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A group 1 hemagglutinin stem vaccine elicits broad humoral responses against influenza in phase 1/2a study

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Vaccines targeting the conserved stem region of influenza hemagglutinin (HA) may induce broad immunity and reduce reliance on strain-matched seasonal formulations. This Phase 1/2a study evaluates safety and immunogenicity of a universal influenza vaccine component comprising a soluble trimeric stabilized group 1 HA-stem protein (INFLUENZA G1 mHA). Healthy adults aged 18-to-45 received one or two doses at 45 μg or 135 μg, with or without $Al(OH)_3$. Primary endpoints were safety and reactogenicity. All regimens were well-tolerated with no vaccine-related serious adverse events. Secondary and exploratory endpoints included binding, neutralizing, and ADCC responses compared to a matched quadrivalent seasonal influenza vaccine cohort. A single dose induced robust binding antibody responses to H5 HA and homologous stem HA, with 6.5–16.4-fold increases at Day 29 versus baseline, exceeding responses elicited by the seasonal influenza vaccine. Additionally, we demonstrate broader binding to a panel of group 1 HAs, with responses maintained for at least one year. Neutralizing antibody and ADCC responses at Day 29 showed 7.9–15-fold and 4.9–8.6-fold increases, respectively. Importantly, serum from vaccinated participants protect mice against lethal heterologous H5N1 challenge. This study provides proof of concept that the HA stem vaccine elicits broad and durable influenza A group 1–directed immunity. This trial is registered with ClinicalTrials.gov (NCT05901636).

Influenza viruses continue to be a seasonal and pandemic threat, causing significant global health and economic burden. Approximately 1 billion people are infected with influenza annually, with an estimated 3 to 5 million cases of severe illness and between 290,000 to 650,000 deaths worldwide[1]. Vaccination is widely recognized as a cost-effective approach to prevent influenza infection[2]. Current seasonal influenza vaccines commonly contain three or four inactivated influenza A and influenza B virus strains to cover the predicted circulating viruses in the population. Influenza A viruses are phylogenetically classified into group 1 and group 2 viruses, and seasonal vaccines are comprised of

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one strain from each of these groups, and either one or two strains from the influenza B Yamagata or Victoria lineages. While these vaccines reduce infections and disease severity, their efficacy is highly variable[3], partly due to antigenic mismatch of predicted vaccine strains to those circulating. This occurs due to antigenic drift, where small genetic changes in the virus necessitates the annual reformulation and administration of influenza vaccines[4]. However, a more concerning phenomenon is zoonotic spillover in combination with antigenic shift, where an influenza virus from an animal reservoir can first infect humans and then gain the propensity for efficient human-to-human transmission. This shift can lead to the emergence of novel influenza viruses to which most people have little to no immunity[4]. Recently, the H5N1 avian influenza virus was identified as a potentially significant threat to cause a devastating pandemic based on infections observed in dairy cows and subsequently in humans[5,6]. The possible transmission within the human population underscores the urgent need for vaccines that can provide broad and durable protection against such drifted strains and those with pandemic potential. The majority of conventional influenza vaccines rely on eliciting antibodies to the globular head domain of the major viral surface protein, hemagglutinin (HA), which is highly variable, typically making vaccine-elicited responses strain-specific[7]. The development of a supra-seasonal or 'universal' influenza vaccine has the potential to protect against a wider variety of strains, alleviating the need for seasonal updates to be effective against drifted variants or strains with pandemic potential. The conserved stem region of influenza HA has been hypothesized to induce a broadly protective immune response[8,9]. We developed an HA stem vaccine which was immunogenic and protective against a variety of influenza A group 1 viruses in animal models[10]. In this work, we present the results of VAC21148FLZ1001 (FLZ1001), a randomized, double-blind, placebo-controlled, first-in-human phase 1/2a study evaluating the safety, reactogenicity, and immunogenicity of this HA stem subunit protein vaccine[10] (INFLUENZA G1 mHA or G1 mHA) with the immunodominant head region removed. The vaccine consists of a soluble, trimeric HA stem antigen based on the influenza A group 1 H1 A/California/07/2009 strain, stabilized by structure-based design[11] without using a membrane-lateral trimerization domain, which was omitted to maximize the effectiveness of the response to antigen and reduce off-target immune responses. We also show findings from FLZ1001 study samples evaluated in a murine passive transfer challenge model to determine if vaccine-induced stem-reactive antibodies mediate protection against H5N1 influenza virus.

Results

Participant demographics

A total of 197 adult participants aged 18 to 45 were screened for eligibility. 170 participants were enrolled in the study between May 31st, 2023 and August 25th, 2023 and were part of the full analysis set, having received at least one dose of study vaccine or placebo (Fig. 1a). Enrollment was executed using a staggered approach, with safety evaluations in place before extending enrollment in Cohort 1 and progressing to Cohort 2 (Supplementary Fig. 1). Participants were randomly assigned into three groups in Cohort 1, and five groups in Cohort 2, to receive either one or two doses of G1 mHA at two different dose levels (45 μg low dose or 135 μg high dose), with or without $Al(OH)_3$. Vaccinations were given on Day 1 and Day 57, and participants were followed up until 1 year after the first vaccination (Fig. 1b).

Participant characteristics at baseline were balanced across the seven treatment groups in FLZ1001 (Supplementary Table 1). Among all participants, the median age was 34 years, 75.9% were white, 60% were not Hispanic or Latino, and 52.4% were female. The median body mass index (BMI) was 28 kg/m^2 and 39.4% of participants had a BMI of ≥30 kg/m^2. A subset of participants from a separate study exploring the co-administration of COVID-19 and seasonal influenza vaccines (VAC31518COV3005 or COV3005)[12], were selected and used to compare immunogenicity of the G1 mHA vaccine regimens to a conventional seasonal influenza vaccine. This randomly selected subset of COV3005 participants aged 18 to 45, who received only a standard dose of the Afluria 2021-2022 Northern Hemisphere quadrivalent seasonal influenza vaccine and referred to as the Q-SD FluVac group, had baseline characteristics comparable to that of the FLZ1001 participants (Supplementary Table 1).

Safety and tolerability

Solicited local and systemic adverse events (AEs) were mostly mild to moderate (Grade 1 or 2) (Fig. 2, Supplementary Table 2). Vaccine groups generally experienced slightly higher AE frequencies than the placebo group. AE frequency did not appear to increase with higher dose levels. Compared to non-adjuvanted groups, those receiving $Al(OH)_3$ adjuvant reported more solicited local AEs, particularly vaccination site pain (Fig. 2a). Solicited systemic AEs including fatigue, myalgia, and headache, varied across treatment arms, with two Grade 3 fatigue events reported post-dose 2 (Fig. 2b). Unsolicited AEs were rare and mostly moderate or mild (Supplementary Table 3), with five participants reporting events post-dose 1 and 12 reporting events post-dose 2. Four Grade 3 unsolicited events included one transient ischemic attack, two influenza-like illnesses, and one case of anemia. None of these were considered related to the study vaccine by the site investigators. Two SAEs (transient ischemic attack and spontaneous abortion) occurred and were evaluated to be unrelated to the study vaccine. Over the course of the study, three confirmed influenza A cases were reported: one in group 4 (135 μg G1 mHA–135 μg G1 mHA) at 123 days post-dose 2, one in group 5 (135 μg G1 mHA/$Al(OH)_3$–135 μg G1 mHA/$Al(OH)_3$) at 16 days post-dose 2, and one in group 6 (135 μg G1 mHA - Placebo) at 86 days post-dose 2. Data on influenza group classification were not collected for these cases, and, as such, it was unknown if infection was caused by a group 1 or group 2 virus. Serological testing was conducted for classification based on anamnestic responses but was inconclusive. There were no fatalities reported in the study, and the vaccine demonstrated an acceptable safety profile.

Vaccine-induced immune responses

To assess immunogenicity, binding antibody responses to the full-length H5 A/Vietnam/1203/2004 (H5 HA) were measured as an indicator of the presence of influenza A group 1-specific stem-binding antibodies, since the low seroprevalence of H5 strains enables a clearer indication of desired vaccine-elicited responses to the conserved HA stem. In addition, we confirmed responses induced by the G1 mHA regimens by measuring vaccine-homologous binding antibodies to the H1 A/California/07/2009-based HA stem antigen (H1 mHA). There was a strong increase in the responses to both the H5 HA (Figs. 3a, 4c, Supplementary Fig. 2a,b, Supplementary Table 4) and the H1 mHA (Figs. 3d, 4c, Supplementary Fig. 3a,b, Supplementary Table 4) across the different regimens, with a 6.5- to 16.4-fold increase in the geometric mean response from baseline detected post-dose 1 (Day 29; Fig. 3b, e), and a percentage of responders with a greater than 4-fold increase from baseline between 64- to 100% across G1 mHA regimens (Fig. 3c, f). In comparison, a lower geometric mean increase from baseline of 2.1 and 2.2-fold was observed for H5 HA- and H1 mHA-specific responses, respectively, for the Q-SD FluVac group at Day 29, with 14% and 17% responders (Fig. 3b,c, e,f). Limited boosting of HA stem responses was observed in participants given a second G1 mHA vaccine dose, regardless of dose level or the addition of adjuvant (Day 85 compared to Day 29), and high antibody levels induced after the first dose were maintained up to 8 months post-dose 1 for the single dose arms or 6 months post-dose 2 (Day 238, Fig. 3a, d, Supplementary Table 4). When responses were presented by gender, there were no differences observed between male and female participants (Supplementary Table 5). There were no clear differences between the regimens, as binding antibody levels were similar between the low dose

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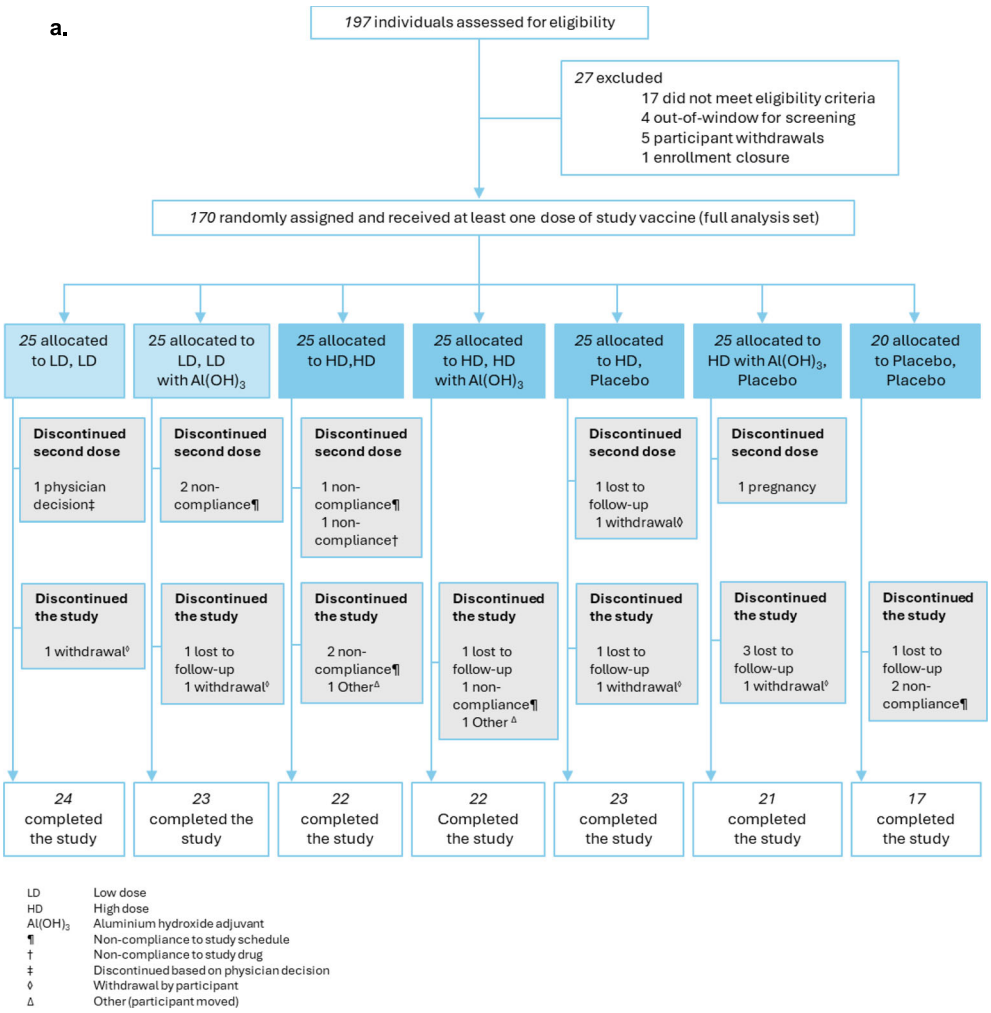

groups and the high dose groups (Group 1 vs. 4 and Group 2 vs. 5), those who received $Al(OH)_3$ and those who did not (Groups 1 vs. 2, Groups 4 vs. 5, and Groups 6 vs. 7), and those who received 1 dose compared to 2 doses (Group 6 vs. 4 and Group 7 vs. 5). However, there was a trend toward stronger responses in the high dose groups compared to the low dose groups.

