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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No custom code was used for data collection or analysis. Data was collected using Microsoft Excel (Mac 16.33) and analyzed using Graphpad Prism 8 (Mac).
Data analysis	No custom code was used for data collection or analysis. Data was collected using Microsoft Excel (Mac 16.33) and analyzed using Graphpad Prism 8 (Mac).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

De-identified data will be made available upon reasonable requests that are fit within the limitations of the informed consent given by study participants.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	This study was a phase I clinical trial, designed to assess the safety of the tested vaccines. Assumptions and power analyses can be found in the attached clinical study protocol (page 132 cont.).
Data exclusions	No data was excluded from analysis.
Replication	The data was consistent between assays and can be replicated. Experiments were performed once unless otherwise stated.
Randomization	Randomization was stratified by site and included a blocking factor. The original allocation ratio for LAIV-LAIV Groups 1, 2 and 3 was 3:3:1, and the original allocation ratio for IIV-IIV Groups 4-5 was 3:2. To preserve comparability of subjects across treatment groups, subjects were randomized to all groups under one allocation sequence. Among LAIV-LAIV subjects, Group 1 was our primary interest, especially for comparison to Group 4. To guard against loss of power from possible drop-out between randomization and admission into the inpatient clinical isolation unit, Group 1 was over-randomized by five subjects, for a final randomization scheme of 4:3:1:3:2. A secure IWRS was utilized for eligibility verification, subject registration, randomization, and treatment assignment. IWRS allowed authorized staff from study centers to perform subject enrollment 24 hours a day, 7 days a week. The system guided users through the process of specifying the subject identifier, completing demographic information, and finally, completing the protocol- specific inclusion-exclusion checklist, which confirmed a subject's eligibility, which was generally predetermined at the site from screening information.
Blinding	The trial was performed in a blinded fashion and samples were tested prior to unblinding.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Mouse anti-human IgG HRP antibody (Southern Biotech, clone JDC-10, # 9040-05) Anti-Human IgG (Fab specific)–Peroxidase antibody produced in goat (Sigma, #A0293) Anti-Human IgG (Fc specific)–Peroxidase antibody produced in goat (Sigma, #A0170) Goat Anti-Human IgA α-chain specific HRP (Sigma, #A0295) Goat Anti-Human secretory component HRP (Nordic-MUbio, # GAHu/SC/PO) Fluorescein-conjugated goat anti-mouse IgG antibody (Chemicon, #AP124F) Pan influenza HA-stalk monoclonal antibody CR9114, HA trimer interface specific monoclonal antibody D1 H1-3/H3-3 and H8 HA specific monoclonal antibody 1A7 were produced in-house or obtained from collaborators. References for the respective antibodies are provided in the main text.
Validation	Secondary antibodies obtained from Sigma-Aldrich, Southern Biotech, Nordic-MUbio or Chemicon were validated by the company and tested for specificity. Primary monoclonal antibodies used in this study (CR9114, D1 H1-3/H3-3, 1A7) were characterized in detail before and the appropriate references are provided in the main text.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Madin Darby canine kidney (MDCK) cells, human embryonic kidney cells (293T) cells, human lung epithelial cell (A549), BTI-TN-5B1-4 (High Five, Trichoplusia ni) cells and Sf9 (Spodoptera frugiperda) cells were obtained from ATCC. Jurkat cells expressing human FcγRIIIa (high affinity version V158) and Jurkat cells engineered to express human FcγRIIa (high affinity version H131) were obtained from Promega. Expi293F mammalian suspension cells were obtained from Thermo Fisher.
Authentication	Cell lines were obtained from a commercial source. After receipt cells were recovered, passaged and not further authenticated.
Mycoplasma contamination	Cells tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	Commonly misidentified cell lines were not used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	6-8 week female BALB/c mice were used for serum transfer
Wild animals	No wild animals were used in this study.
Field-collected samples	No field-collected samples were used.
Ethics oversight	Animal experiments were approved by the Icahn School of Medicine IACUC.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	In Groups 1, 2, and 4 about two-thirds of the subjects were female (70.0%, 66.7%, and 62.5%, respectively), compared to 2/5 (40.0%) in Group 3 and 5/10 (50.0%) in Group 5. Most were non-Hispanic or Latino (80% to 100% per group) and black or African American (66.7% to 87.5%). The median age at enrollment ranged from 26 to 31 years across groups, with minimum age ranging from 18 to 22 and maximum age from 29 to 38. The median weight ranged from 74.7 kg to 84.2 kg and the median height ranged from 165.5 cm to 174.0 cm. The most common pre- existing conditions across all treatment groups were immune system disorders (24/66, 36.4%), the majority being allergies (seasonal and food), followed by drug hypersensitivity. No subjects in the placebo Groups 3 and 5 reported taking prior medications and more subjects in Group 1 (5/19, 26.3%) reported taking prior medications compared to Group 2 (1/14, 7.1%) and Group 4 (2/15, 13.3%). The proportions of subjects taking concomitant medications in Groups 1 to 5 were 84.2%, 78.6%, 100%, 60.0% and 80.0%, respectively. The most common, by therapeutic subgroup, were analgesics (20/61, 32.8%), anti-inflammatory and antirheumatic products (19/61, 31.1%) and sex hormones and modulators of the genital system (18/61, 29.5%).
Recruitment	Participants were recruited from the local community. The target population reflected the community at large at each of the participating study sites. Information regarding this trial was provided to potential subjects who have previously participated in vaccine trials conducted at the participating study sites. The local IRB approved all materials prior to use. Careful recruitment and communication about all aspects of the study was critical to ensuring eligible subjects who eventually enroll and participate in the trial were committed to participate for the full length of the study.
Ethics oversight	The study protocol was approved by the IRBs of the Icahn School of Medicine at Mount Sinai, Duke University and Cincinnati Children's Hospital Medical Center (CCHMC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT03300050
Study protocol	Attached in submission.
Data collection	Study Centers:

