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Safety and immunogenicity of a live-attenuated influenza virus vector-based intranasal SARS-CoV-2 vaccine in adults: randomised, double-blind, placebo-controlled, phase 1 and 2 trials

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Summary

Background All currently available SARS-CoV-2 vaccines are administered by intramuscular injection. We aimed to evaluate the safety and immunogenicity of a live-attenuated influenza virus vector-based SARS-CoV-2 vaccine (dNS1-RBD) administered by intranasal spray in healthy adults.

Methods We did double-blind, randomised, placebo-controlled phase 1 and 2 trials, followed by a phase 2 extension trial, at a single centre in Jiangsu, China. Healthy adults (≥18 years) who had negative serum or fingertip blood total antibody tests for SARS-CoV-2 (in phases 1 and 2), with no prevalent SARS-CoV-2 infection or history of infection and no SARS-CoV-2 vaccination history (in all three trials reported here), were enrolled. Participants were randomly allocated (4:1 in phase 1, 2:1 in phase 2, and 1:1 in the extension trial) to receive two intranasal doses of the dNS1-RBD vaccine or placebo on days 0 and 14 or, for half of the participants in phase 2, on days 0 and 21. To avoid cross-contamination during administration, vaccine and placebo recipients were vaccinated in separate rooms in the extension trial. The phase 1 primary outcome was safety (adverse events recorded on days 0–44; serious adverse events recorded from day 0 until 12 months after the second dose). In the phase 2 and extension trials, the primary immunogenicity outcomes were SARS-CoV-2-specific T-cell response in peripheral blood (measured by IFN-γ ELISpot), proportion of participants with positive conversion for SARS-CoV-2 receptor-binding domain (RBD)-specific IgG and secretory IgA (s-IgA) antibodies, and concentration of SARS-CoV-2 RBD IgG in serum and SARS-CoV-2 RBD s-IgA in the nasopharynx (measured by ELISA) at 1 month after the second dose in the per-protocol set for immunogenicity. χ^2 test and Fisher's exact test were used to analyse categorical data, and *t* test and Wilcoxon rank sum test to compare the measurement data between groups. These trials were registered with the Chinese Clinical Trial Registry (ChiCTR2000037782, ChiCTR2000039715, and ChiCTR2100048316).

Findings Between Sept 1, 2020, and July 4, 2021, 63, 724, and 297 participants without a history of SARS-CoV-2 vaccination were enrolled in the phase 1, phase 2, and extension trials, respectively. At least one adverse reaction after vaccination was reported in 133 (19%) of 684 participants in the vaccine groups. Most adverse reactions were mild. No vaccine-related serious adverse event was noted. Specific T-cell immune responses were observed in 211 (46% [95% CI 42–51]) of 455 vaccine recipients in the phase 2 trial, and in 48 (40% [31–49]) of 120 vaccine recipients compared with one (1% [0–5]) of 111 placebo recipients (p<0·0001) in the extension trial. Seroconversion for RBD-specific IgG was observed in 48 (10% [95% CI 8–13]) of 466 vaccine recipients in the phase 2 trial (geometric mean titre [GMT] 3·8 [95% CI 3·4–4·3] in responders), and in 31 (22% [15–29]) of 143 vaccine recipients (GMT 4·4 [3·3–5·8]) and zero (0% [0–2]) of 147 placebo recipients (p<0·0001) in the extension trial. 57 (12% [95% CI 9–16]) of 466 vaccine recipients had positive conversion for RBD-specific s-IgA (GMT 3·8 [95% CI 3·5–4·1] in responders) in the phase 2 trial, as did 18 (13% [8–19]) of 143 vaccine recipients (GMT 5·2 [4·0–6·8]) and zero (0% [0–2]) of 147 placebo recipients (p<0·0001) in the extension trial.

Interpretation dNS1-RBD was well tolerated in adults. Weak T-cell immunity in peripheral blood, as well as weak humoral and mucosal immune responses against SARS-CoV-2, were detected in vaccine recipients. Further studies are warranted to verify the safety and efficacy of intranasal vaccines as a potential supplement to current intramuscular SARS-CoV-2 vaccine pools. Steps should be taken in future studies to reduce the potential for cross-contamination caused by the vaccine strain aerosol during administration.

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For the **WHO COVID-19 vaccine tracker** see https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines

Research in context

Evidence before this study

We searched PubMed for clinical trials published from database inception to Dec 13, 2021, with the search terms "COVID-19" or "SARS-CoV-2", "vaccine", "intranasal" or "nasal spray", and "clinical trial"; no language restrictions were applied. To our knowledge, no data from human clinical trials of SARS-CoV-2 vaccine administered intranasally had been reported at the time of the search. According to WHO's COVID-19 vaccine tracker and landscape for SARS-CoV-2 candidate vaccines (updated on Dec 10, 2021), and in addition to dNS1-RBD reported in this Article, there were seven intranasal SARS-CoV-2 vaccine candidates in ongoing clinical trials, including two viral vector vaccines, two protein subunit vaccines, two live-attenuated vaccines, and one inactivated vaccine.

Added value of this study

To our knowledge, this study is the first to report clinical data for an intranasal SARS-CoV-2 vaccine. We evaluated the safety and immunogenicity of dNS1-RBD with a two-dose regimen in three randomised, double-blind, placebo-controlled clinical trials, including phase 1, phase 2, and a phase 2 extension trial. The results showed that two doses of dNS1-RBD were well tolerated, with no vaccine-related serious adverse events reported. Weak cellular immunity in peripheral blood, as well as weak humoral and mucosal immune responses against SARS-CoV-2, were detected in vaccine recipients. This study is also the first to provide evidence of cross-contamination caused by aerosol of the intranasal vaccine produced during administration, which could help to pave the way for clinical development of other intranasal vaccines in the future.

Implications of all the available evidence

Based on the available evidence, dNS1-RBD is well tolerated but only weakly immunogenic in peripheral blood, which is concordant with the observation in animals of a weaker immune response in the circulation than in the respiratory tract. SARS-CoV-2 receptor-binding domain-specific IgG (serum) and secretory IgA (nasopharynx) antibody responses were also weak in our clinical trials. Because the immune mechanism underlying the strong and broad-spectrum protective effect of dNS1-RBD in animals, we argue that this intranasal spray vaccine warrants further study. Considering the complementarity of the potential protective effects of intranasal vaccines with intramuscularly administered SARS-CoV-2 vaccines, dNS1-RBD and other intranasal vaccines in development could be an important supplement to current SARS-CoV-2 vaccine pools. The efficacy and cost-effectiveness of intranasal vaccination will be evaluated in the future studies.

Introduction

COVID-19, a highly contagious disease that affects the respiratory system, had caused more than 5 million deaths globally as of Dec 10, 2021.[1] As the pandemic continues, vaccination remains the most cost-effective intervention to prevent the disease. Several different forms of licensed SARS-CoV-2 vaccine are available worldwide, but no intranasal vaccine has been approved for use to date. As SARS-CoV-2 vaccination coverage and the quantity of clinical and real-world research have increased, vaccine effectiveness against mild or asymptomatic disease has been shown to be well below expectations,[2–6] as has the ability of available vaccines to interrupt human-to-human transmission.

Prevention of respiratory diseases through intranasal vaccination has been shown previously. Cold-adapted, live, attenuated influenza vaccine (CAIV; FluMist, AstraZeneca, London UK) was licensed as a safe and effective vaccine by the US Food & Drug Administration in 2003 and is approved for use in people aged 2–49 years.[7] Compared with intramuscular vaccines, intranasal vaccines are considered to provide two additional layers of protection: resident memory B cells and T cells in the respiratory mucosa, and secretory IgA (s-IgA).[8] In the face of the COVID-19 pandemic and the constant mutation of SARS-CoV-2, establishing comprehensive immunisation protection through multiple pathways might be the best approach.