To evaluate the breadth of vaccine-induced binding antibody responses, we used a panel of 8 ELISAs focused on influenza A group 1 (Main Breadth panel), selected to cover the vaccine-homologous H1 subtype, the H1-, H9-, and H11 clades, and additional strains from group 1 with pandemic potential (Supplementary Table 6, Supplementary Table 7). All participant samples up to Day 238 for FLZ1001 and up to

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Fig. 1 | Study CONSORT diagram and overview of clinical study design. **a** CONSORT diagram showing the flow of healthy adult participants through the FLZ1001 study between 17th May 2023 and 19th Aug 2024. Participants were randomly assigned to either a low dose (LD; 45 μg INFLUENZA G1 mHA) or high dose (HD; 135 μg INFLUENZA G1 mHA) with or without aluminum hydroxide adjuvant ($Al(OH)_3$). ¶: Discontinued due to non-compliance with study schedule; †: Discontinued due to non-compliance with study drug; ‡: Discontinued vaccination due to physician decision; ◇: Withdrawal by participant; Δ Participant moved. **b** Overview of FLZ1001 study regimens and sampling scheme. Participants were vaccinated at Day 1 and Day 57 with either a placebo dose or active vaccine. Participants in the active vaccine groups received two doses of INFLUENZA G1 mHA at 45 μg with or without $Al(OH)_3$ or one or two doses of INFLUENZA G1 mHA at 135 μg, with or without $Al(OH)_3$. Groups 3 and 8 received 2 doses of placebo at Day 1 and Day 57 and these two groups were combined for analysis. Participants were followed up until 1 year after the first vaccination. Vaccination and sampling time points are indicated with vertical lines (top). Vaccination (syringe), and safety and immunogenicity visits are indicated by symbols (bottom).

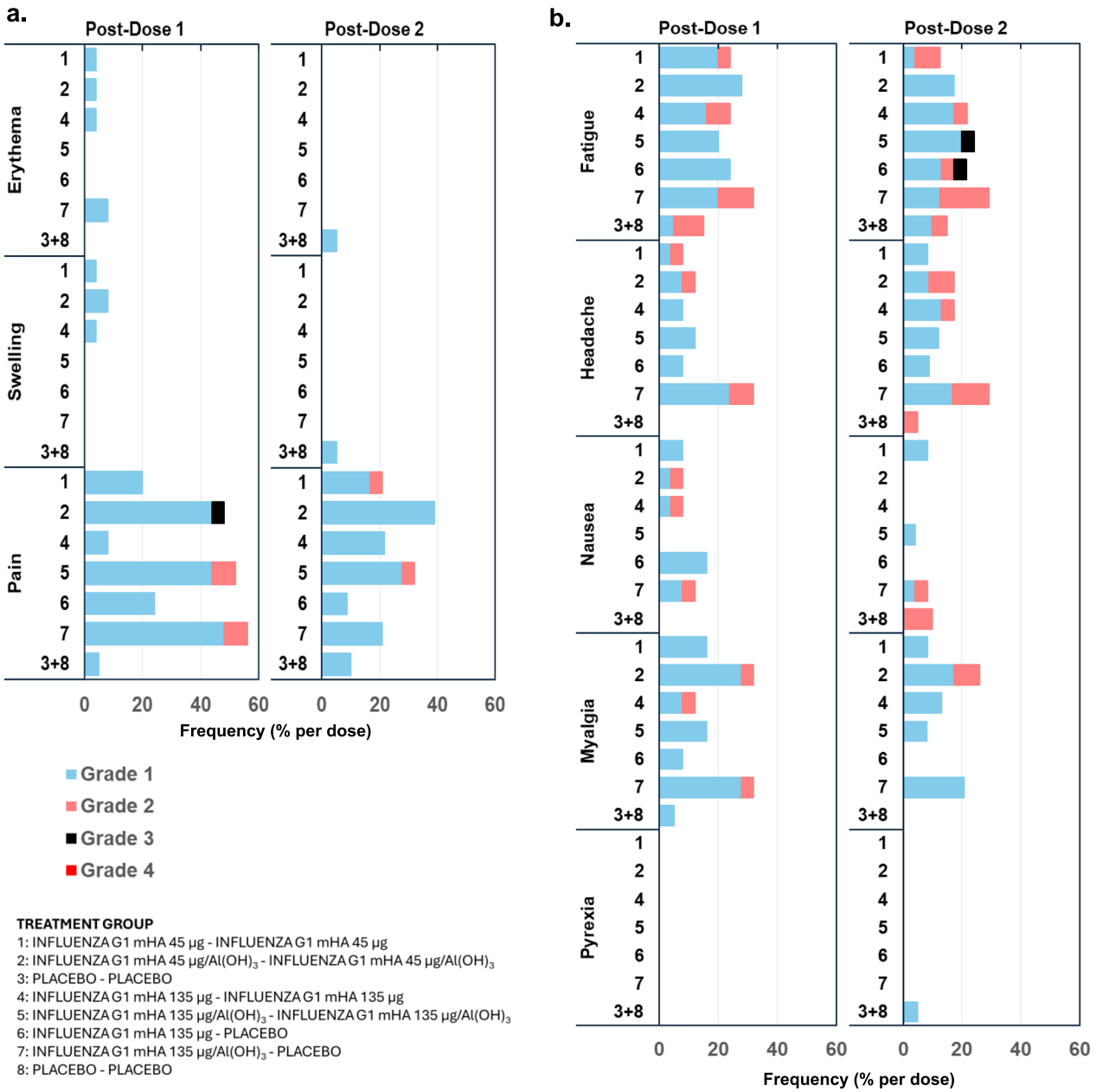

Fig. 2 | Frequency of solicited local and systemic adverse events following INFLUENZA G1 mHA vaccination. **a** Solicited local adverse events by grade. **b** Solicited systemic adverse events by grade. Placebo groups (3 and 8) were pooled for analysis. Frequency is given as the percentage of participants who experienced an adverse event per dose. Sample sizes used for statistical analysis can be found in Supplementary Table 2 and 3. Source data are provided as a Source Data file.

Day 29 for the Q-SD FluVac group were measured (Fig. 4a). Responses to the breadth panel showed marked increases, ranging from a 2.5- to 15-fold increase from baseline across G1 mHA groups at Day 29, compared to a 1.6- to 7.4-fold increase for the Q-SD FluVac group, with the higher responses associated with strains antigenically closest to the vaccine strains (Fig. 4d). Geometric mean increases from baseline for the G1 mHA groups were similar at Day 85 compared to Day 29 and decreased slightly at Day 238 (Supplementary Fig. 4). To further assess the durability of the binding antibody responses, binding antibody levels to H1 A/Victoria/2570/2019 were evaluated at Day 365 post-dose

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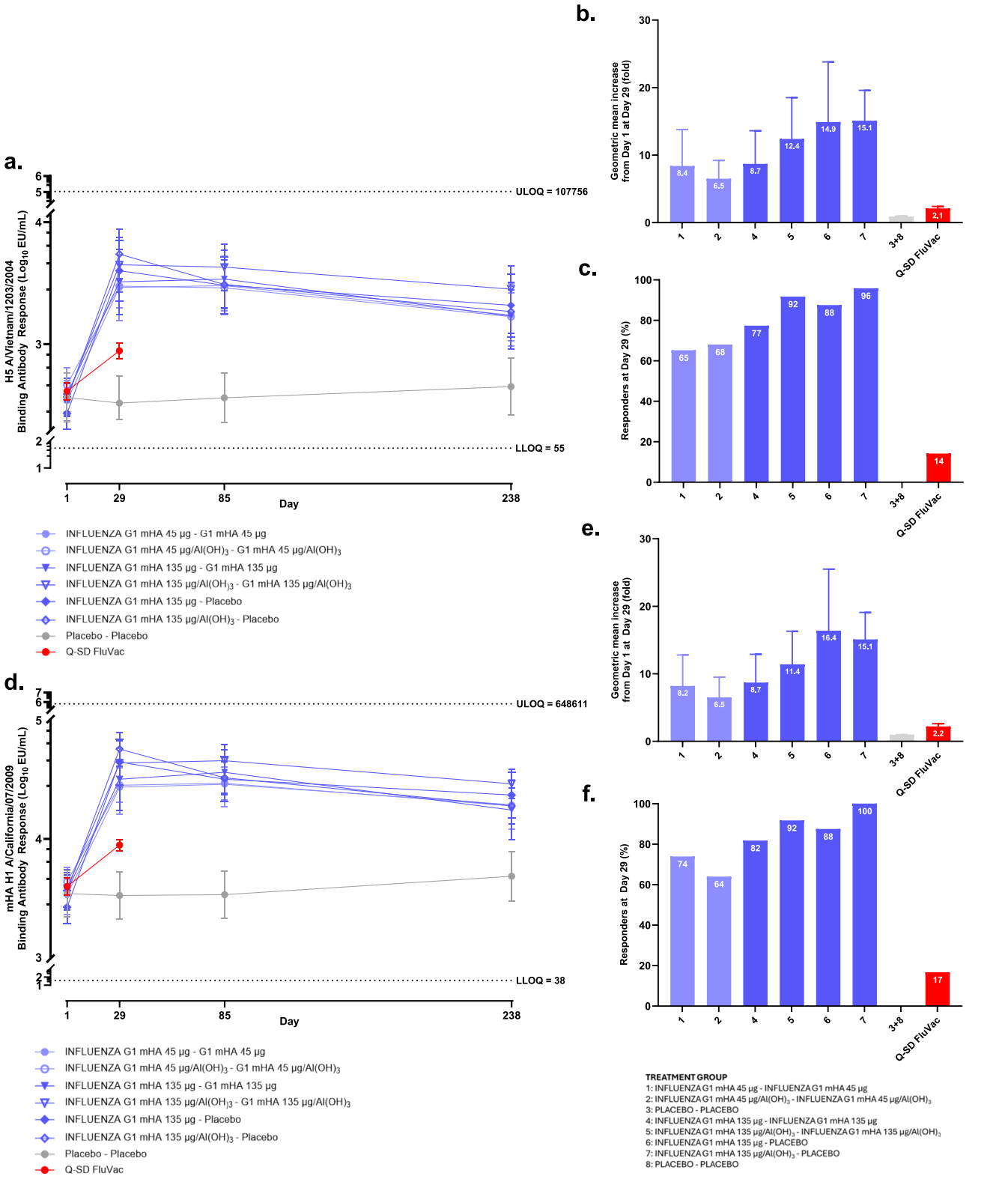

