowling BJ, Gao HZ, Thompson MG. Comparative immunogenicity of enhanced seasonal influenza vaccines in older adults: a systematic review and meta-analysis. *J Infect Dis* 2019; **219:** 1525–35.

6 Domnich A, Arata L, Amicizia D, Puig-Barberà J, Gasparini R, Panatto D. Effectiveness of MF59-adjuvanted seasonal influenza vaccine in the elderly: a systematic review and meta-analysis. *Vaccine* 2017; **35:** 513–20.

7 Lee JKH, Lam GKL, Yin JK, Loiacono MM, Samson SI. High-dose influenza vaccine in older adults by age and seasonal characteristics: systematic review and meta-analysis update. *Vaccine X* 2023; **14:** 100327.

8 McElhaney JE, Xie D, Hager WD, et al. T cell responses are better correlates of vaccine protection in the elderly. *J Immunol* 2006; **176:** 6333–39.

9 Monto AS, Petrie JG, Cross RT, et al. Antibody to influenza virus neuraminidase: an independent correlate of protection. *J Infect Dis* 2015; **212:** 1191–99.

10 Couch RB, Atmar RL, Franco LM, et al. Antibody correlates and predictors of immunity to naturally occurring influenza in humans and the importance of antibody to the neuraminidase. *J Infect Dis* 2013; **207:** 974–81.

11 Gould VMW, Francis JN, Anderson KJ, Georges B, Cope AV, Tregoning JS. Nasal IgA provides protection against human influenza challenge in volunteers with low serum influenza antibody titre. *Front Microbiol* 2017; **8:** 900.

12 Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis* 2000; **181:** 1133–37.

13 Wilkinson TM, Li CK, Chui CS, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nat Med* 2012; **18:** 274–80.

14 Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med* 2013; **19:** 1305–12.

15 Atmar RL, Keitel WA, Cate TR, Munoz FM, Ruben F, Couch RB. A dose-response evaluation of inactivated influenza vaccine given intranasally and intramuscularly to healthy young adults. *Vaccine* 2007; **25:** 5367–73.

16 Giurgea LT, Morens DM, Taubenberger JK, Memoli MJ. Influenza neuraminidase: a neglected protein and its potential for a better influenza vaccine. *Vaccines (Basel)* 2020; **8:** 409.

17 McElhaney JE, Verschoor CP, Haynes L, et al. Key determinants of cell-mediated immune responses: a randomized trial of high dose vs standard dose split-virus influenza vaccine in older adults. *Front Aging* 2021; **2:** 2.

18 Sarawar S, Hatta Y, Watanabe S, et al. M2SR, a novel live single replication influenza virus vaccine, provides effective heterosubtypic protection in mice. *Vaccine* 2016; **34:** 5090–98.

19 Eiden J, Gordon G, Fierro C, et al. Safety and immunogenicity of M2-deficient, single replication, live influenza vaccine (M2SR) in adults. *Vaccines (Basel)* 2021; **9:** 1388.

20 Eiden J, Volckaert B, Rudenko O, et al. M2-deficient single-replication influenza vaccine-induced immune responses associated with protection against human challenge with highly drifted H3N2 influenza strain. *J Infect Dis* 2022; **226:** 83–90.

21 Eiden J, Fierro C, Schwartz H, et al. Intranasal M2SR (M2-deficient single replication) H3N2 influenza vaccine provides enhanced mucosal and serum antibodies in adults. *J Infect Dis* 2022; **227:** 103–12.

22 Biuso F, Palladino L, Manenti A, et al. Use of lentiviral pseudotypes as an alternative to reassortant or Triton X-100-treated wild-type influenza viruses in the neuraminidase inhibition enzyme-linked lectin assay. *Influenza Other Respir Viruses* 2019; **13:** 504–16.

23 Keitel WA, Cate TR, Nino D, et al. Immunization against influenza: comparison of various topical and parenteral regimens containing inactivated and/or live attenuated vaccines in healthy adults. *J Infect Dis* 2001; **183:** 329–32.

24 Evans TG, Bussey L, Eagling-Vose E, et al. Efficacy and safety of a universal influenza A vaccine (MVA-NP+M1) in adults when given after seasonal quadrivalent influenza vaccine immunisation (FLU009): a phase 2b, randomised, double-blind trial. *Lancet Infect Dis* 2022; **22:** 857–66.

25 Kumar A, McElhaney JE, Walrond L, et al. Cellular immune responses of older adults to four influenza vaccines: results of a randomized, controlled comparison. *Hum Vaccin Immunother* 2017; **13:** 2048–57.

26 Cowling BJ, Perera RAPM, Valkenburg SA, et al. Comparative immunogenicity of several enhanced influenza vaccine options for older adults: a randomized, controlled trial. *Clin Infect Dis* 2020; **71:** 1704–14.

27 Treanor JJ, Mattison HR, Dumyati G, et al. Protective efficacy of combined live intranasal and inactivated influenza A virus vaccines in the elderly. *Ann Intern Med* 1992; **117:** 625–33.

28 Gorse GJ, O'Connor TZ, Young SL, et al. Efficacy trial of live, cold-adapted and inactivated influenza virus vaccines in older adults with chronic obstructive pulmonary disease: a VA cooperative study. *Vaccine* 2003; **21:** 2133–44.

29 Rudenko LG, Arden NH, Grigorieva E, et al. Immunogenicity and efficacy of Russian live attenuated and US inactivated influenza vaccines used alone and in combination in nursing home residents. *Vaccine* 2000; **19:** 308–18.