The intranasal SARS-CoV-2 vaccine candidate CA4-dNS1-nCoV-RBD (dNS1-RBD) is manufactured with a cold-adapted influenza strain without non-structural protein 1 (NS1) as the genetic backbone, into which receptor-binding domain (RBD) genes from SARS-CoV-2 are inserted by gene reassortment.[9] In the preclinical study, currently reported as a preprint,[10] the vaccine showed rapid (onset of action after 24 h), long-lasting, and broad protection against SARS-CoV-2 challenge in hamsters by inducing strong innate and adaptive local immune responses in the respiratory tract, with weaker responses in the circulation, even when administered 24 h after SARS-CoV-2 infection. 9 months after two doses of dNS1-RBD, the protective effect against the SARS-CoV-2 beta variant provided by vaccination remained as good as that against the original strain of the virus.[10] dNS1-RBD is currently being assessed in an international, multicentre, phase 3 efficacy study (ChiCTR2100051391), with the first participant enrolled on Dec 16, 2021. Additionally, seven other intranasal spray SARS-CoV-2 vaccines are under clinical development worldwide,[11] although no human data have yet been published. The clinical development of an adenovirus-based intranasal SARS-CoV-2 vaccine, AdCOVID (Altimmune, Gaithersburg, MD, USA), was discontinued because of poor results from early clinical trials in June, 2021.

Although the route of vaccine delivery is an important determinant of immune priming at the site of vulnerability, clinical evaluation of the respiratory mucosal immune response is difficult. In this Article, we report on phase 1 and 2 clinical trials and a phase 2

extension clinical trial of dNS1-RBD, in which the vaccine candidate's safety and immunogenicity were assessed.

Methods

Study design and participants

Our studies, including phase 1 (from Sept 1, 2020, to Oct 5, 2021), phase 2 (from Nov 11, 2020, to Dec 31, 2021), and an extension phase 2 trial initiated after the interim analysis of phase 1 and 2 (from July 2, 2021), were all single-centre, double-blind, randomised, placebo-controlled studies conducted at Dongtai Center for Disease Control and Prevention (Dongtai, Jiangsu, China). Healthy adult participants aged 18 years and older without a history of or current SARS-CoV-2 infection were recruited through local village health centres. Before enrolment, participants in phases 1 and 2 were screened for SARS-CoV-2 infection history using serum or fingertip blood SARS-CoV-2 total IgG antibody test, and required to have had no suspected exposure to SARS-CoV-2 infection. Participants confirmed to have no SARS-CoV-2 infection history by inquiry in the extension trial were screened for SARS-CoV-2 vaccination history through the local health system, because mass SARS-CoV-2 vaccination campaigns were running at that time. In the extension trial, volunteers both with and without SARS-CoV-2 vaccination history were recruited, but this Article focuses only on the participants who had not yet received a SARS-CoV-2 vaccine. Female participants who were able to conceive agreed to use effective contraception for the duration of these trials; individuals with a chronic illness (eg, hypertension, diabetes, asthma, thyroid disease) that was stable or controlled, without deterioration, hospitalisation, or major changes in treatment within 3 months before enrolment, were able to participate. Participants were excluded if they had an axillary temperature greater than 37·0°C at the time of screening; were pregnant or breastfeeding; had an acute illness requiring systemic antibiotic or antiviral therapy, an immunodeficiency condition, a primary disease of the vital organs, cancer, or an immune disease; or had a history of severe allergy or history of SARS or Middle East respiratory syndrome. Screening, randomisation and the first vaccination for participants enrolled were required to be completed at the same day. The duration of the study period was estimated as 13 months. Full details of the eligibility criteria are listed in the protocols for phase 1, phase 2, and the extension trial.

The studies were approved by the ethics committee of Jiangsu Provincial Centre for Disease Control and Prevention. Written informed consent from all participants was obtained before screening, and all the trials were done in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice, and Chinese regulatory requirements. Safety oversight for specific vaccination pause rules and for advancement was done by an independent data monitoring committee.

Randomisation and masking

Block randomisation (with block sizes of ten for phase 1 and 12 for phase 2) was used in all three trials; randomisation codes were computer-generated before the trials by an independent statistical company (Nanjing CR Medicon Technology, Nanjing, Jiangsu, China). All participants, investigators, and laboratory staff were masked to treatment allocation.

In phase 1, participants were randomly assigned in a 4:1 ratio to either the vaccine group or the placebo group. In phase 2, participants were randomly assigned in a 2:1 ratio, with stratification by age (18–39, 40–59, and ≥60 years) and sex, to receive vaccine or placebo. In the extension trial, participants were randomly assigned in a 1:1 ratio, with stratification by age (18–59 and ≥60 years), to one of the two groups, and then assigned a vaccination room (A, B, C, or D), which could only be viewed in the randomisation system by the designated investigator for a limited time.

The randomisation code was assigned sequentially to each participant in order of enrolment, and participants received investigational products labelled with the corresponding code. The vaccine and placebo were identical in appearance. In the extension trial, participants went to designated rooms for vaccination.

Procedures

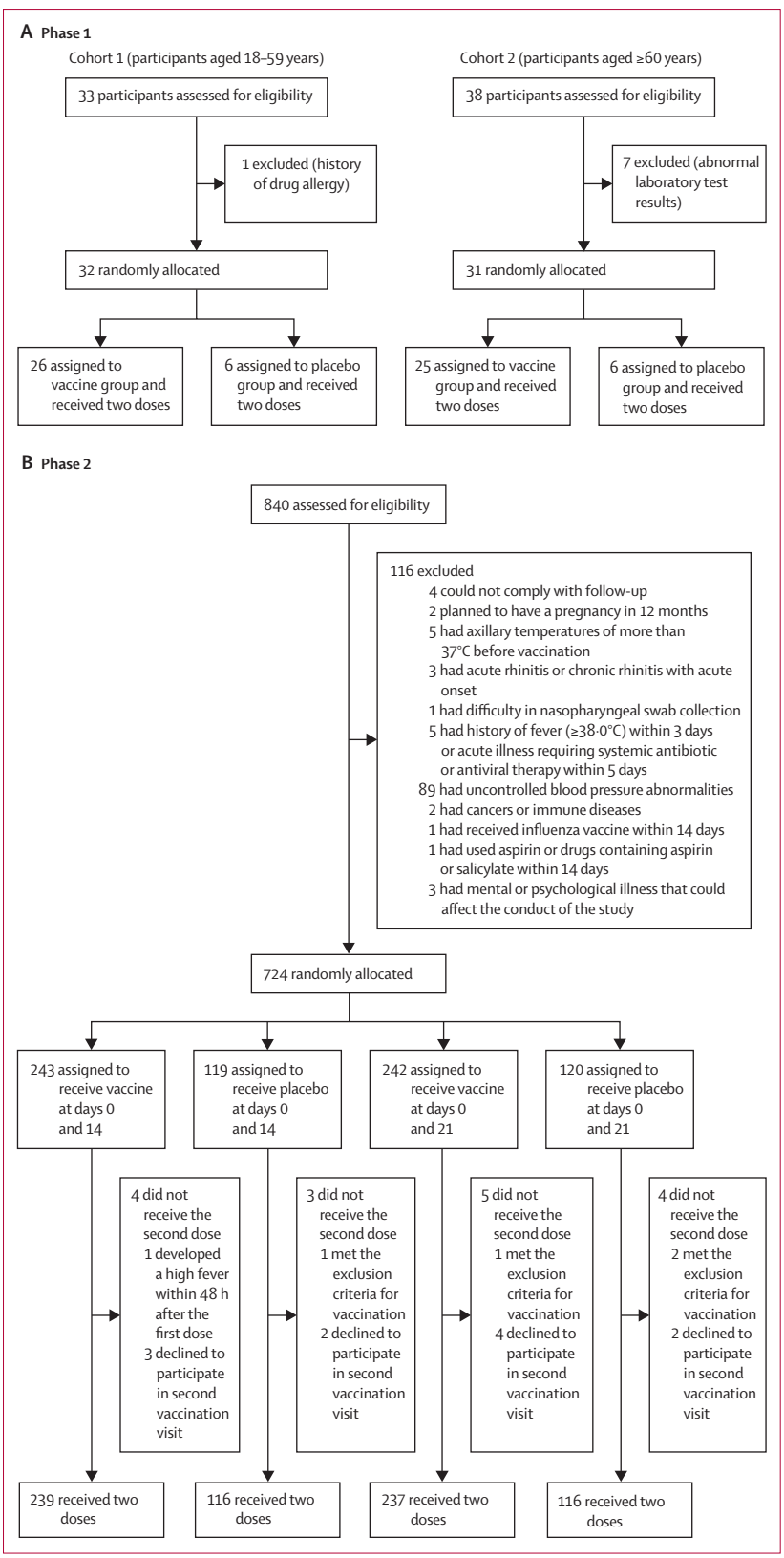
The vaccine was jointly developed by Xiamen University (Fujian, China), the University of Hong Kong (Hong Kong Special Administrative Region, China), and Beijing Wantai Biological Pharmacy Enterprise (Beijing, China). The vaccine is a liquid preparation containing 1×10^6 plaque-forming units (PFUs) of CA4-dNS1-nCoV-RBD per mL, which is stored in vials with a long-term storage temperature of –15° or lower. The vaccine was administered with a specific sprayer (item number NSM01; NEST Biotechnology, Wuxi, Jiangsu, China) that atomises the liquid into a fine mist of droplets with a diameter of 10–70 μm. The volume of each dose was 0·2 mL (0·1 mL per nasal cavity). The placebo was composed of diluent without vaccine virus components and was administered in the same way as the vaccine.