1 (Supplementary Table 8). Results demonstrated that antibody levels were maintained, with fold increases from baseline at Day 365 ranging from 1.7 to 3.4 across G1 mHA groups. All regimes induced robust breadth of binding antibodies, and the addition of $Al(OH)_3$ adjuvant or a second dose of vaccine did not strongly affect the magnitude, breadth, or durability of binding antibody responses. This analysis showed that binding antibody responses in the G1 mHA vaccine groups achieved greater breadth than those induced by a quadrivalent seasonal influenza vaccine and were greater in magnitude against all antigens except for two H1 strains that were seasonal vaccine-matched or genetically similar to the H1 strain included in the seasonal vaccine (H1 A/Victoria/2570/2019 and H1 California/07/2009, respectively, Fig. 4f). Importantly, the G1 mHA vaccine based on the H1 California/07/2009 strain elicited similar levels of binding antibodies to these two strains compared to the seasonal influenza vaccine. The H1 responses measured were specific to strains representative of H1N1 viruses that

Fig. 3 | Binding antibody responses to core binding panel assessed by ELISA. **a** Binding antibody responses to H5 A/Vietnam/1203/2004 assessed by ELISA. Geometric mean concentrations (ELISA Units per mL) and 95% confidence intervals are shown for all regimens and the quadrivalent seasonal influenza vaccinated comparator cohort (Q-SD FluVac). Placebo groups were pooled for analysis. The lower limit of quantification (LLOQ) for the assay is 55, the upper limit of quantification (ULOQ) for the assay is 107756, EU: ELISA Units. **b** Fold increase in H5 A/Vietnam/1203/2004 geometric mean concentrations with 95% CI at Day 29 from Day 1 baseline. Treatment groups are labeled 1-8. **c** Percentage of responders at Day 29 defined as those with a > 4-fold increase in H5 A/Vietnam/1203/2004 binding antibody response from Day 1 baseline or > 4-fold increase from LLOQ in case baseline ≤LLOQ. INFLUENZA G1 mHA treatment groups are labeled 1-8. **d** Binding antibody responses to the INFLUENZA G1 mHA-homologous antigen mHA H1 A/California/07/2009 assessed by ELISA. Geometric mean concentrations (ELISA Units per mL) and 95% confidence intervals are shown for all regimens and the quadrivalent seasonal influenza vaccinated comparator cohort (Q-SD FluVac). Placebo groups were pooled for analysis. LLOQ for the assay = 38, ULOQ for the assay = 648611, EU: ELISA Units. **e** Fold increase in mHA H1 A/California/07/2009 geometric mean concentrations with 95% CI at Day 29 from Day 1 baseline. **f** Percentage of responders at Day 29 defined as those with a > 4-fold increase in mHA H1 A/California/07/2009 binding antibody response from Day 1 baseline or > 4-fold increase from LLOQ in case baseline ≤LLOQ. INFLUENZA G1 mHA treatment groups are labeled 1–8. Sample sizes used for statistical analysis can be found in Supplementary Table 4. Source data are provided as a Source Data file.

have circulated globally and include prevalent variants evolving from the H1 A/South Carolina/1/1918 strain first documented in humans[13–15] (Fig. 4f).

To further explore the breadth of binding responses and potential vaccine coverage, we randomly selected a subset of participants to measure responses to an extended breadth panel (Supplementary Table 6) which included an additional 6 antigens covering further group 1 HAs, another stem-based H1 mHA, and an influenza A group 2 and influenza B strain (Fig. 4b, Supplementary Table 6). The group 1 responses showed geometric mean increases of 6- to 13.7-fold from baseline at Day 29 across all tested G1 mHA regimens, compared to a lower 2.1- to 2.3-fold observed in the Q-SD FluVac group (Fig. 4e). In addition, responses to the H5 A/Cambodia/NPH230032/2023 strain were measured at Day 365 post-dose 1, with fold increases from baseline ranging from 1.9 to 5.1 across G1 mHA groups (Supplementary Table 9). Furthermore, binding antibody responses for the G1 mHA regimens to an influenza A group 2 strain (H7 A/Anhui/1/2013), increased 3- to 4.8-fold following vaccination at Day 29, compared to 1.6-fold for the Q-SD FluVac group (Fig. 4b, e), while no response to H3 A/Darwin/9/2021 was detected (Supplementary Table 10). As expected for the G1 mHA vaccine studied, no responses to influenza B HA (B/Iowa/06/2017) were observed after vaccination while the Q-SD FluVac vaccine, containing an influenza B component, induced a 3.7-fold geometric mean increase over baseline at Day 29 (Fig. 4b, e).

To evaluate whether the vaccine-induced humoral responses included functionally active antibodies that can induce a diverse range of protective responses, we first assessed responses using a H5N1-specific neutralization assay. Samples from participants who received the G1 mHA high-dose regimens with or without $Al(OH)_3$ were analyzed for neutralizing antibody responses up to Day 238 alongside a subset of the Q-SD FluVac cohort up to Day 29. Similarly to the binding antibody responses, large increases in neutralizing antibodies were observed across the G1 mHA high dose groups (Fig. 5a, Supplementary Table 11). Geometric mean increases from baseline at Day 29 ranged from 7.9- to 15-fold across the G1 mHA high dose groups, compared to a considerably lower increase of 1.9-fold observed for the Q-SD FluVac subset (Fig. 5b, Supplementary Fig. 5a), corresponding to 75–92% of participants with a greater than 4-fold increase from baseline at Day 29 for the G1 mHA groups compared to 12% of participants in the Q-SD FluVac subset (Fig. 5c, Supplementary Table 11). Durable neutralizing antibody responses were observed whereby levels measured after the first dose only slightly decreased by 8 months post-dose 1 (Day 238, Fig. 5a, Supplementary Table 11). There were no distinct differences in neutralizing antibody levels for all high dose groups at Day 29 or Day 238, where the addition of $Al(OH)_3$ did not strongly impact levels, nor did receiving an additional dose.

Next, we used a Human FcγRIIIa Reporter Assay[16] to measure antibody-dependent cellular cytotoxicity (ADCC) responses. We observed induction of ADCC responses across all G1 mHA regimens (Fig. 5d, Supplementary Table 12), with fold-increases from baseline at Day 29 ranging from 4.9 to 8.6, which was higher than that observed with the Q-SD FluVac group, where the fold increase was 2.2 (Fig. 5e, Supplementary Fig. 5b). 50 to 84% of participants in the G1 mHA groups had a greater than 4-fold increase over baseline at Day 29 compared to 21.7% of participants in the Q-SD FluVac group (Fig. 5f, Supplementary Table 12). Responses slightly decreased at Day 85 and Day 238, and as was observed with binding and neutralizing antibody titers, a second dose of vaccine or addition of $Al(OH)_3$ adjuvant did not change the levels at these later time points, since no differences between the treatment groups were observed. Correlation analysis revealed that there was a strong positive association between binding antibody levels with neutralizing antibody, and ADCC levels, confirming that most binding antibodies elicited are functional (Supplementary Fig. 6b).

Protective capacity of vaccine-induced stem-reactive antibodies in a passive transfer mouse challenge model

To determine if vaccine-induced antibodies could mediate protection against influenza, we employed a stringent passive transfer model in BALB/c mice[17]. To evaluate whether the use of adjuvant increased protective capacity, we randomly selected a subset of 50 FLZ1001 participants from the high-dose groups that received G1 mHA protein at 135 μg, either with or without $Al(OH)_3$. Mice were infused with either Day 1 or Day 29 sera and subsequently challenged with an H5N1 (A/Hong Kong/156/1997) influenza strain (Fig. 6a, Supplementary Fig. 7a), where pre-vaccination sera served as a control. To confirm successful transfer, blood samples from mice were collected one day after transfer to measure HA-specific antibody levels and to compare to levels measured in the corresponding participant sample. 6 out of 50 mice (12%) with serum binding antibody titers more than 100-fold lower than those observed in human sera were considered a failed transfer and excluded from further analysis (Supplementary Fig. 7b). The overall survival proportion of mice infused with human post-vaccination sera from the FLZ1001 study was significantly higher compared to mice infused with pre-vaccination sera from the same participants (67% versus 0% survival, $p < 0.001$, Fig. 6b), indicating that the vaccine-induced humoral response was protective against a vaccine-heterologous H5N1 strain. Additional clinical readout parameters including clinical scores (Fig. 6c) and relative bodyweight loss over time (Fig. 6d) were also significantly different between mice infused with post- compared to pre-vaccination sera when assessing bodyweight change across the follow-up period per mouse as area-under-the-curve. When comparing the survival proportions of mice injected with serum from adjuvanted participants versus non-adjuvanted participants, we observed a trend for better protection in the adjuvanted group based on all clinical readout parameters, although this difference did not reach statistical significance (78% versus 55%) (Supplementary Fig. 7c). To verify that antibody levels present in human sera were linked to survival, we first compared binding antibody levels to H1, H1 mHA, and H5 HA in mice that survived H5N1 challenge versus those that did not, which revealed that mice surviving challenge had higher human binding antibody titers. This result was also confirmed in mouse sera evaluated post-transfer (Supplementary Fig. 7d). To examine whether the H5-specific ELISA,

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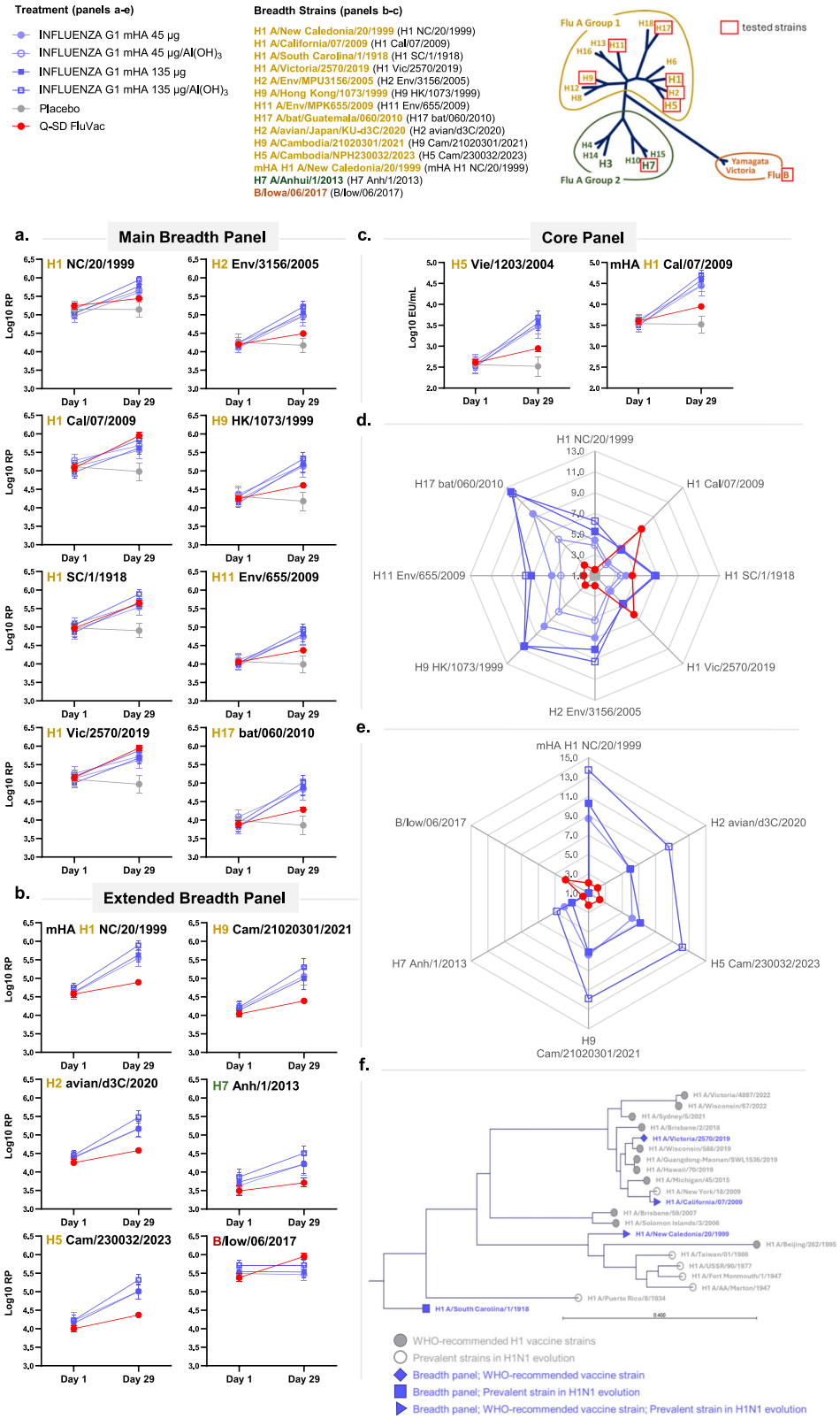