Data collection

Gamble Program for Clinical Studies, Cincinnati Children's Hospital Medical Center 3333 Burnet Avenue, Cincinnati, OH 45229-3039
Duke Early Phase Clinical Research Unit, Duke Clinical Research Institute 40 Duke Medicine Circle, Durham, NC 27710

Studied period:
Date of first enrollment: 10 October 2017
Date of last subject completion: 09 August 2019

Outcomes

Primary Objectives
To assess the reactogenicity and safety through 28 days after each priming dose of cH8/1N1 LAIV (or placebo) and the booster dose of cH5/1N1 IIV +/- AS03A (or placebo) and through 28 days after each dose of IIV (cH8/1N1 IIV + AS03A and cH5/1N1 IIV + AS03A) (or placebo) in terms of rates of solicited local and general adverse events (AEs) through 7 days post-vaccination, unsolicited AEs through 28 days post vaccination, hematological and biochemical laboratory abnormalities up to Visit 13, and medically attended event (MAEs), laboratory-confirmed influenza-like illness (LC-ILI), potential immune-mediated disease (pIMDs), and serious adverse events (SAEs) through Visit 13.
SSecondary Objectives
Safety
To assess the safety of each treatment group during the entire study period in terms of rates of primary endpoints and additionally hematological and biochemical laboratory abnormalities up to Visit 15, and MAEs, LC-ILIs, pIMDs, and SAEs through Visit 16.
Viral shedding – post-prime dose
To describe the shedding of vaccine virus through 5 days after administration of cH8/1N1 LAIV (Groups 1, 2, and 3 only) in terms of the proportions of subjects with influenza type A vaccine virus ribonucleic acid (RNA) detected by reverse transcription polymerase chain reaction (RT-PCR) in nasal and oropharyngeal (OP) swabs and the proportion of subjects with vaccine virus isolated in cell culture each day post-vaccination.
Immunogenicity - descriptive, post-boost dose
To describe the anti-H1 hemagglutinin (HA)-stalk humoral immune responses (anti-H1 HA-stalk serum immunoglobulin G [IgG], anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum immunoglobulin A [IgA], and antibody-dependent cell-mediated cytotoxicity [ADCC] activity) 28 days after the booster dose of cH5/1N1 IIV +/- AS03A (or placebo) (Groups 1, 2, and 3) and 28 days after the second dose of IIV (cH5/1N1 IIV + AS03A) (or placebo) (Groups 4 and 5) in terms of seropositivity rates, geometric mean titers (GMTs), percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and mean geometric increases (MGIs) from Day 1.
To describe the anti-H1 HA-stalk mucosal immune responses (anti-H1 HA-stalk salivary total IgA, anti- H1 HA-stalk secretory IgA in saliva, and anti-H1 HA-stalk salivary IgG) 28 days after the booster dose of cH5/1N1 IIV +/- AS03A (or placebo) (Groups 1, 2, and 3) and 28 days after the second dose of IIV (cH5/1N1 IIV + AS03A) (or placebo) (Groups 4 and 5) in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.
Immunogenicity - descriptive, post-boost dose - breadth
To describe the breadth of the anti-H1 HA-stalk humoral immune responses (anti-H2 HA-full length serum IgG, anti-H9 HA-full length serum IgG, anti-H18 HA-full length serum IgG, anti-H5N8 serumneutralizing antibodies, anti-avian swine H1N1 serum neutralizing antibodies, and anti-H1pdm09-like serum neutralizing antibodies) to Group 1 influenza A viruses 28 days after the booster dose of cH5/1N1 IIV +/- AS03A (or placebo) (Groups 1, 2, and 3) and 28 days after the second dose of IIV (cH5/1N1 IIV + AS03A) (or placebo) (Groups 4 and 5) in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.