The initial and booster doses were administered on days 0 and 14, respectively, except in phase 2, in which half of the participants received the booster dose on day 21. All participants were monitored for 30 min post-vaccination for immediate adverse reactions, and were followed up for any adverse events within 30 days (in phase 1) or 42 days (in the phase 2 and extension trials) after vaccination. All participants were trained and required to record the vaccination-site adverse events (eg, rhinorrhoea, itchy nose, nasal congestion) or systemic adverse events (eg, fever, headache, cough) on paper diary cards. Serious adverse events were collected throughout the study (from day 0 until 12 months after the second dose) by spontaneous report from participants combined with regular visits. The reported adverse events were graded

For the **phase 1 study protocol** see http://www.chictr.org.cn/showproj.aspx?proj=55421

For the **phase 2 study protocol** see http://www.chictr.org.cn/showproj.aspx?proj=63754

For the **extension trial protocol** see http://www.chictr.org.cn/showproj.aspx?proj=55455



(Figure 1 continues on next page)

according to the China National Medical Products Administration guidelines. The causal association between adverse events and vaccination was determined by the investigators. In addition, participants in phase 1 were assessed for changes in laboratory parameters 3 days after each vaccination.

The detailed methods of the assays are provided in the appendix (pp 1–4). In brief, serum and nasopharyngeal swab samples were collected at baseline and at 28 days and 6 months after the second dose for measurement of IgG and s-IgA antibody responses with ELISA kits (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China). For the measurement of specific cellular immune responses, peripheral blood mononuclear cells (PBMCs) were collected at baseline (day 0) and at 14 days (in phase 2), 1 month, and 6 months after the second dose; following stimulation by a SARS-CoV-2 spike peptide pool (GL Biochem, Shanghai, China), PBMC IFN-γ expression was quantified using an enzyme-linked immunospot (ELISpot) assay (Dakewe Biotech, Shenzhen, China [phases 1 and 2]; Mabtech, Stockholm, Sweden [extension trial]), as described previously.[10] For the measurement of vaccine virus strain shedding in phase 1, nasopharyngeal swab samples were collected on days 0, 1, 7, 14, 15, and 21, and serum samples on days 0, 3, 14, and 17. Vaccine virus strain RNA was quantified using a real-time PCR assay, which was also used to measure vaccine virus strain in the environment in the extension trial.

Outcomes

The primary outcomes of the phase 1 trial and secondary endpoints of the phase 2 and extension trials were local and systemic adverse events within 30 days (0–44 days, phase 1) or 42 days (0–56 or 0–63 days, phase 2; 0–56 days, extension trial) after each vaccination; and serious adverse events throughout the observation period. The secondary safety endpoint of the phase 1 trial was occurrence of any abnormal changes in laboratory measurements at day 3.

In the phase 2 and extension trials, the primary immunogenicity endpoints were the proportion of responders (ie, those who were negative at baseline and post vaccination had ≥10 [phases 1 and 2] or ≥30 [extension trial] spot-forming cells per 1×10^6 PBMCs and a number of spot-forming cells in stimulated well at least 2·1-times that in unstimulated control well) and the response intensity of specific T-cell immunity (measured by IFN-γ ELISpot) in peripheral blood; and the proportion of participants with positive conversion (negative at baseline with a post-vaccination titre ≥2, or a post-vaccination titre ≥2-times the baseline titre) and the geometric mean titres (GMTs) of SARS-CoV-2 RBD IgG antibodies in serum and s-IgA antibodies in nasopharyngeal swab samples (measured by ELISA). Except for the phase 2 primary endpoint of T-cell immunity (which was assessed at 14 days after the second dose), all primary immunogenicity endpoints

were assessed at 1 month after the second dose in the per protocol set for immunogenicity (PPS-I). Additional exploratory endpoints reported in this Article are the proportion of participants who shed vaccine virus in phase 1; the proportion of participants with positive conversion (as defined above) and the GMTs of anti-H1N1 IgG and s-IgA antibodies by ELISA in phase 1 and 2; the proportion of vaccinators who shed vaccine virus and the proportion of responders among vaccinators in the extension trial; and the proportion of air and surface samples positive for vaccine virus strain RNA from the four vaccination rooms in the extension trial.

Other secondary endpoints or exploratory endpoints are listed in the protocols, including some outcomes that are not available yet, which will be reported elsewhere. Protocol deviations are listed in the appendix (pp 29–37).

Statistical analysis

The sample size for the trials was based on clinical and practical considerations rather than a formal statistical power calculation. The populations used for each analysis were prespecified. The intention-to-treat population for immunogenicity (ITT-I) was defined as participants who received at least one dose and had any available result for the objective endpoint. The analysis population for primary immunogenicity endpoints (ie, the PPS-I) was defined as participants in the ITT-I who completed the full course of vaccination without any major protocol violations and who tested negative for the objective endpoint at baseline. All analyses conducted in the PPS-I were repeated in the ITT-I.

Safety and immunogenicity data were analysed descriptively using SAS (version 9.4). χ^2 test and Fisher's exact test were used to analyse categorical data. Comparisons of antibody responses or the intensity of cellular immunity between baseline and post-vaccination in individuals were done with paired *t* tests, and comparisons between the groups were analysed with *t* tests or Wilcoxon rank sum tests, depending on the normality of the data. The number and proportion of participants with adverse events after vaccination were described. Antibodies against SARS-CoV-2 or H1N1 were presented as the proportion of positive responders and GMTs with 95% CIs. The cellular immune responses were shown as a proportion of positive responders and the median number of IFN-γ-secreting cells (with IQR). Significance was set at $p<0·05$ (two-sided).

These trials were registered with the Chinese Clinical Trial Registry (ChiCTR2000037782 [phase 1], ChiCTR2000039715 [phase 2], and ChiCTR2100048316 [extension trial]).