H5N1-specific neutralization assay, and the H5-specific ADCC titers in humans could be used to predict survival of mice after serum transfer and subsequent challenge with H5N1 virus, a logistic regression model was used to fit survival status with assay titer as a covariable (Fig. 6e). The slope estimates of 5.979, 4.782 and 2.139 for binding, neutralization, and ADCC assay titers were statistically significant (z-test: $p < 0.001$, $p = 0.001$, and $p = 0.002$, respectively) and showed that all assay titers predict survival in the H5N1 passive serum transfer challenge model. A $\log_{10}$ titer of 3.41, 1.94 and 2.48 for binding, neutralization and ADCC assay titers, respectively, predicted 50% survival in the model. In addition, Receiver Operating Characteristic (ROC) curves for the three assays were generated and summarized as Area Under the Curve (AUC) with 95% confidence intervals (Fig. 6f). The AUC confidence intervals were above 0.50, with a statistically

Fig. 4 | Binding antibody responses to the Main Breadth and Extended Breadth Panel assessed by ELISA at Day 1 and Day 29. a Binding antibody responses at Day 1 and Day 29 to the main breadth panel and **b** extended breadth panel measured by ELISA are shown as geometric mean titer (log_{10} relative potency (RP)) with 95% confidence interval. The INFLUENZA G1 mHA groups receiving the same dose 1 vaccination were pooled for analysis. Placebo groups were pooled for analysis. **c** Responses (geometric mean concentration with 95% confidence interval) to the core binding panel (H5 Vietnam/1203/2004 and mHA H1 A/California/07/2009) shown in Fig. 3a and d are included for reference. Responses to the main breadth panel (**a**) were measured for all available samples whereas responses to the extended breadth panel (**b**) were measured on a subset of samples. **d** Fold increase in relative potency binding antibody responses at Day 29, from Day 1 baseline for all groups assessed with the main breadth panel. Placebo groups were pooled for analysis. The INFLUENZA G1 mHA groups receiving the same dose 1 vaccination were pooled for analysis. **e** Fold increase in relative potency binding antibody responses at Day 29, from Day 1 baseline for the subset of participants assessed with the extended breadth panel. The INFLUENZA G1 mHA groups receiving the same dose 1 vaccination were pooled for analysis. **f** Phylogenetic analysis of HA protein for H1 strains tested in the main breadth ELISA panel (blue symbols) against H1 strains recommended by the World Health Organization since 1999 for seasonal influenza vaccine formulation in the northern and southern hemispheres (gray filled circles, blue diamond, blue triangle), and the prevalent strains in the H1N1 virus evolution documented in humans since 1918 (gray open circles, blue square, blue triangles). Full length H1 HA amino acid sequences obtained from the NCBI[54] and GISAID[55] influenza databases were aligned with the k-mer (15) based tree construction tool using the Neighbor-Joining method and Mahalanobis distance measure. Sample sizes used for statistical analysis can be found in Supplementary Fig. 6a. Source data are provided as a Source Data file.

significant positive correlation between titers and survival (Mann-Whitney test, $p < 0.001$), indicating that higher H5-specific binding and functional antibody levels are associated with protection from H5N1 challenge in mice. Building a correlate model to predict survival based on a combination of assays, including baseline and Day 29 H1- and G1 mHA-specific antibody titers revealed that using the H5-specific neutralization assay by itself was already sufficient as a predictor (Supplementary Fig. 9).

Discussion

Efforts to develop a universal influenza vaccine effective against a broad variety of circulating and emerging viral variants have been a long-term focus for the field and more recently guided by the isolation of broadly neutralizing antibodies targeting the conserved HA stem region[18–22]. This first-in-human study demonstrates proof of immunological concept that an influenza A group 1 universal vaccine component[8] comprised of a stabilized HA stem antigen is able to induce robust HA-directed immune responses across a wide range of group 1 subtypes. The vaccine was safe and well-tolerated, and the humoral responses elicited were greater in magnitude, durable, and more broadly reactive than those elicited by a conventional quadrivalent seasonal influenza vaccine. Strong humoral responses against a broad panel of group 1 strains were induced following the initial vaccination, regardless of the regimen tested, indicating that a single dose of vaccine even at a low dose level would suffice to elicit effective and durable immune responses in adults, potentially without the need for addition of an adjuvant. All participants had HA-specific immune responses before receiving the first vaccination, and responses after a second dose were maintained but not strongly increased, which is suggestive of an anamnestic response that boosted levels of pre-existing stem-binding antibodies after the first dose. This is in agreement with earlier work in non-human primates vaccinated with the same construct, which showed that vaccine-induced responses were only modest after a single dose in influenza-naïve animals, but reached peak levels when pre-boost antibody titers were similar to pre-vaccination titers observed in humans in this study[10]. Our findings confirm that targeting the conserved HA stem could be an effective approach to overcome the variable vaccine efficacy observed when vaccine antigens are mismatched to circulating strains and could be effective against emerging variants with pandemic potential.

Since this was a phase 1/2a study that was not powered to assess vaccine efficacy, it is unclear whether the responses elicited would be protective in humans. Hemagglutinin inhibition (HAI) assay titers are generally correlated with protection against influenza infection, with a titer of 1:40 often cited as a threshold for 50% protection[23]. However, this assay relies on antibodies directed at the globular head domain of HA and is therefore not suitable for assessing protective titers of stem-binding antibodies. In the absence of efficacy data in humans or an established correlate of protection, we employed a stringent passive transfer model where mice were challenged with a vaccine-heterologous H5N1 strain and demonstrated high protective capacity of human G1 mHA vaccinee sera that correlated with binding antibody titers. This result contrasted with earlier studies using the same model which showed limited efficacy from responses elicited by seasonal influenza vaccination, verifying that seasonal vaccines eliciting head-binding antibodies with potent neutralization capacity are not protective in such a vaccine-heterologous setting[17]. In addition, the functionality of these antibodies was demonstrated by H5-specific ADCC and neutralizing antibody responses, which have been correlated with protection in previous studies[24–26]. Furthermore, a household transmission study[27] showed that stem-binding antibodies were an independent correlate of protection against influenza infection in humans and correlated with protection from lower respiratory tract infection[28]. These human data are in line with earlier observations that these antibodies were protective in animal models[28–32]. There is nonetheless still a need to directly establish a correlate of protection against influenza infection and disease for an HA stem vaccine candidate.

Only a few clinical studies have been performed to evaluate vaccine antigens that aim to elicit stem-binding antibodies. In a recently conducted phase 1 study, participants were immunized with a stabilized H1 stem antigen displayed on a ferritin nanoparticle, which induced cross-binding and neutralizing antibodies to H1, H2, and H5 HA subtypes and cross-reactive B cells[33,34]. Although a direct comparison with other studies is difficult, the fold increases in the post-vaccination responses were lower than those observed in our study, and a proportion of the response was directed against the *H. pylori* ferritin used to display and further stabilize the trimeric structure of the H1 stem antigen. Such trimerization domains are now often omitted from vaccine antigen designs to avoid off-target responses. Others have used a chimeric HA approach, that combines the stem domain with a divergent head domain to which no pre-existing immunity is expected[29,35,36]. Results for this vaccine concept evaluated in a phase 1 study resulted in humoral responses directed against both the head and the H1 HA stem domain[35,37], potentially limiting the induction of stem-directed responses. The breadth of responses elicited in these studies can only be directly compared in future studies that make use of standardized assays or reagents, such as the broadly-reactive influenza A group 1, group 2, and influenza B multi-domain antibody[38] used for binding antibody assays in this study.

Immune responses to an HA stem-based vaccine may be more broadly neutralizing if administered to influenza naïve individuals such as children, who could then establish broader initial immunity to influenza compared to those who mount a strain-specific response at first encounter with a circulating strain. The immunodominance of the HA head region limits the breadth of a recall response, which was recently confirmed in a study using an H2 HA antigen in H2-naïve and H2-exposed individuals, where the H2-exposed individuals had limited HA stem responses post-vaccination compared to H2-naïve participants[39]. Immune imprinting from initial exposure to an HA stem-based antigen in influenza-naïve individuals could bias future

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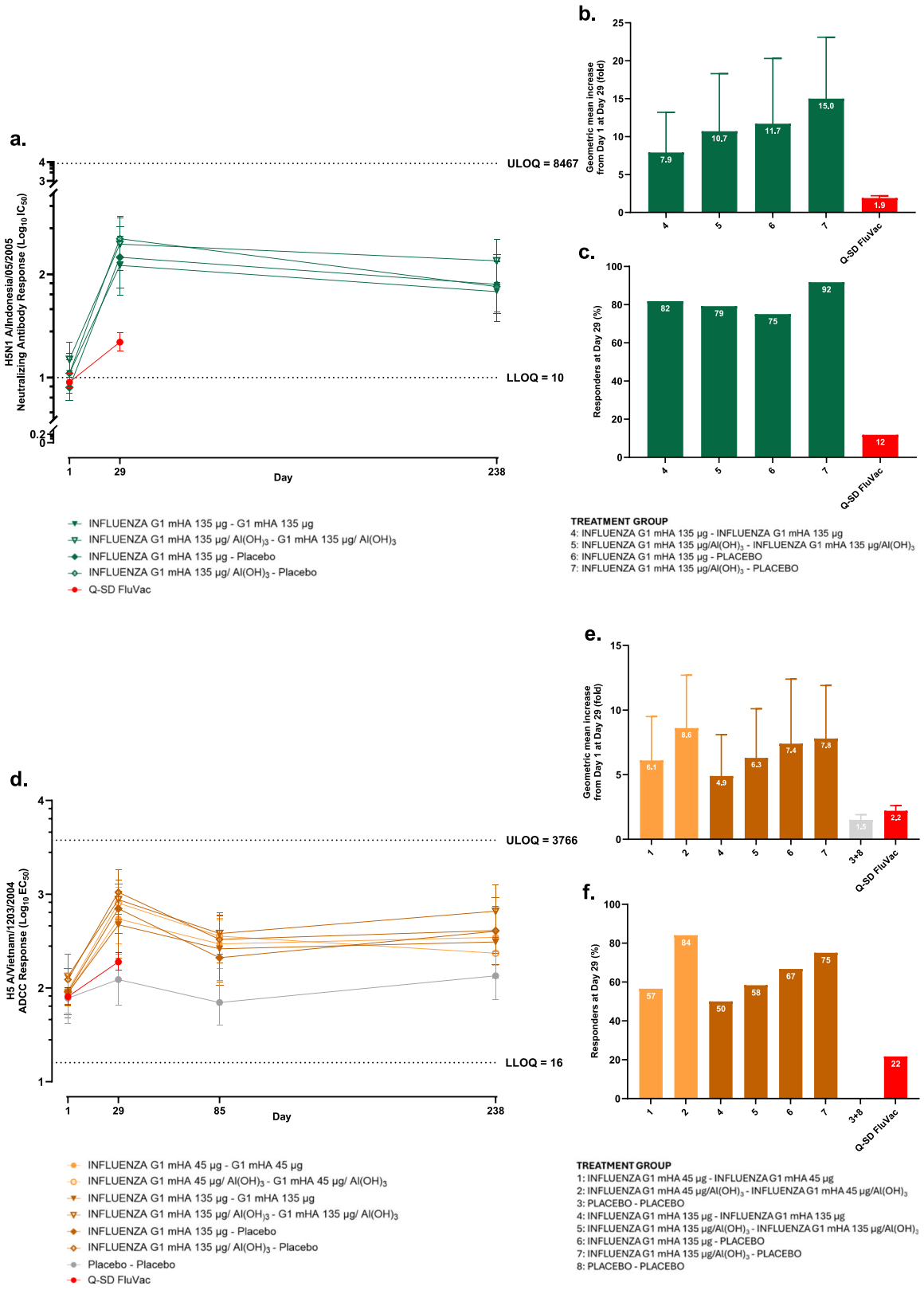

responses toward greater breadth upon subsequent encounters with virus[40].