Immunogenicity - descriptive, post-boost dose - persistence
To describe the persistence of the anti-H1 HA-stalk humoral immune responses (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) up to 12 months after the booster dose of cH5/1N1 IIV +/- AS03A (or placebo) (Groups 1, 2, and 3) and up to 12 months after the second dose of IIV (cH5/1N1 IIV + AS03A) (or placebo) (Groups 4 and 5) in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.
To describe the persistence of the anti-H1 HA-stalk mucosal immune responses (anti-H1 HA-stalk salivary total IgA, anti-H1 HA-stalk secretory IgA in saliva and anti-H1 HA-stalk salivary IgG) up to 12 months after the booster dose of cH5/1N1 IIV +/- AS03A (or placebo) (Groups 1, 2, and 3) and up to 12 months after the second dose of IIV (cH5/1N1 IIV + AS03A) (or placebo) (Groups 4 and 5) in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.
Immunogenicity - descriptive, by vaccine regimen, post-prime dose
To describe the anti-H1 HA-stalk humoral immune responses (anti-H1 HA-stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) 28 days after the prime dose of cH8/1N1 LAIV (or placebo) (Groups 1, 2, and 3) and 28 days after the first dose of IIV (cH8/1N1 IIV + AS03A) (or placebo) (Groups 4 and 5) in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.
To describe the anti-H1 HA-stalk mucosal immune responses (anti-H1 HA-stalk salivary total IgA, anti- H1 HA-stalk secretory IgA in saliva, anti-H1 HA-stalk salivary IgG) 28 days after the prime dose of cH8/1N1 LAIV or placebo (Groups 1, 2, and 3) and 28 days after the first dose of IIV (cH8/1N1 IIV + AS03A) or placebo (Groups 4 and 5) in terms of seropositivity rates, GMTs, percentages of subjects with a 4-fold or greater increase in titer from Day 1, percentages of subjects with a 10-fold or greater increase in titer from Day 1, and MGIs from Day 1.
Immunogenicity - comparative, post-boost dose
To compare the anti-H1 HA-stalk humoral immune responses (anti-H1 HA-stalk serum IgG, anti-H1 HA- stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) 28 days after the booster dose with cH5/1N1 IIV +/- AS03A after previous receipt of cH8/1N1 LAIV (Groups 1 or 2) to that after priming with two doses of IIV (cH8/1N1 IIV + AS03A and cH5/1N1 IIV + AS03A) (Group 4) in terms of the adjusted GMT ratio and the seroresponse (≥4-fold) rate difference.
To compare the anti-H1 stalk mucosal immune responses (anti-H1 HA-stalk salivary IgA, anti-H1 HA- stalk secretory IgA in saliva, and anti-H1 HA-stalk salivary IgG) 28 days after the booster dose with cH5/1N1 IIV +/- AS03A after previous receipt of cH8/1N1 LAIV (Groups 1 or 2) to that after priming with two doses of IIV (cH8/1N1 IIV + AS03A and cH5/1N1 IIV + AS03A) (Group 4) in terms of the adjusted GMT ratio and the seroresponse (≥4-fold) rate difference.

To evaluate the adjuvant effect of AS03A on the anti-H1 stalk humoral immune response (anti-H1 HA- stalk serum IgG, anti-H1 HA-stalk serum neutralizing antibodies, anti-H1 HA-stalk serum IgA, and ADCC activity) after one booster dose of cH5/1N1 IIV + AS03A after priming with cH8/1N1 LAIV (Group 1) compared to boosting with cH5/1N1 IIV, unadjuvanted (Group 2) in terms of the adjusted GMT.