Role of the funding source

The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

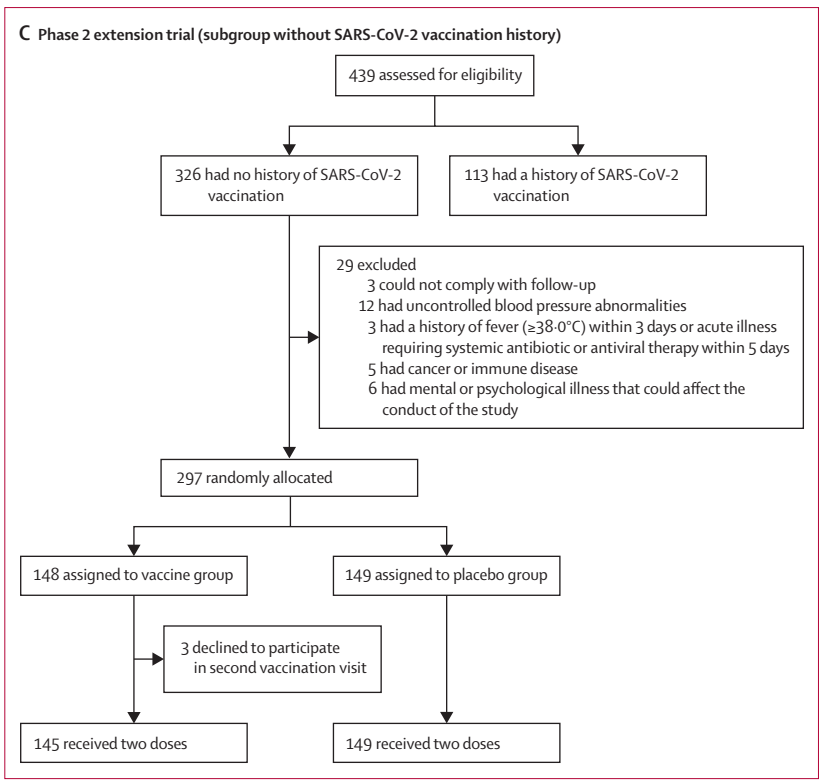


Figure 1: **Trial profile**

(A) In phase 1, participants were separated into two age cohorts with a sequential design. Enrolment of cohort 2 (participants aged ≥60 years) did not begin until a 7-day safety observation following the initial vaccination in cohort 1, supervised by the data monitoring committee, had been completed. (B) In phase 2, among the 16 participants who did not receive the second dose, one developed a transient high fever (39·9°C) within 48 h after the initial vaccination from the vaccine group; four met the exclusion criteria for vaccination (one from the vaccine group, three from the placebo group); and the other participants refused to attend the second vaccination visit. (C) In the phase 2 extension trial, subgroups of volunteers with and without a history of SARS-CoV-2 vaccination were recruited. Results for the subgroup who had previously received a SARS-CoV-2 vaccination will be reported elsewhere. Three participants in the vaccine group refused to attend the second vaccination visit.

Results

See Online for appendix

Between Sept 1 and Sept 18, 2020, 71 volunteers underwent eligibility screening for phase 1, of whom 63 were enrolled and randomly allocated, with 51 (81%) assigned to the vaccine group and 12 (19%) to the placebo group; all participants in phase 1 received two doses (figure 1A). 840 volunteers were assessed for eligibility in phase 2 between Nov 18 and Nov 22, 2020, of whom 724 participants were enrolled. Among these participants, half were assigned to receive vaccine (243 [34%] participants) or placebo (119 [16%]) on days 0 and 14, and the other half to receive vaccine (242 [33%]) or placebo (120 [17%]) on days 0 and 21 (figure 1B). 708 (98%) participants in phase 2 received two doses, and the rate of loss to follow-up (2–3%) was similar across groups. Between July 3 and July 4, 2021, from a screen of 326 volunteers without a history of SARS-CoV-2 vaccination, 297 participants were enrolled in the extension trial, of whom 148 (50%) were assigned to the vaccine group and 149 (50%) to the placebo group. Three (2%) participants in the vaccine group did not receive the second dose (figure 1C).

	Phase 1 trial		Phase 2 trial		Phase 2 extension trial (subgroup)	
	Vaccine group (n=51)	Placebo group (n=12)	Vaccine group (n=485)	Placebo group (n=239)	Vaccine group (n=148)	Placebo group (n=149)
Sex						
Male	23 (45%)	7 (58%)	233 (48%)	115 (48%)	69 (47%)	67 (45%)
Female	28 (55%)	5 (42%)	252 (52%)	124 (52%)	79 (53%)	82 (55%)
Age, years						
18–59	26 (51%)	6 (50%)	320 (66%)	157 (66%)	77 (52%)	77 (52%)
≥60	25 (49%)	6 (50%)	165 (34%)	82 (34%)	71 (48%)	72 (48%)
Mean	52·8 (14·7), 26–75	47·2 (20·5), 19–70	49·1 (14·7), 19–86	48·8 (14·5), 19–73	55·0 (11·9), 21–76	55·7 (11·8), 25–71
Pre-existing influenza A virus (H1N1-CA4) IgG antibody						
GMT	4681·9 (3833·2–5718·5)	4031·7 (2513·3–6467·5)	4625·0 (4228·2–5059·1)	4781·5 (4482·3–5100·6)	··	··
Titre						
<1:6400	23 (45%)	7 (58%)	233 (48%)	116 (49%)	··	··
≥1:6400	28 (55%)	5 (42%)	252 (52%)	123 (51%)	··	··

Data are n (%); mean (SD), range; or mean (95% CI). GMT=geometric mean titre. CA4=A/California/4/2009.

Table 1: **Baseline characteristics of enrolled participants**

The mean age of participants was 51·7 years (SD 15·9, range 19–75; 31 [49%] participants aged ≥60 years) in phase 1, 49·0 years (14·6, 19–86; 247 [34%]) in phase 2, and 55·3 years (11·8, 21–76; 143 [48%]) in the extension trial. The distributions of age and sex were similar between the vaccine and placebo groups. Baseline titres of serum IgG antibodies against H1N1 influenza virus (A/California/4/2009 [CA4]) were similar in phases 1 and 2; 33 (52%) participants in phase 1 and 375 (52%) in phase 2 had an H1N1 IgG titre of at least 1:6400 (table 1).

Data regarding vaccination-site and systemic adverse events and serious adverse events were available for all the participants. Overall, adverse reactions were largely absent or mild: 133 (19%) of 684 participants in the vaccine group had any adverse reactions within 30 days (phase 1) or 42 days (phase 2 and extension trial) after any dose, including 55 (8%) with local reactions and 103 (15%) with systemic reactions (table 2; appendix p 5). Common local adverse reactions mainly consisted of influenza-like symptoms, such as rhinorrhoea (34 [5%]) and itchy nose (13 [2%]), while common systemic reactions included fever (42 [6%]), fatigue (20 [3%]), headache (19 [3%]), and cough (19 [3%]). Four participants in the vaccine group (phase 2) had adverse reactions of grade 3 (three with fever and one with diarrhoea), and all recovered within 3 days of their occurrence. No vaccine-related serious adverse events were reported throughout the study period in phases 1 and 2, and no vaccine-related serious adverse events had been reported in the extension trial as of May 10, 2022. We found no significant difference in the incidence of adverse reactions or overall adverse events between the groups. Details of the safety data for each trial are provided in the appendix (pp 10–15).

In phase 1, transient laboratory abnormalities were observed in two (4%) of 51 participants in the vaccine group on the third day after vaccination, and were considered to be self-limiting events. One participant had a grade 1 blood glucose increase from baseline, and the other had grade 1 proteinuria after vaccination (appendix p 16).