During the study, three laboratory-confirmed influenza A cases were reported by participants receiving active regimens. This incidence (3/150 or 2%) was lower than the CDC case number (11.7/100 or 11.7%) in the same age category recorded in the 2023-2024 season in the USA[41], where the FLZ1001 study was conducted in the corresponding time frame[42]. Due to differences in methodologies for case definition and since 33.8% of 18–49-year-olds were vaccinated with seasonal influenza vaccine while participants in the FLZ1001 study were not, direct comparisons cannot be made. Nonetheless, due to the low number of cases observed, there is limited concern of previously

Fig. 5 | Neutralizing antibody and antibody-dependent cellular cytotoxicity responses. a Neutralizing antibody responses assessed by H5N1 A/Indonesia/05/2005 Plaque Reduction Neutralization Test (PRNT). Geometric mean titer (expressed as 90% Inhibitory Concentration (IC_{90})) and 95% confidence intervals are shown for all high dose INFLUENZA G1 mHA regimens and the quadrivalent seasonal influenza vaccinated comparator cohort (Q-SD FluVac). The lower limit of quantification (LLOQ) for the assay is 10; the upper limit of quantification (ULOQ) for the assay is 8467. **b** Fold increase with 95% confidence intervals in H5N1 A/Indonesia/05/2005 geometric mean titers at Day 29 from Day 1 baseline. INFLUENZA G1 mHA treatment groups are labeled 4-7. **c** Percentage of responders at Day 29 defined as those with a > 4-fold increase in H5N1 A/Indonesia/05/2005 neutralizing antibody response from Day 1 baseline or > 4-fold increase from LLOQ in the case where baseline ≤LLOQ. INFLUENZA G1 mHA treatment groups are labeled 4-7. Sample sizes used for statistical analysis can be found in Supplementary Table 11. **d** Antibody Dependent Cellular Cytotoxicity (ADCC) responses assessed by Human FcγRIIIa Reporter Assay. Geometric mean titer (expressed as 50% effective concentration (EC_{50})) and 95% confidence intervals are shown for all INFLUENZA G1 mHA regimens and the quadrivalent seasonal influenza vaccinated comparator group (Q-SD FluVac). The LLOQ for the assay is 16; the ULOQ for the assay is 3766. **e** Fold increase with 95% confidence intervals in Antibody Dependent Cellular Cytotoxicity Response at Day 29 from Day 1 baseline. INFLUENZA G1 mHA treatment groups are labeled 1-8. **f** Percentage of responders at Day 29 defined as those with a > 4-fold increase in ADCC responses from Day 1 baseline or > 4-fold increase from LLOQ in the case where baseline value ≤ LLOQ. INFLUENZA G1 mHA treatment groups are labeled 1-8. Sample sizes used for statistical analysis can be found in Supplementary Table 12. Source data are provided as a Source Data file.

reported antibody-dependent enhancement of respiratory disease in animal models following vaccination with HA stem antigens[43,44].

There are a few limitations to this study. Since the study served as a proof of immunological principle, only an influenza A group 1 antigen was evaluated. Based on current evidence, a universal influenza vaccine would require additional components to cover the common influenza A group 1 and group 2, and influenza B subtypes circulating every season. The G1 mHA vaccine increased the magnitude of influenza A group 2- specific antibodies against H7, indicating that the vaccine elicits antibodies towards conserved epitopes in the stem domain, however, this was not at the magnitude achieved for group 1, and not observed against H3. Of note, this binding profile was observed infrequently for HA stem-specific neutralizing monoclonal antibodies. The lack of an increased response to H3 also highlights the need for a group 2 antigen. A recent Phase 1 study with a conceptually similar group 2 stabilized HA stem ferritin nanoparticle demonstrated that such a vaccine component could increase group 2-specific antibody levels[45,46]. It would also be of interest to perform additional studies including PBMC isolation to further characterize the antibody and B-cell response to vaccination. HA stem vaccines have been shown to induce antibodies that clonally converge into lineages targeting two supersites in the central and anchor stem region[34,47].

Another limitation was that the study was not powered to detect differences between the regimens evaluated. However, since immune responses were similar across groups with overlapping confidence intervals, clearly distinctive responses may only be detected between regimens if much larger group sizes were enrolled. Furthermore, use of an external immunogenicity comparator group to compare our vaccine responses to a seasonal influenza vaccine has drawbacks since study conduct could not be fully controlled. However, the demographics of the participants selected were balanced, and immune responses were measured simultaneously in the same assays. While comparisons to seasonal influenza vaccine performance was insightful, durability measurements could not be compared, as only baseline and Day 29 samples from the comparator group were available. However, the durability of the response in this study is at least similar to, if not superior to durability reported for seasonal influenza vaccines[48].

Future studies are needed to further develop a universal vaccine as additional components are required to cover all circulating seasonal strains. Ideally, prior to conducting complex efficacy studies, vaccine effectiveness could be evaluated by means of a human challenge study, providing early insights into the value of new vaccine components to accelerate development efforts, which is especially relevant in the context of ongoing pandemic threats by, for example, avian H5N1 viruses.

To conclude, this phase 1 study showed that the INFLUENZA G1 mHA candidate is a promising vaccine component capable of eliciting a strong and broad range of group 1 binding and functional antibody responses that were protective in a murine H5N1 passive transfer challenge model, and is superior as a single dose to a quadrivalent seasonal influenza vaccine. These findings can be taken forward to possibly further develop a universal influenza vaccine. A broadly protective vaccine against a variety of influenza strains has the potential to reduce the global health burden from influenza disease and could be critical to support preparedness for a future pandemic response.

Methods

Study design

This randomized, double-blind, placebo-controlled Phase 1/2a study (ClinicalTrials.gov:NCT05901636) was conducted at four sites in the USA. The study protocol and amendments were reviewed and approved by the institutional review boards at each study site. The study adhered to the current Declaration of Helsinki and ICH Good Clinical Practice guidelines. The objective was to evaluate safety, reactogenicity and immunogenicity of a universal Influenza vaccine component consisting of one or two vaccinations with INFLUENZA G1 mHA in healthy adults, administered at a low-dose (LD) of 45 μg protein or a high-dose (HD) of 135 μg protein with or without 0.75 mg aluminum hydroxide adjuvant ($Al(OH)_3$). The choice of the adjuvant was based on its well-established safety profile[49], immediate availability, and preclinical data that indicated that vaccine immunogenicity in combination with $Al(OH)_3$ was improved[10]. Sex or gender was not considered in the study design.

Sample size

This was a first-in-human study with no formal statistical hypothesis testing for safety or immunogenicity outcomes between the groups. However, given 25 participants assigned to each vaccine regimen, the observation of no adverse reactions after the first vaccination would be associated with a 95% confidence that the true rate of adverse reactions is less than 11.3% for this sample size. Across active regimens with a sample size of 150, the observation of no adverse reactions after the first vaccination would be associated with a 95% confidence that the true rate is less than 2.0%.

Vaccine

The INFLUENZA G1 mHA vaccine was manufactured under Good Manufacturing Practice and comprised a soluble, trimeric, stabilized HA stem protein based on the H1 A/California/07/2009 strain. This stem HA antigen was created by excision of the variable and immunedominant head domain and insertion of stabilizing mutations and a GCN4 trimerization domain, as previously described in Swart et al[10]. and depicted in Supplementary Fig. 8. To summarize, combining rational design with high-throughput screening resulted in a single-chain protein formed of a headless HA ectodomain, which assembles into a soluble, covalently bound trimeric stem HA when expressed in mammalian cells. The head domain was replaced with a glycine linker, reconnecting the remaining HA1 ends post-deletion. Additionally, several mutations were introduced to enhance stability, along with a sequence to promote trimer formation in the absence of the head and transmembrane domain.

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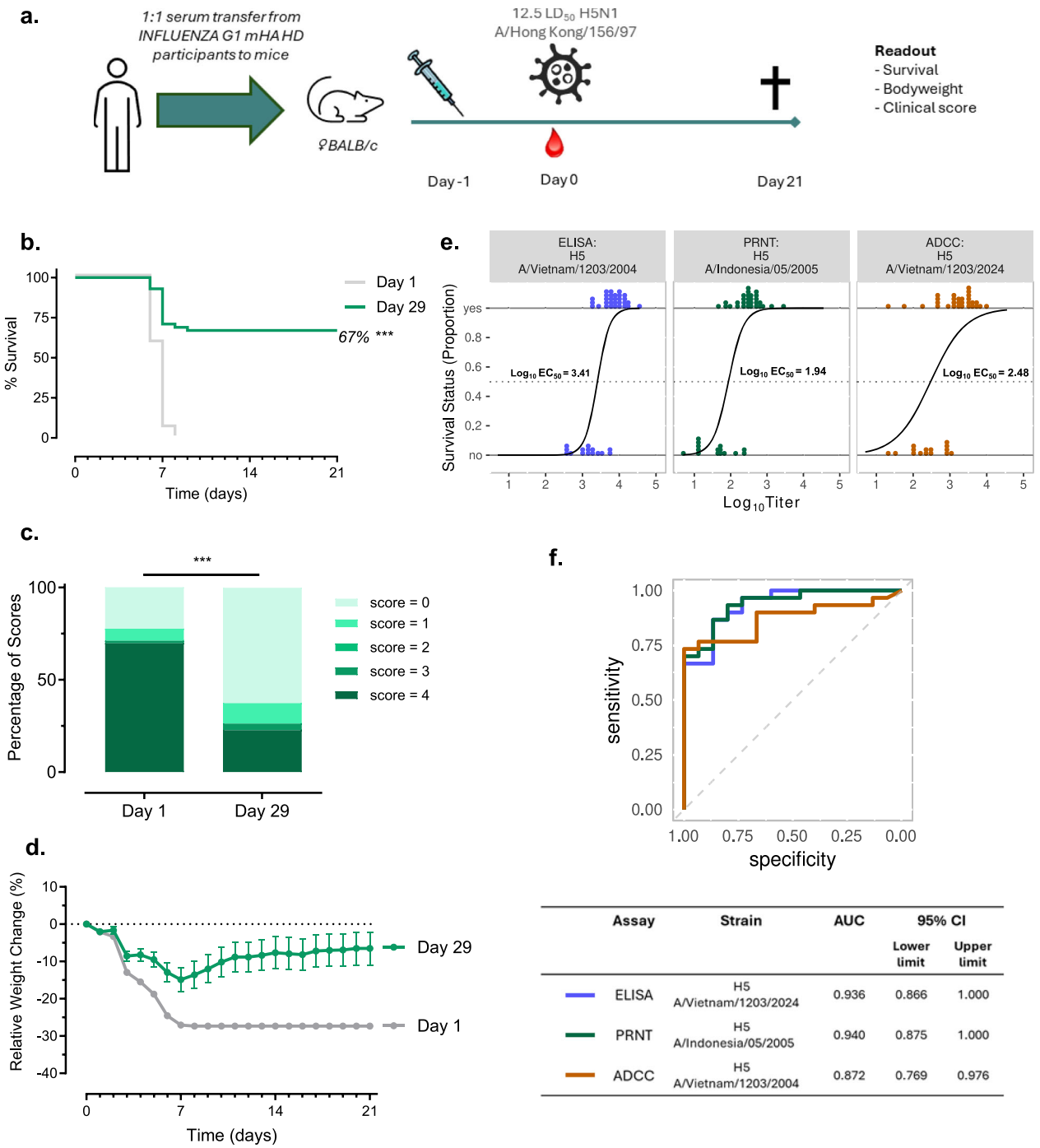

Assay	Strain	AUC	95% CI	
			Lower limit	Upper limit
ELISA	H5 A/Vietnam/1203/2024	0.936	0.866	1.000
PRNT	H5 A/Indonesia/05/2005	0.940	0.875	1.000
ADCC	H5 A/Vietnam/1203/2004	0.872	0.769	0.976