Vaccine virus strain shedding was rare in phase 1 (appendix p 6). Among 63 participants, the vaccine strain was recovered from only one nasopharyngeal swab (from a vaccine recipient) collected 24 h after the first dose, although the RNA concentration was low (Ct value 32·7; 50% cell culture infectious dose per mL 14·8). No viral shedding was detected in blood or nasopharyngeal swab samples more than 24 h after the first vaccination or any time after the second vaccination.

The results of phases 1 and 2 were similar (appendix p 7). 1 month after the second dose, the proportion of responders in the vaccine group was 38% (95% CI 24–53; 18 of 48 participants) in phase 1 and 46% (42–51; 211 of 455 participants) in phase 2. At 6 months after the second dose, the proportion of responders in the vaccine group remained at 47% (95% CI 32–62; 22 of 47 participants) in phase 1 and 33% (29–38; 146 of 436) in phase 2. Among vaccine recipients in phase 2, there were no significant differences in IFN-γ ELISpot responses between age groups (18–59 years *vs* ≥60 years). There was only weak evidence of difference between vaccination schedules; at 14 days after the second dose, the response rate in the 0–21 days schedule group (28% [95% CI 23–35], 65 of 229 participants) was significantly higher than that of the 0–14 days schedule group (20% [15–25], 45 of 229; p=0·029), but there was no statistically significant difference between the groups at 28 days after the second dose (49% [42–56; 111 of 226] *vs* 44% [37–50; 100 of 229], p=0·24; appendix pp 17, 19, 22–23). Additionally, the pre-existing H1N1-IgG antibody titres of participants in

	Vaccine group (n=684)				Placebo group (n=400)			
	Any	Grade 1	Grade 2	Grade 3	Any	Grade 1	Grade 2	Grade 3
All adverse events	166 (24%)	113 (17%)	44 (6%)	9 (1%)	109 (27%)	73 (18%)	34 (9%)	2 (1%)
All adverse reactions	133 (19%)	112 (16%)	17 (2%)	4 (1%)	86 (22%)	75 (19%)	10 (3%)	1 (0%)
Any local reactions	55 (8%)	48 (7%)	7 (1%)	0 (0%)	37 (9%)	33 (8%)	4 (1%)	0 (0%)
Rhinorrhoea	34 (5%)	30 (4%)	4 (1%)	0 (0%)	27 (7%)	25 (6%)	2 (1%)	0 (0%)
Itchy nose	13 (2%)	13 (2%)	0 (0%)	0 (0%)	6 (2%)	6 (2%)	0 (0%)	0 (0%)
Nasal congestion	10 (1%)	9 (1%)	1 (0%)	0 (0%)	15 (4%)	14 (4%)	1 (0%)	0 (0%)
Pharyngalgia	8 (1%)	5 (1%)	3 (0%)	0 (0%)	9 (2%)	7 (2%)	2 (1%)	0 (0%)
Sneezing	3 (0%)	2 (0%)	1 (0%)	0 (0%)	1 (0%)	1 (0%)	0 (0%)	0 (0%)
Epistaxis	1 (0%)	1 (0%)	0 (0%)	0 (0%)	2 (1%)	2 (1%)	0 (0%)	0 (0%)
Rhinalgia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (1%)	3 (1%)	0 (0%)	0 (0%)
Oropharyngeal discomfort	1 (0%)	1 (0%)	0 (0%)	0 (0%)	1 (0%)	1 (0%)	0 (0%)	0 (0%)
Any systemic reactions	103 (15%)	84 (12%)	15 (2%)	4 (1%)	69 (17%)	59 (15%)	9 (2%)	1 (0%)
Fever	42 (6%)	38 (6%)	1 (0%)	3 (0%)	30 (8%)	28 (7%)	1 (0%)	1 (0%)
Headache	19 (3%)	16 (2%)	3 (0%)	0 (0%)	20 (5%)	19 (5%)	1 (0%)	0 (0%)
Cough	19 (3%)	15 (2%)	4 (1%)	0 (0%)	15 (4%)	11 (3%)	4 (1%)	0 (0%)
Fatigue	20 (3%)	19 (3%)	1 (0%)	0 (0%)	17 (4%)	15 (4%)	2 (1%)	0 (0%)
Dizziness	11 (2%)	11 (2%)	0 (0%)	0 (0%)	8 (2%)	7 (2%)	1 (0%)	0 (0%)
Nausea	7 (1%)	6 (1%)	1 (0%)	0 (0%)	5 (1%)	4 (1%)	1 (0%)	0 (0%)
Diarrhoea	8 (1%)	5 (1%)	2 (0%)	1 (0%)	5 (1%)	4 (1%)	1 (0%)	0 (0%)
Vomiting	5 (1%)	3 (0%)	2 (0%)	0 (0%)	1 (0%)	1 (0%)	0 (0%)	0 (0%)
Myalgia	7 (1%)	5 (1%)	2 (0%)	0 (0%)	10 (3%)	9 (2%)	1 (0%)	0 (0%)
Abdominal pain	3 (0%)	0 (0%)	3 (0%)	0 (0%)	2 (1%)	2 (1%)	0 (0%)	0 (0%)
Allergic reaction	3 (0%)	1 (0%)	2 (0%)	0 (0%)	2 (1%)	2 (1%)	0 (0%)	0 (0%)
Arthralgia	1 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Upper respiratory tract infection	1 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Leg aches	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	1 (0%)	0 (0%)	0 (0%)
Anorexia	1 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pruritus	1 (0%)	0 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Chest discomfort	1 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Palpitations	1 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Blurred vision	1 (0%)	1 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Data are n (%), where n is the number of participants reporting an adverse event. All adverse events were graded according to the guidelines for grading standards of adverse events in clinical trials of preventive vaccines issued by China National Medical Products Administration.

Table 2: **Adverse events occurring from the first dose to 30 days (phase 1) or 42 days (phase 2 and extension trials) after the second dose**

phases 1 and 2 had no effect on the vaccine-induced T-cell responses (appendix pp 17–21). Unexpectedly, IFN-γ ELISpot responses were detectable in both vaccine and placebo recipients in phases 1 and 2 at various timepoints; at 1 month after the second dose in phase 2, responses were observed in 211 (46% [95% CI 42–51]) of 455 vaccine recipients and 117 (53% [46–60]) of 220 placebo recipients (p=0·097; appendix pp 17–21). Investigations on the process of administration and animal experiments (data not yet published) indicate that cross-contamination caused by the vaccine strain aerosol might have occurred in the phase 1 and phase 2 studies. The extension trial was designed to confirm this cross-contamination hypothesis after the results from phases 1 and 2 had been revealed in the interim analysis.

As shown in figure 2, more participants were assigned to vaccine than to placebo in phases 1 (ratio of 4:1) and 2 (2:1). All participants of both groups were vaccinated in a small shared room, where vaccine droplets or aerosol in the air could be produced during administration of the intranasal spray vaccine. Additionally, recipients had to hold an upward-facing position for 10–20 sec following vaccination to facilitate full absorption in the nasopharynx. Thus, placebo recipients might have had exposure to air containing the vaccine. In the extension trial, to avoid cross-contamination, vaccine and placebo recipients were vaccinated in four separate rooms, with two rooms each for vaccine and placebo. 1 month after the second dose, the proportion of responders in the vaccine group (PPS-I) was 40% (95% CI 31–49; 48 of 120 participants), which was, as expected, significantly higher than that in the placebo group (1% [0–5]; one of 111; p<0·0001; figure 3; appendix p 23).

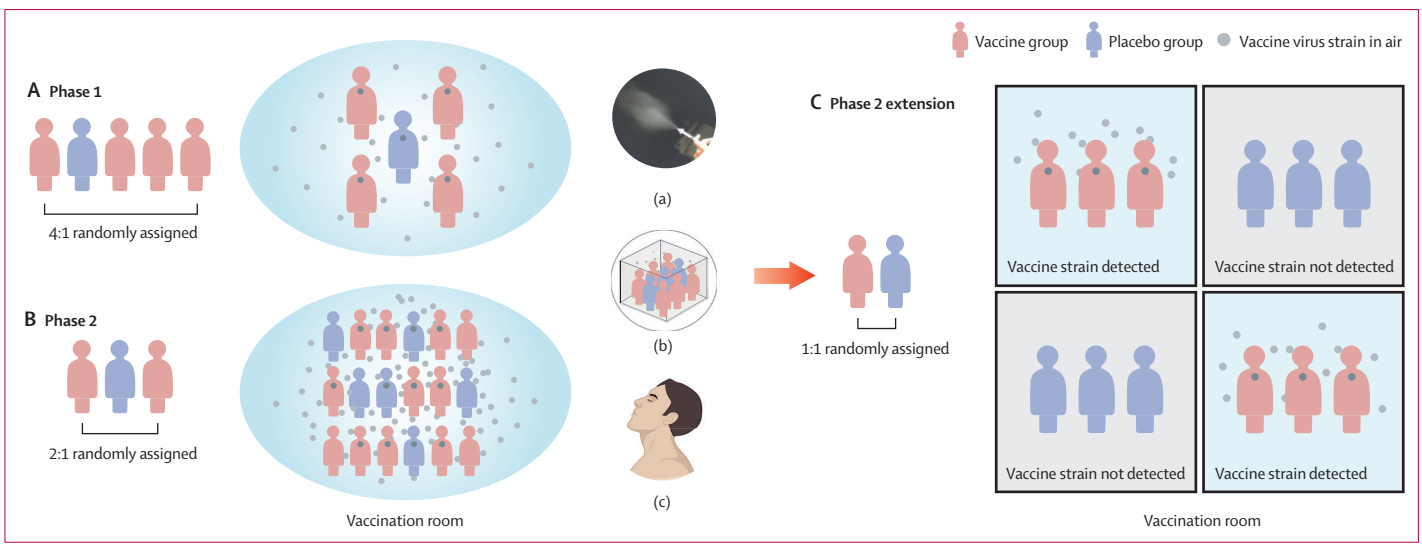


Figure 2: **Cross-contamination caused by the vaccine virus strain during administration**
As an intranasal spray vaccine (a), it is difficult to ensure zero leakage of the vaccine into the environment during administration; vaccine droplets or aerosol might be released into the air when the vaccinator removes bubbles from the spray syringe or when the medical waste recycling bin is repeatedly opened, especially in a small and enclosed vaccination room (b). During administration, participants must take off their mask and hold their face upwards at an angle of roughly 30–45° for 10–20 sec following vaccination for full absorption of the vaccine in the nasopharynx (c). A block randomisation design was used, with ratios of assignment of vaccine to placebo of 4:1 in phase 1 (A), 2:1 in phase 2 (B), and 1:1 in the extension trial (C). In phases 1 and 2, participants in both groups received the allocated treatment in the same room. In phase 1 (A), vaccine or placebo administration was completed within half a day for each dose so all participants were vaccinated at a similar time. In phase 2 (B), vaccine or placebo administration was done over 5 days for each dose, with 100–200 participants vaccinated per day. In the extension trial (C), participants were randomly assigned to one of four rooms for administration, with each room used for only one type of investigational product (vaccine or placebo). According to the results of environmental detection studies, only the two rooms used for vaccine administration had detectable vaccine virus strain in the air and on surfaces.

At baseline, specific serum IgG or nasopharyngeal s-IgA antibody responses against SARS-CoV-2 were not detectable in any participants in phases 1 and 2, and detectable in only two participants (one per group) in the extension trial. At 1 month after the second dose, the proportion of participants in the vaccine group (PPS-I) with seroconversion was 25% (95% CI 14–40; 13 of 51 participants) in phase 1, 10% (8–13; 48 of 466) in phase 2, and 22% (15–29; 31 of 143) in the extension trial, whereas none of the placebo recipients seroconverted. Significant differences were noted between the two groups in the phase 2 and extension trials (both p<0·0001). The GMTs of SARS-CoV-2 RBD IgG antibodies in serum samples from positive responders were 3·2 (95% CI 2·5–4·2) in phase 1, 3·8 (3·4–4·3) in phase 2, and 4·4 (3·3–5·8) in the extension trial (table 3). Positive conversion of nasopharyngeal s-IgA at 1 month after the second dose was found in six (12% [95% CI 4–24]) of 51 participants in phase 1, 57 (12% [9–16]) of 466 in phase 2, and 18 (13% [8–19]) of 143 in the extension trial, with significant differences observed between the two groups (phase 1 p=0·057; phase 2 p<0·0001; extension p<0·0001). The GMTs of SARS-CoV-2 RBD s-IgA antibodies in nasopharyngeal swab samples from positive responders were 4·0 (95% CI 2·1–7·7) in phase 1, 3·8 (3·5–4·1) in phase 2, and 5·2 (4·0–6·8) in the extension trial (table 3; appendix pp 24–25). Comparison between age groups showed no effect of age on antibody response (appendix p 26). s-IgA was detectable post-vaccination in seven participants from the placebo group (two in phase 1 and five in phase 2), in line with our hypothesis that there was passive immunity among placebo recipients.

In phase 2, anti-H1N1 IgG antibodies were detectable in all participants at baseline, since H1N1 influenza virus is common and all populations are susceptible to infection. However, only 11 (2%) of 715 participants in the ITT-I population were positive for anti-H1N1 s-IgA at baseline. GMTs for anti-H1N1 IgG antibodies in the vaccine group were significantly increased at 1 month after the second dose (5660 [95% CI 5360·7–5976·4) compared with baseline (4791 [4489·9–5112·4]; p<0·0001), whereas no such increase was observed in the placebo group. In both groups, GMTs for s-IgA tended to increase from baseline, although the GMT was significantly higher in the vaccine group than in the placebo group (p<0·0001). The proportion of participants with seroconversion (≥2-times increase in titre from baseline) of anti-H1N1 IgG antibodies was significantly higher in the vaccine group (33% [95% CI 29–37]; 156 of 474 participants) than in the placebo group (20% [15–26]; 47 of 232; p=0·0005), as was the proportion of participants with a positive response (negative at baseline and positive post-vaccination, or ≥2-times increase in titre from baseline) for s-IgA antibodies (38% [95% CI 34–43; 180 of 474 participants] in the vaccine group *vs* 19% [14–24; 43 of 232 participants] in the placebo group; p<0·0001]. Additionally, the geometric mean increase (defined as the ratio of post-vaccination GMT to pre-vaccination GMT) for IgG and s-IgA antibodies differed significantly

between the two groups, but the values were of limited clinical significance (appendix p 27).

During administration in the extension trial, 57 indoor air samples and 75 surface samples in each administration room were collected independently by professional investigators who were masked to study group allocation. Real-time PCR analysis of the samples collected over 6 days (3 days per dose) showed that samples positive for vaccine virus strain RNA were found in the two rooms where vaccines were administered, whereas no positive samples were found in the two placebo administration rooms. In the two vaccine rooms, the proportions of positive air samples were 46% (26 of 57 samples) and 32% (18 of 57), and the proportions of positive surface samples were 27% (20 of 75) and 12% (nine of 75; appendix p 28).

To assess the risk of vaccinators with primary personal protective equipment (including white gown, disposable isolation gown, surgical mask, disposable medical cap, and latex gloves) being infected with the vaccine virus strain during administration, nasopharyngeal swabs and blood samples were collected from four vaccinators before and after administration of each dose in the extension trial. No vaccine virus was recovered from any swabs, and no specific T-cell responses were detected in PBMCs from vaccinators 1 month after the participants had received the second dose.