Fig. 6 | Passive transfer challenge data and protective capacity of induced stem-reactive antibodies in mice. a Experimental design. Mice received FLZ1001 serum via intraperitoneal injection on Day −1 followed by intranasal challenge with 12.5 LD_{50} of H5N1 A/Hong Kong/156/1997 on Day 0 and were followed up for 21 days post infection. **b** Survival curves of mice receiving Day 29 (green) or Day 1 (baseline; gray) human sera from FLZ1001 participants who received 135 µg of INFLUENZA G1 mHA. Data for all high-dose (HD) participants were pooled for analysis. Comparison of survival proportion for combined Day 29 vs Day 1 groups by a two-sided sign test showed the difference was statistically significant (*** $p < 0.001$). **c** Clinical scores for mice receiving Day 1 ($N = 49$) and Day 29 ($N = 45$) FLZ1001 sera at Day −1 followed by 12.5 LD_{50} H5N1 A/Hong Kong/156/1997 challenge at Day 0, and followed up for 21 days post infection until a humane endpoint was reached. Comparison of survival proportion for combined Day 29 vs Day 1 groups by a two-sided z-test showed difference was statistically significant (*** $p < 0.001$). **d** Bodyweight data, expressed as relative weight loss compared to Day 0, of mice receiving Day 29 (green) and Day 1 (gray) human sera at Day −1 followed by 12.5 LD_{50} H5N1 A/Hong Kong/156/1997 challenge at Day 0, and followed up for 21 days post infection. Mean relative bodyweight loss relative to Day 0 during follow-up is shown. Dotted horizontal line references no relative weight change. Error bars denote 95% confidence interval. **e** Logistic regression model applied on survival status with H5-specific ELISA, PRNT and ADCC assay titers as a covariate. Titers predicting 50% survival are indicated as 50% Effective Concentration (EC_{50}). The slope estimates of 5.979, 4.782 and 2.139 for H5-specific ELISA, PRNT and ADCC assay titers were statistically significant (two-sided z-test: $p < 0.001$, $p = 0.001$, and $p = 0.002$, respectively). **f** The Receiver Operating Characteristics (ROC) curve summarized as Area Under the Curve (AUC) for the H5-specific ELISA, PRNT, and ADCC assays with 95% confidence intervals. A two-sided Mann-Whitney test showed a statistically significant association for all three assays with survival: ELISA, PRNT, and ADCC ($p < 0.001$). Source data are provided as a Source Data file.

Participants

Eligible participants were healthy adults aged 18-45 years, who had not received vaccination with a pandemic influenza vaccine (other than H1N1) in a vaccine study prior to randomization. Full inclusion and exclusion criteria are listed in the Study Protocol (Supplementary Information). Participants provided written informed consent to participate in the study.

Randomization and procedures

Participants were randomly assigned into vaccine groups, based on a computer-generated randomization schedule using randomly permuted blocks prepared under the sponsor's supervision. Participants were assigned into 3 groups in Cohort 1 (60 adults) and 5 groups in Cohort 2 (110 adults), at an overall ratio of 7.5:1 to receive study vaccine or placebo (0.9% saline) (Fig. 1b) using a staggered approach (Supplementary Fig. 1). An independent internal data review committee (DRC) was responsible for oversight of the study and regularly reviewed safety and reactogenicity data. Participants in Cohort 1 were enrolled prior to progressing to Cohort 2. Initially, 5 sentinel participants were enrolled and randomized into Groups 1, 2 or 3 (Supplementary Fig. 1, Fig. 1b). Participants then received the first dose of vaccine on Day 1 and were contacted 48 hours post-vaccination to collect safety information. Blinded post-vaccination safety data for these sentinel participants was reviewed by the principal investigator (PI) and study-responsible physician (SRP). In the absence of any clinically significant findings in the sentinel cohort, an additional 12 participants (safety ramp-up subset) were enrolled and randomized to receive the first dose of vaccine. The DRC reviewed post-vaccination blinded safety data of all 17 participants enrolled (7 days after the first vaccination). Further enrollment and randomization of the remaining 43 participants in Cohort 1 was initiated following completion of the 7-day DRC safety evaluation and determination that there were no clinically significant findings. This was initiated in parallel with the first 5 participants to be enrolled into Groups 4 to 8 of Cohort 2. Enrollment in Cohort 2 followed the same procedure as for Cohort 1 (Supplementary Fig. 1), where an additional 10 participants were randomized following absence of clinical findings at the 48-hour post-vaccination safety review of the sentinel group, and then the remaining 95 participants randomized following the 7-day DRC safety evaluation of the sentinel and safety ramp-up subset. Safety evaluations 7-days after administration of the second dose were also carried out in the same manner as performed following dose 1.

INFLUENZA G1 mHA was administered intramuscularly at a dose of either 45 μg or 135 μg. Participants in Cohort 1 received two doses of placebo or study vaccine at 45 μg per dose on Day 1 and Day 57, either with or without $Al(OH)_3$ adjuvant. Participants in Cohort 2 received either two doses of placebo or two doses of study vaccine at 135 μg per dose on Day 1 and Day 57, administered with or without $Al(OH)_3$ adjuvant, or one dose of study vaccine at 135 μg per dose on Day 1 (with or without $Al(OH)_3$ adjuvant) followed by a placebo dose on Day 57 (Fig. 1b).

Safety assessments

Participants were monitored for adverse events (AEs) for at least 30 min post-vaccination for any acute reactions. Diary cards were then used to record solicited local (pain or tenderness, erythema, and swelling) and systemic (pyrexia or fever, fatigue, headache, nausea, myalgia, and nausea) adverse events on the day of injection and for the next 7 days. Unsolicited adverse events were recorded until 28 days after each study injection (post-dose 1 and post-dose 2 phase) and 29 days after each vaccination until the date of the next vaccination (follow-up 1 phase) or until the date of last contact (follow-up 2 phase). Serious adverse events, adverse events leading to discontinuation from the study, and adverse events of special interest were recorded until the end of the study. AEs were graded based on the Food and Drug Administration (FDA) toxicity grading scale[50].

Immunogenicity assessments

Serum was obtained from blood samples collected on study days 1 (pre-vaccination), 29 (28 days post-dose 1), 85 (28 days post-dose 2), 238 (6 months post-dose 2) and 365 (1 year post-dose 1) for humoral immunogenicity assessments. Day 29 and Day 85 were selected as peak antibody levels and seroconversion are commonly observed approximately 28 days following vaccination. The 6 months post-dose 2, and 1-year post-dose 1 time points were selected for long-term evaluation of response durability, and, since this time frame covers the typical duration of an influenza season. Analysis was based on the Per Protocol Immunogenicity (PPI) set. The PPI population included all randomized and vaccinated participants for whom immunogenicity data were available, excluding samples taken on or after the date when a participant experienced a major protocol deviation expected to impact immunogenicity outcomes.

Subsets. Q-SD FluVac: A subset of participants from the clinical study VAC31518COV3005 (NCT05091307)[12], exploring the coadministration of a seasonal influenza vaccine on the safety, reactogenicity and immunogenicity of the Ad26.COV2.S COVID-19 vaccine, were included for immunogenicity assessments and used as an external immunogenicity comparator group. 120 participants from COV3005 who received the Afluria (Seqirus, Australia) 2021-2022 Northern Hemisphere quadrivalent seasonal influenza vaccine, containing inactivated A/Victoria/2570/2019 (H1N1) pdm09-like virus, A/Cambodia/e0826360/2020 (H3N2)-like virus, B/Washington/02/2019- like virus (B/Victoria lineage) and B/Phuket/3073/2013-like virus (B/Yamagata lineage), were randomly selected from the group which received only the standard dose (60 μg hemagglutinin) of unadjuvanted seasonal influenza vaccine at Day 1 together with placebo (0.9% saline), and who had serum samples available at Day 1 and Day 29. Participants were matched to FLZ1001 participants for age (≥18–≤45 years) and referred to as the Quadrivalent Standard Dose (Q-SD) FluVac group. Participants in the Q-SD FluVac group were recruited to the COV3005 study in the USA and Poland.

Extended Breadth: A subset of 75 participants from the FLZ1001 study eligible for per-protocol immunogenicity analysis and 76 participants from the COV3005 Q-SD FluVac group were randomly selected for additional analysis at Day 1 and Day 29 by ELISA using the Extended Breadth panel. The 75 participants from FLZ1001 were randomly selected from the high-dose G1 mHA groups and the low-dose G1 mHA group without $Al(OH)_3$.

Plaque Reduction Neutralization Test (PRNT): All available FLZ1001 participants eligible for per-protocol immunogenicity analysis from the G1 mHA high-dose groups were included for assessment of neutralizing antibody responses. The same 76 participants from the Q-SD FluVac group analyzed by the Extended Breadth panel of ELISAs were included for PRNT analysis.

For an overview of the planned number of participants and subsets analyzed per assay, see Supplementary Fig. 6a.

Full-length H5 HA and H1 stem HA (mHA) enzyme-linked immunosorbent assays. HA-specific IgG binding antibody responses were measured by Enzyme Linked Immunosorbent Assay (ELISA) for both the H5 A/Vietnam/1203/2004 full-length HA (H5-HA) and the H1 A/California/04/2009 stem HA (mHA). The assays were validated at Nexelis, Canada, based on the U.S. Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance for Industry[51] and the European Medicines Agency (EMA) ICH M10 guidelines[52]. Briefly, streptavidin (Southern Biotech) was coated at 1 μg/mL in PBS onto 96-well microtiter plates (ThermoFisher Scientific) for a minimum of 16 h at 4 °C, then subsequently washed with wash buffer (PBS + 0.05%

Tween-20) and blocked with Blocker™ Casein (ThermoFisher Scientific) for 1 h at room temperature (RT). Biotinylated H5-HA or H1 mHA (Janssen Vaccines & Prevention) was added to the blocked plates at 0.25 μg/mL and 0.125 μg/mL, respectively, and incubated for 1 h at room temperature to create an antigen-streptavidin complex. Test samples were 2-fold serially diluted eight times in duplicate at a start dilution of 1:100. Diluted serum samples (consisting of test samples, standard, and quality controls) were then allowed to bind for 1 h at RT. Unbound sample was then washed from the wells with wash buffer and HRP conjugated anti-human IgG (Jackson Immuno Research) was added at a 1:16,000 dilution for 1 h at RT. Excess conjugate was washed away and a colorimetric 3,3′,5,5′-Tetramethylbenzidine (TMB) peroxidase (BioRad) substrate was added for 30 min at RT in the dark. After development, the reaction was stopped using 2 N sulfuric acid solution (Millipore). Plates were read on a spectrophotometer (Molecular Devices) to report optical densities (OD) at 450 nm and 620 nm (450–620 nm). IgG antibody concentration (ELISA Units/mL) in the serum sample was calculated against a standard curve prepared with purified anti-HA multidomain antibody (MD3606[38], Janssen Vaccines & Prevention).