Discussion

To our knowledge, this is the first report of findings on the safety and immunogenicity of an intranasal SARS-CoV-2 vaccine trialled in humans. Primary safety analyses from three trials with a total of 1084 participants indicate that dNS1-RBD administered as two doses was safe and well tolerated in healthy adults aged 18–86 years, with no serious adverse reactions. Compared with intramuscular SARS-CoV-2 vaccines,[12–16] the needle-free administration of dNS1-RBD might be associated with a reduced incidence of adverse events following vaccination. The incidence of vaccine-related adverse events associated with those intramuscular vaccines ranged from 29% to 100%,[12–16] compared with less than 20% for the intranasal vaccine in the study. Most adverse reactions were mild or moderate flu-like symptoms, such as rhinorrhoea, fever, and fatigue, and there was no difference in the incidence of adverse reactions between the vaccine and placebo groups. It is possible that some side-effects of nasopharyngeal swabbing were indistinguishable from local adverse reactions induced by the vaccine and were thus counted as adverse reactions.

In our trials, results from ELISpot assays showed that 277 (44%) of 623 vaccine recipients had a detectable SARS-CoV-2-specific cellular immune response in peripheral blood samples at 1 month after the second dose, and this proportion was 35% (168 of 483 participants) at 6 months after the second dose. However, IFN-γ ELISpot responses in PBMCs are not the most suitable index to evaluate an intranasal vaccine that

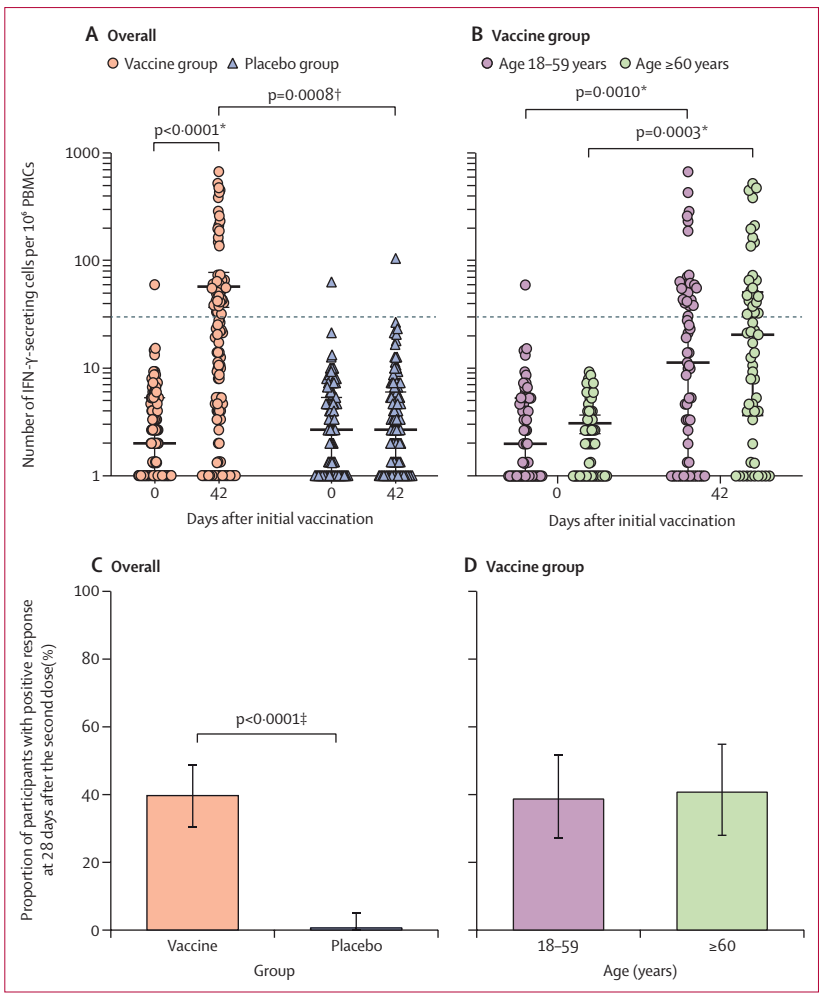


Figure 3: **SARS-CoV-2 spike protein-specific cellular immune responses following vaccination in the phase 2 extension trial**

(A, B) Number of IFN-γ-secreting cells per 10^6 PBMCs overall (A) and per age group in the vaccine group (B). Each datapoint represents the mean number of spots from triplicate stimulated wells for one participant after subtraction of the unstimulated control, with values less than 1 corrected to 1. Dotted lines indicate the cutoff for positive responses, defined as those in which the number of IFN-γ-secreting cells per 10^6 PBMCs was more than 30 (and in which the number of spots in stimulated wells increased to at least 2·1-times that in unstimulated control). (C, D) Proportion of participants with positive responses to vaccination overall (C) and by age group within the vaccine group (D). Error bars are IQRs. PBMCs=peripheral blood mononuclear cells. *Analysed by paired *t* test. †Analysed by Wilcoxon rank-sum test. ‡Analysed by Fisher's exact test.

mainly induces local immune responses in the upper respiratory tract and lungs. Several studies have suggested that intranasal live-attenuated influenza vaccines can induce specific IFN-γ $CD4^+$ and $CD8^+$ T cells in lung tissue,[17,18] and that these cellular immune responses are more robust than those induced by intramuscular trivalent inactivated vaccines (TIVs), with TIVs predominantly inducing humoral immunity.[17,19] Furthermore, the robust and durable tissue-resident memory $CD8^+$ T cells generated by intranasal vaccination with MCMV-M (a respiratory syncytial virus [RSV] M protein vaccine based on a murine cytomegalovirus vector) respond rapidly upon antigen re-exposure, leading to lower viral loads after RSV challenge.[20] Data

	IgG antibody					s-IgA antibody				
	Positive*			GMT		Positive*			GMT	
	n/N	% (95% CI)	p value†	Mean (95% CI)	p value‡	n/N	% (95% CI)	p value†	Mean (95% CI)	p value‡
Phase 2 trial	··	··	<0·0001	··	NA	··	··	<0·0001	··	0·51
Vaccine group	48/466	10% (8–13)	··	3·8 (3·4–4·3)	··	57/466	12% (9–16)	··	3·8 (3·5–4·1)	··
Placebo group	0/227	0% (0–2)	··	NA	··	5/227	2% (1–5)	··	3·5 (1·4–8·9)	··
Phase 2 extension trial (subgroup)§	··	··	<0·0001	··	NA	··	··	<0·0001	··	NA
Vaccine group	31/143	22% (15–29)	··	4·4 (3·3–5·8)	··	18/143	13% (8–19)	··	5·2 (4·0–6·8)	··
Placebo group	0/147	0% (0–2)	··	NA	··	0/147	0% (0–2)	··	NA	··

GMT=geometric mean titre (for positive responders in PPS-I). IgG=immunoglobulin G. NA=not applicable. PPS-I=per-protocol set for immunogenicity. s-IgA=secretory immunoglobulin A. *Cut-off titre is 2. †Analysed with χ^2 test or Fisher's exact test. ‡Analysed with *t* test or *t'* test. §Subgroup of participants with no history of SARS-CoV-2 vaccination.

Table 3: **Anti-SARS-CoV-2 IgG and s-IgA antibodies at 1 month after the second vaccine dose (PPS-I)**

from the preclinical study of dNS1-RBD showed that lung-resident memory RBD-specific CD4+ and CD8+ T cells could be induced by vaccination, and the T-cell immune response produced in lung tissue was about 26-times stronger than that in PBMCs in mice immunised with a single dose.[10] Nevertheless, it is difficult to observe the immune response in the lungs in clinical trials when human lung sampling is impractical, which might lead to underestimation of the intensity of cellular immunity of such vaccines clinically.