Breadth enzyme-linked immunosorbent assays. HA-specific IgG binding antibody levels were measured by ELISA using a panel of HA antigens (Janssen Vaccines & Prevention, The Netherlands, Supplementary Table 6). The assays were developed and qualified at Janssen Vaccines & Prevention, assessing various assay parameters (Supplementary Table 7). Briefly, ½-area 96-well plates (Perkin Elmer) were coated with 5 μg/mL streptavidin (ThermoFisher Scientific) for 2 h at 37 °C in a humidified incubator. Plates were washed with Wash Buffer (PBS + 0.05% Tween-20) and subsequently blocked with Blocker™ Casein (ThermoFisher Scientific) for 1 h at RT. Plates were incubated with 0.25 μg/mL of the biotinylated HA antigen to be assessed in the breadth ELISA. After incubation for 1 h at RT, reference standard (MD3606[38]) was added to each plate and samples were tested in duplicate using plate pairs. Samples and reference standard evaluated on each plate, were 5-fold serially diluted 4 times in block buffer and incubated for 1 h at RT. Following a wash with Wash Buffer, a 1:3750 dilution of HRP conjugated anti-human IgG (Jackson ImmunoResearch) was added for 1 h at RT, the plates were washed again, and then detection was performed with Enhanced Chemiluminescence (ECL, Bio-Rad) substrate. The luminescence signal, expressed as relative luminescence units (RLU), was read on an Envision plate reader (Perkin Elmer) after 10 min. RLU signals were converted to relative potency titers. This methodology was chosen to report data based on improved assay robustness towards environmental changes[53], resulting in less variability in reference standard titers over time. RLU values were first $\log_{10}$ transformed after the addition of 10,000 as an offset to all values to stabilize variance across the dilution series. Per plate pair, a four-parameter logistic (4-PL) regression curve was fitted through the $\log_{10}$ RLU of the MD3606 in-plate reference against the $\log_{10}$ dilution. Subsequently, the estimated parameters of the reference curve fit (slope, upper asymptote, and lower asymptote) were applied to the sample curves (by fixing the slope, upper, and lower asymptote of an assay plate to those estimated from the reference curve and only estimating the EC_{50} parameter for each sample in an assay plate), which was acceptable based on demonstration of parallelism of the reference standard and samples during assay qualification. The effective concentration of each sample at 50% (EC_{50}) was then calculated and expressed as $\log_{10}$ relative potency ($\log_{10}$ RP = $\log_{10}$ [EC_{50} sample] − $\log_{10}$ [EC_{50} reference]) with a constant value of +4 on the reportable value to shift the majority of the $\log_{10}$ RP values to the positive scale for ease of visualization.

Phylogenetic tree construction. Full length H1 HA amino acid sequences were obtained from the NCBI[54] and GISAID[55] influenza databases. Sequences were aligned with the k-mer (15) based tree construction tool in CLC Main Workbench 23.0.5 (Qiagen Aarhus, Denmark) using the Neighbor-Joining method and Mahalanobis distance measure. Distances of the H1 HA sequences in the breadth panel were shown with H1 sequences recommended by the World Health Organization for seasonal influenza formulations since 1999[14] and dominant H1N1 strains documented in the evolutionary history in humans since 1918[13,15].

Virus neutralization assay measured by Plaque Reduction Neutralization Test. H5-specific neutralizing antibody responses were measured by Plaque Reduction Neutralization Test (PRNT). The assay was qualified at Nexelis, Canada, assessing assay parameters including precision, dilutional linearity and relative accuracy, specificity, matrix interference, sample freeze-thaw stability, and robustness. Prior to analysis, serum samples were heat-inactivated in a water bath for 60 min at 56 ± 1 °C. Samples were two-fold serially diluted seven times in duplicate in 96-well tissue culture plates from a 1:10 starting dilution in Growth medium (Dulbecco's Minimal Essential Medium (DMEM) supplemented with 2 mM L-glutamine, 1 mM Sodium Pyruvate, 1X MEM Non-Essential Amino Acids, 25 mM HEPES and 1X Penicillin-Streptomycin). Each plate included a virus control and cell control to monitor virus infectivity and cell growth, respectively. Positive and negative controls were evaluated on each assay plate. H5N1 A/Indonesia/05/2005 virus was added to the diluted serum at a dilution resulting in 175-200 plaque-forming units (PFU) per well and incubated for 1 h at 35 °C, 5% CO_2. The virus-serum mix was then added to Madin-Darby Canine Kidney cells expressing human 2,6-sialtransferase (MDCK-SIAT1) and incubated for 1 h at 35 °C, 5% CO_2 before the inoculum was removed and 150 μL Maintenance medium (DMEM with 1X MEM Non-Essential Amino Acids, 1X Penicillin-Streptomycin, 25 mM HEPES, 1.5 μg/mL Trypsin (TPCK-treated), and 0.5% Methylcellulose) was added to the plates and incubated for a further 23 ± 1 h at 35 °C, 5% CO_2. After overnight incubation, medium was removed, and wells were fixed with 4% paraformaldehyde (PFA) + 0.2% Triton-X-100 for 20 min at RT. Fixative was then removed and plates were washed and stained with mouse anti-Influenza A H5-specific monoclonal antibody (Rockland, USA) at 1:2000 dilution for 1 h at RT. Plates were then washed and incubated with goat anti-mouse IgG conjugated-horseradish peroxidase (Sigma, USA) at 1:1000 dilution for 30 min at RT. After a final wash, 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate was added for 10 min at RT and plaques were counted in a Cytation plate reader with an automated image analysis system (Agilent Technologies, USA). 50% inhibitory concentration titers (IC_{50}) were calculated as the reciprocal of the highest sample dilution corresponding to 50% reduction in plaques compared to the virus control.

Antibody-dependent cellular cytotoxicity assay. H5 A/Vietnam/1203/2004-specific antibodies inducing Antibody-Dependent Cellular Cytotoxicity (ADCC) responses were measured in a developed assay at Nexelis, Canada. 50 μL of a clonal MDCK cell line stably transfected with inducible Influenza Hemagglutinin (HA) from strain H5 A/Vietnam/1203/2004 (MDCK-H5-HA) at 7×10^4 cells/mL were seeded into 96-well half-area white assay plates for 22 ± 2 h at 37 °C + 5% CO_2 in DMEM containing 10% HI-FBS, 2mM L-Glutamine, and 0.25 μg/mL Doxycycline (Induction Medium). Serum samples were heat-inactivated in a water bath for 30 min at 56 ± 2 °C. Samples were 3-fold serially diluted 11 times in duplicate in RPMI 1640 HEPES Medium without further additions (Assay Buffer) from a 1:10 starting dilution in 96-well pre-dilution plates. Cell and Quality Control (QC) samples were included in each plate. Assay plates containing the seeded MDCK-H5-HA cells were washed twice with 100 μL of Assay Buffer. 25 μL of Assay Buffer was then added to the plates, followed by 12.5 μL of Jurkat effector cells expressing human FcγRIIIa, high-affinity version V158 (Promega) at a concentration of 2.8×10^6 cells/mL, and 12.5 μL of

the serial diluted samples from the pre-dilution plate. Assay plates were incubated at 37 °C + 5% CO_2 for 18 ± 1 h to allow for ADCC responses to develop. The ADCC reaction was detected by the addition of 50 μL Bio-Glo Luciferase substrate (Promega) and the luminescence signal was measured after 10 min incubation using a SpectraMax i3x plate reader (Molecular Devices). EC_{50} titers were determined using a four-parameter logistic model with a fixed upper and lower asymptote and no constraints applied on the slope.

Passive transfer challenge model

A BALB/c AnNCrl (SPF) mouse challenge model was used to evaluate antibody-mediated protective responses in serum samples from FLZ1001 by passive transfer of human sera to mice. Female BALB/c AnNCrl (SPF) mice (N = 116) were obtained from Charles River Laboratories (Germany) at approximately 6 weeks old on the day of arrival. The mouse strain was previously used in a serum transfer study to show protection from H5N1 infection[17]. The Animal Welfare Application for this study was approved by the Dutch Central Authority for Scientific Procedures on Animals (CCD) prior to study start and registered under the number AVD4010020185285. The study protocol was approved by the Wageningen BioVeterinary Research Animal Welfare Body under permit number 2017.D-0030.017.

Environmental control. Mice were housed under environmentally controlled conditions between a minimum and maximum temperature of 21.0 °C and 21.7 °C, respectively, and between 55% and 67% relative humidity. The room was maintained under negative pressure and both incoming and outgoing air was filtered by HEPA filters. Artificial lighting was used with 12 h of light and 12 h of dark per 24 h.

Serum samples. The same subset of 50 participants randomly selected from the FLZ1001 high-dose G1 mHA groups for Extended Breadth ELISA analysis was included in the passive transfer challenge study evaluating samples at Day 1 and Day 29, where the largest difference in protective capacity could be expected based on available binding antibody data.

Serum transfer. 24 h prior to challenge (Day −1), 400 μL human serum was transferred into mice via intraperitoneal (i.p) administration into 1 of 4 groups (N = 25 mice/group, Supplementary Fig. 7a). 15 mg/kg of a broadly protective HA-specific monoclonal antibody (CR6261)[18] and PBS were used as positive and negative controls, respectively (N = 8/group, Supplementary Fig. 7a). Approximately 24 h later, prior to challenge on Day 0, a blood sample of 200 μL was taken from the mice. Serum was analyzed to determine the efficiency of the serum transfer by the presence of binding antibodies to mHA H1 A/New Caledonia/20/1999, H1 A/Victoria/2570/2019 and H5 A/Cambodia/NPH230032/2023 HA at Janssen Vaccines & Prevention. Successful transfer was assessed based on the correlation between HA-specific antibody titers in the human serum and mouse pre-challenge serum. When titers in the serum from recipient mice were >100 fold below the corresponding human serum titers, this was considered as a failed transfer and excluded from further analysis.

Challenge. Stocks of H5N1 A/Hong Kong/156/1997 (Wageningen BioVeterinary Research, Wageningen University) were grown on embryonated chicken eggs. On Day 0, anaesthetized mice were challenged intranasally with 12.5 LD_{50} virus using a volume of 50 μL (25 μL per nostril). Challenges were considered valid when there was a statistically significant difference in survival proportion (Fisher's exact-test, 2-sided) between the CR6261 and PBS positive and negative control groups. Bodyweight and clinical scores were monitored daily for up to 21 days or until a humane endpoint was reached (clinical score 4 or 25% body weight loss), to limit animal discomfort. Clinical scores were defined as: 0 = no clinical signs, 1 = rough coat, 2 = rough coat, less-reactive, passive during handling, 3 = rough coat, rolled up, labored breathing, passive during handling, 4 = rough coat, rolled up, labored breathing, unresponsive.

Statistical analysis. The mice infused with human post-vaccination sera (Day 29) from the FLZ1001 study were compared to mice infused with pre-vaccination sera (Day 1) from the same participants. For survival proportion, the two-sided sign test was used on the survival status of each pair of mice that received sera from the same human donor. For bodyweight, the change across the follow-up period was first summarized per mouse as area-under-the-curve (AUC) with last-observation-carried-forward (LOCF) after dropout. Next, the AUC values were compared between the two groups (Day 1 vs Day 29) with a two-sided t-test in an analysis-of-variance with group and human donor as factors. For the clinical score, each level of the score was first counted across the follow-up period per mouse. Next, the counts were compared between the two groups with a two-sided z-test in a cumulative logistic regression model with score level, group, and human donor as explanatory factors and mouse as experimental unit.

Correlates of protection. A correlate of protection analysis was conducted to evaluate whether binding antibody, neutralizing antibody, and ADCC titers in human serum samples were associated with the survival of mice in the passive transfer challenge study. Logistic regression was applied to assess the survival status of the mice based on the log-transformed titers measured in human post-vaccination sera from the H5-specific ELISA, PRNT, or ADCC assays, and to estimate the titer that provides 50% protection (EC_{50}). Furthermore, Receiver Operating Characteristic (ROC) curves for the three assays were presented and summarized as Area Under the Curve (AUC) with a 95% confidence interval. Statistical analysis was performed with SAS (version 9.04) and R (version 4.5.1) software.