The RBD-binding antibody levels induced by dNS1-RBD were not strong, with low response rates for IgG (14%) and s-IgA (12%) in vaccine recipients. However, a human challenge trial of FluMist, conducted by Treanor and colleagues,[21] suggested that a low antibody response was not directly associated with low protective efficacy. In that study, among 103 adults aged 18–45 years who received a single dose, the seroresponse rates of haemagglutination-inhibiting antibodies for influenza A/H1N1, A/H3N2, and B/Harbin were 23%, 33%, and 3%, and the response rates of IgA antibodies in nasal wash were 14%, 32%, and 18%, respectively. Encouragingly, the virus challenge results indicated that the protective effects of FluMist for A/H1N1, A/H3N2, and B/Harbin were 80%, 78%, and 100%, respectively, which were higher than those of intramuscular TIV (60%, 67%, and 100%), despite TIV inducing higher seroresponse rates (91%, 76%, and 76%). Samples used to evaluate nasal mucosal immunogenicity commonly include nasal wash, nasal swabs, and nasopharyngeal swabs, the latter of which was used in our studies. However, unlike IgG and neutralising antibody assays, which have been validated by a large amount of data, the results of evaluation of s-IgA lack comparability in the absence of standardised methods of mucosal secretion sampling and mucosal antibody detection.[22–24] Furthermore, the effect of pre-existing local anti-H1N1 s-IgA antibodies (which had a low detection rate at baseline in our phase 2 study) or T cells on specific s-IgA antibody induction has not been clarified, although there was no evidence that pre-existing serum anti-H1N1 IgG antibodies had a negative effect on the cellular immune response in our study. Differences in vaccine-induced immune responses in the local mucosal environment of populations with diverse immune states need to be further explored.

We found that dNS1-RBD could cause vaccine virus strain aerosol to be released during administration, which might be responsible for the specific cellular immune response and weak s-IgA antibody response observed in placebo recipients in phases 1 and 2. Any cross-contamination was interrupted by physical separation in the extension trial. To our knowledge, our study is also the first to provide evidence of cross-contamination caused by the virus strain of the intranasal vaccine during administration, with definite evidence of environmental detection. This finding will help to pave the way for clinical development of other intranasal vaccines.

Assuming that T-cell responses in placebo recipients were due to passive inhalation of the vaccine strain aerosol, it is hard to understand why the intensity of the T-cell response in the placebo group was almost the same as that in the vaccine group despite the antibody response being significantly weaker or absent. One published study[25] showed that some patients with asymptomatic or mild COVID-19 have low or even undetectable levels of neutralising antibodies but a strong T-cell response. Another study[26] also found SARS-CoV-2-specific memory T cells in exposed seronegative healthy individuals, indicative of asymptomatic infection. These findings suggest that it might be easier to elicit overt cellular immunity than humoral immunity in mild infection. Based on the results of phases 1 and 2, in which the intensity of cellular immune response was almost the same in both groups, we cannot infer a lack of dose–response relationship because the breadth of sites that the vaccine acts on and the extent of absorption of dNS1-RBD in vaccinees and placebo recipients who inadvertently inhaled the vaccine remain unclear. Learning from this phenomenon, we plan to explore further the combination of multiple modes of mucosal vaccination in the future.

Based on the viral shedding results reported in phase 1, the probability of vaccine strain transmission through

close contact with a vaccinated person is believed to be very low. A previous study of a live-attenuated influenza vaccine also suggested a low rate of viral transmission, with only one (1%) of 99 placebo recipients confirmed to be infected through close contact with a FluMist-vaccinated person.[27] In addition, according to the assessment of viral shedding and specific immune responses in vaccinators in our extension trial, there is a low probability of infection with the vaccine virus strain from the administration environment in the context of work, provided that primary protection measures (eg, use of personal protective equipment) are taken.

Older individuals are at a disproportionately high risk of severe COVID-19,[28,29] but results from numerous clinical trials of licensed SARS-CoV-2 vaccines have shown lower efficacy in older adults than in younger people.[30,31] In this study, dNS1-RBD was well tolerated in all participants aged 18–86 years, and immunogenicity in older adults (aged ≥60 years) was similar to that in younger participants (aged 18–59 years; appendix pp 17, 20, 23, 26).

The development of mucosal vaccines is made more difficult by the fact that little is known about the related immunological mechanisms. Cellular immune responses, including innate cell responses and adaptive T-cell responses, are thought to be pivotal in clearing viral infection. However, induction of T-cell-mediated protection through vaccination with intranasal administration has remained elusive, and the precise T-cell subsets or cytokines involved in protection are still being defined. Notably, the levels of several cytokines or chemokines, including those from innate and adaptive immune responses, were significantly altered in the lung tissues of mice after dNS1-RBD immunisation in the preclinical study.[10] Additionally, vaccine-induced innate responses have been shown to be protective against viral infection in several studies. An important limitation of our study is the absence of an evaluation of local immune responses in humans, both innate and acquired immune responses; which might support the findings in animal studies that dNS1-RBD can act rapidly in the upper respiratory tract or nasal mucosa.[10] Of note, dNS1-RBD provided broad-spectrum protection in the preclinical study: the protective effect against the SARS-CoV-2 beta variant after vaccination was as good as the effect against the original strain of the virus, which might be attributable to the innate immune response in the nasal epithelium and the local cross-variant specific T-cell immune response. The translational gap between animals and humans cannot be ignored: promising results in preclinical animal studies do not necessarily predict safety and efficacy in humans. The human immune system is more sophisticated than that of animal subjects, and the local environment of the human nasal or respiratory tract is likely to have been exposed to a variety of pathogens before trial participation, whereas that of an animal raised in a controlled laboratory environment is likely to be naive to such exposures, which might affect immune responses to vaccination.

On the basis of data from our three human clinical trials, dNS1-RBD is well tolerated and can activate multiple, albeit weak immune responses. The efficacy of dNS1-RBD will be confirmed by the results of the ongoing phase 3 trial. Considering the complementarity of protective immune mechanisms (ie, eliciting local immunity in the respiratory tract) potentially offered by intranasal vaccines with those of intramuscularly administrated SARS-CoV-2 vaccines, intranasally administered vaccines might become an important supplement to current SARS-CoV-2 vaccine pools.

Contributors

FZ, CZ, KC, LZ, and HZ were co-first authors of this manuscript. FZ, CZ, JZ, TW, and N-SX designed the trials and study protocols. FZ and HP were co-principal investigators, and CY, HJ, XZ, and DL were investigators of this trial. KC was the site coordinator. CZ, SH, HL, YH, XL, QC, QS, JQ, ZH, GZ, and JL supervised the trials. LZ, HZ, TZ, and CL were responsible for the laboratory analysis. HS and LC were responsible for the environmental sampling and detection work. CZ and YS did the statistical analysis. CZ drafted the manuscript. TZ, TW, and JZ critically reviewed and revised the manuscript. JC, YC, and TZ interpreted the data. JH, XY, TW, JZ, and N-SX monitored the trials. FZ, CZ, SH, YS, TW, JZ, and N-SX accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

QS was an employee of Beijing Wantai Biological Pharmacy Enterprise during the conduct of the study. JH and XY are employees of and have stock options in Beijing Wantai Biological Pharmacy Enterprise. All other authors declare no competing interests.

Data sharing

The study protocols are available for review. The extension trial is still ongoing, and the data in this Article will be available after publication and finalisation of the complete clinical study report for at least 6 months. Researchers who provide a scientifically sound proposal will be allowed to access the de-identified individual participant data. Proposals should be sent to corresponding author N-SX (nsxia@xmu.edu.cn). Proposals will be reviewed and approved by the sponsor, investigator, and collaborators. To gain access, data requestors will need to sign a data access agreement.

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