Data analysis and statistical methods

The full analysis set for safety comprised all randomized participants who received at least 1 dose of study vaccine. The primary analysis set for immunogenicity included all randomized and vaccinated participants for whom immunogenicity data were available, excluding samples taken on or after the date when a participant experienced a major protocol deviation expected to impact the immunogenicity outcomes. Samples obtained after missed doses or samples obtained after natural infection were excluded from the immunogenicity analysis. All clinical data were analyzed using descriptive statistics without formal hypothesis testing. For immunogenicity endpoints, all values below the lower limit of quantification (LLOQ) were imputed with half the lower limit of quantification for calculation of Geometric Mean Titers or Geometric Mean Concentrations (GMT or GMC), and values greater than the upper limit of quantification (ULOQ) were truncated with the ULOQ. For the calculation of fold changes, the values below LLOQ were truncated with the corresponding LLOQ and values above the ULOQ were truncated with the ULOQ. A sample was considered positive if the value was above the assay LLOQ. A response was defined as >4-fold increase from baseline or >4-fold increase from LLOQ (if the baseline sample was ≤LLOQ). Log_{10} transformation was applied to most of the immunogenicity endpoints. Geometric mean ratios (GMR) between active G1 mHA groups versus placebo and the Q-SD FluVac external comparator group, with corresponding 95% confidence intervals (CI) estimated via an analysis of variance (ANOVA), using log-transformed post-baseline values as a dependent variable and study group as an independent variable. Statistical analysis was performed with SAS (version 9.04) and R (version 4.5.1) software. Further details can be found in the Statistical Analysis Plan (SAP, Supplementary Information).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Source data are provided with this paper. Johnson & Johnson has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for evaluation of requests for clinical study reports and participant-level data from investigators and physicians for scientific research that will advance medical knowledge and public health. Data will be made available following publication and approval by YODA of any formal requests with a defined analysis plan. YODA aims to handle requests within 30 days of receipt and make data available for approved requests within 30 days from the signing of a Data Use Agreement. For more information on this process or to make a request, please visit The YODA Project site at yoda.yale.edu. The data sharing policy of Johnson & Johnson is available online at https://www.janssen.com/clinical-trials/transparency. Source data are provided with this paper.

References

1. WHO. The burden of influenza, https://www.who.int/news-room/feature-stories/detail/the-burden-of-influenza#:~:text=Thankfully%2C%20although%20there%20are%20hundreds,650%20000%20respiratory%20deaths%20annually> (2024).
2. Remy, V., Zollner, Y. & Heckmann, U. Vaccination: the cornerstone of an efficient healthcare system. *J. Mark. Access. Health Policy* **3**, https://doi.org/10.3402/jmahp.v3.27041 (2015).
3. CDC. *Seasonal Flu Vaccine Effectiveness Studies*, https://www.cdc.gov/flu-vaccines-work/php/effectiveness-studies/index.html (2024).
4. CDC. *How Flu Viruses Can Change: "Drift" and "Shift"*, https://www.cdc.gov/flu/php/viruses/change.html (2024).
5. CDC. CDC A(H5N1) *Bird Flu Response Update March 19, 2025*, https://www.cdc.gov/bird-flu/spotlights/h5n1-response-03192025.html (2025).
6. CDC. *H5 Bird Flu: Current Situation*, https://www.cdc.gov/bird-flu/situation-summary/index.html (2025).
7. Nuwarda, R. F., Alharbi, A. A. & Kayser, V. An overview of influenza viruses and vaccines. *Vaccines* **9**, https://doi.org/10.3390/vaccines9091032 (2021).
8. Impagliazzo, A. et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science* **349**, 1301–1306 (2015).
9. Yassine, H. M. et al. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat. Med.* **21**, 1065–1070 (2015).
10. Swart, M. et al. Enhancing breadth and durability of humoral immune responses in non-human primates with an adjuvanted group 1 influenza hemagglutinin stem antigen. *NPJ Vaccines* **8**, 176 (2023).
11. Milder, F. J. et al. Universal stabilization of the influenza hemagglutinin by structure-based redesign of the pH switch regions. *Proc. Natl. Acad. Sci. USA* **119**, https://doi.org/10.1073/pnas.2115379119 (2022).
12. Tapia-Calle, G. et al. Safety, reactogenicity, and immunogenicity of Ad26.COV2.S co-administered with a quadrivalent standard-dose or high-dose seasonal influenza vaccine: a non-inferiority randomised controlled trial. *EClinicalMedicine* **79**, 103016 (2025).
13. Huang, S. S. et al. Immunity toward H1N1 influenza hemagglutinin of historical and contemporary strains suggests protection and vaccine failure. *Sci. Rep.* **3**, 1698 (2013).
14. *Wikipedia. Historical Annual Reformulations of Influenza Vaccine* https://en.wikipedia.org/wiki/Historical_annual_reformulations_of_the_influenza_vaccine (2025).
15. Nelson, M. I. et al. Multiple reassortment events in the evolutionary history of H1N1 influenza A virus since 1918. *PLoS Pathog.* **4**, e1000012 (2008).
16. Chromikova, V. et al. Activity of human serum antibodies in an influenza virus hemagglutinin stalk-based ADCC reporter assay correlates with activity in a CD107a degranulation assay. *Vaccine* **38**, 1953–1961 (2020).
17. Roozendaal, R. et al. Transient humoral protection against H5N1 challenge after seasonal influenza vaccination of humans. *PLoS One* **9**, e103550 (2014).
18. Throsby, M. et al. Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. *PLoS One* **3**, e3942 (2008).
19. Ekiert, D. C. et al. Antibody recognition of a highly conserved influenza virus epitope. *Science* **324**, 246–251 (2009).
20. Ekiert, D. C. et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science* **333**, 843–850 (2011).
21. Dreyfus, C. et al. Highly conserved protective epitopes on influenza B viruses. *Science* **337**, 1343–1348 (2012).
22. Corti, D. et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science* **333**, 850–856 (2011).
23. Hobson, D., Curry, R. L., Beare, A. S. & Ward-Gardner, A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J. Hyg.* **70**, 767–777 (1972).
24. Weiss, C. D. et al. Neutralizing and neuraminidase antibodies correlate with protection against influenza during a late season A/H3N2 outbreak among unvaccinated military recruits. *Clin. Infect. Dis.* **71**, 3096–3102 (2020).
25. Edgar, J. E. et al. Antibodies elicited in humans upon chimeric hemagglutinin-based influenza virus vaccination confer FcgammaR-dependent protection in vivo. *Proc. Natl. Acad. Sci. USA* **120**, e2314905120 (2023).
26. Verschoor, C. P. et al. Microneutralization assay titres correlate with protection against seasonal influenza H1N1 and H3N2 in children. *PLoS One* **10**, e0131531 (2015).
27. Ng, S. et al. Novel correlates of protection against pandemic H1N1 influenza A virus infection. *Nat. Med.* **25**, 962–967 (2019).
28. Aydillo, T. et al. Pre-existing hemagglutinin stalk antibodies correlate with protection of lower respiratory symptoms in flu-infected transplant patients. *Cell Rep. Med.* **1**, 100130 (2020).
29. Nachbagauer, R. et al. A universal influenza virus vaccine candidate confers protection against pandemic H1N1 infection in preclinical ferret studies. *NPJ Vaccines* **2**, 26 (2017).
30. Jacobsen, H. *et al*. Influenza Virus Hemagglutinin Stalk-Specific Antibodies in Human Serum are a Surrogate Marker for In Vivo Protection in a Serum Transfer Mouse Challenge Model. *mBio*. **8**, https://doi.org/10.1128/mBio.01463-17 (2017).
31. van der Lubbe, J. E. M. et al. Mini-hemagglutinin vaccination induces cross-reactive antibodies in pre-exposed NHP that protect mice against lethal influenza challenge. *NPJ Vaccines* **3**, 25 (2018).
32. van der Lubbe, J. E. M. et al. Mini-HA Is Superior to Full Length Hemagglutinin Immunization in Inducing Stem-Specific Antibodies and Protection Against Group 1 Influenza Virus Challenges in Mice. *Front Immunol.* **9**, 2350 (2018).
33. Widge, A. T. et al. An influenza hemagglutinin stem nanoparticle vaccine induces cross-group 1 neutralizing antibodies in healthy adults. *Sci. Transl. Med.* **15**, eade4790 (2023).
34. Andrews, S. F. et al. An influenza H1 hemagglutinin stem-only immunogen elicits a broadly cross-reactive B cell response in humans. *Sci. Transl. Med.* **15**, eade4976 (2023).
35. Nachbagauer, R. et al. A chimeric haemagglutinin-based influenza split virion vaccine adjuvanted with AS03 induces protective stalk-reactive antibodies in mice. *NPJ Vaccines* **1**, 16015 (2016).

36. Choi, A. et al. Chimeric hemagglutinin-based influenza virus vaccines induce protective stalk-specific humoral immunity and cellular responses in mice. *Immunohorizons* **3**, 133–148 (2019).
37. Folschweiller, N. et al. Reactogenicity, safety, and immunogenicity of chimeric haemagglutinin influenza split-virion vaccines, adjuvanted with AS01 or AS03 or non-adjuvanted: a phase 1-2 randomised controlled trial. *Lancet Infect. Dis.* **22**, 1062–1075 (2022).
38. Laursen, N. S. et al. Universal protection against influenza infection by a multidomain antibody to influenza hemagglutinin. *Science* **362**, 598–602 (2018).
39. Houser, K. V. et al. Safety and immunogenicity of a ferritin nanoparticle H2 influenza vaccine in healthy adults: a phase 1 trial. *Nat. Med* **28**, 383–391 (2022).
40. Rioux, M. et al. The power of first impressions: can Influenza Imprinting during infancy inform vaccine design? *Vaccines* **8**, https://doi.org/10.3390/vaccines8030546 (2020).
41. CDC. *Preliminary Estimated Flu Disease Burden 2023–2024 Flu Season* https://www.cdc.gov/flu-burden/php/data-vis/2023-2024.html (2024).
42. CDC. *Flu Disease Burden: Past Seasons*, https://www.cdc.gov/flu-burden/php/data-vis/past-seasons.html (2025).
43. Winarski, K. L. et al. Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics. *Proc. Natl. Acad. Sci. USA* **116**, 15194–15199 (2019).
44. Khurana, S. et al. Vaccine-induced anti-HA2 antibodies promote virus fusion and enhance influenza virus respiratory disease. *Sci. Transl. Med.* **5**, 200ra114 (2013).
45. Casazza, J. P. et al. Phase 1 dose-escalation trial evaluating a group 2 influenza hemagglutinin stabilized stem nanoparticle vaccine. *NPJ Vaccines* **9**, 171 (2024).
46. Mantus, G. E. et al. Vaccination with different group 2 influenza subtypes alters epitope targeting and breadth of hemagglutinin stem-specific human B cells. *Sci. Transl. Med.* **17**, eadr8373 (2025).
47. Guthmiller, J. J. et al. Long-lasting B cell convergence to distinct broadly reactive epitopes following vaccination with chimeric influenza virus hemagglutinins. *Immunity* **58**, 980–996.e987 (2025).
48. Krammer, F. The human antibody response to influenza A virus infection and vaccination. *Nat. Rev. Immunol.* **19**, 383–397 (2019).
49. Jefferson, T., Rudin, M. & Di Pietrantonj, C. Adverse events after immunisation with aluminium-containing DTP vaccines: systematic review of the evidence. *Lancet Infect. Dis.* **4**, 84–90 (2004).
50. CBER. *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*, https://www.fda.gov/media/73679/download (2007).
51. CDER. *Bioanalytical Method Validation Guidance for Industry* https://www.fda.gov/media/70858/download (2018).
52. CHMP. *ICH guideline M10 on bioanalytical method validation and study sample analysis*, https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf (2022).
53. Carreno, J. M. et al. Development and assessment of a pooled serum as candidate standard to measure influenza A virus group 1 hemagglutinin stalk-reactive antibodies. *Vaccines* **8**, https://doi.org/10.3390/vaccines8040666 (2020).
54. NCBI. *Influenza Virus Database*, https://www.ncbi.nlm.nih.gov/genomes/FLU/Database/nph-select.cgi?go=database (2025).
55. GISAID. *GISAID Influenza Database*, https://gisaid.org/ (2025).

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Author contributions

N.H. and C.T. drafted the manuscript. V.P., J.S., H.K., J.T., R.Z., B.B., Z.A., J.H., S.vd.B, C.M., N.H. and C.T. contributed to the overall study conception. Z.A., L.Y., M.W. and S.K. contributed to data generation and study oversight. V.P., H.K., D.L., W.H., N.H., C.T., M.J., S.vd.B, C.M., J.T., K.K. and S.K. assisted with laboratory analyses and data analyses. All co-authors reviewed the manuscript, approved the final version, and are fully responsible for all content and editorial decisions.

Competing interests

All authors are or were employees of Johnson & Johnson and may hold Johnson & Johnson stock.B.B. and M.J. are named inventors on a patent granted for the HA antigen (US11905314B2).

Additional information

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