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A randomized phase I trial of intranasal SARS-CoV-2 vaccine dNS1-RBD in children aged 3–17 years

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The intranasal SARS-CoV-2 vaccine dNS1-RBD (Pneucolin®), based on a live-attenuated influenza virus vector, has obtained Emergency Use Authorization in China for individuals aged 18 years and older. Here, we conducted a single-center, double-blind, placebo-controlled, age de-escalation phase 1 clinical trial to evaluate the safety of the dNS1-RBD in children aged 3–17 years (ChiCTR2300068044). Sixty-three participants received 2 intranasal doses of the vaccine or placebo at days 0 and 14. Safety assessments included adverse events/reactions within 30 days and serious adverse events (SAEs) over 12 months. Blood and nasal secretion samples were collected to further monitor blood indices and viral shedding. The vaccine group showed similar adverse reaction rates to the placebo group (39.0% vs 36.4%), with no SAEs related to vaccination. Data suggested that the dNS1-RBD vaccine is well-tolerated in children aged 3–17 years, and warrants further studies on its safety, immunogenicity and efficacy in this population.

Coronavirus Disease 2019 (COVID-19), an exceedingly contagious disease that primarily targets the respiratory system, has inflicted severe damage on public health and the global economy[1,2]. Since the initial identification of the ancestral strain of SARS-CoV-2 in 2019, the virus has undergone rapid evolution, resulting in mutations that increase viral fitness, enhance transmission kinetics, and facilitate immune evasion[3]. Despite the World Health Organization (WHO) determining in May 2023 that COVID-19 no longer constitutes a public health emergency of international concern, numerous cases continue to occur worldwide, primarily due to the emergence of new variants[4].

Although the incidence of SARS-CoV-2-related hospitalizations and deaths in children and adolescents is lower compared to those in adults, severe disease can still occur, resulting in hospitalizations, life-threatening complications such as multisystem inflammatory syndrome in children (MIS-C), and long COVID symptoms post-infection[5–7]. It is noteworthy that COVID-19 vaccination not only protects individuals from COVID-19 but also reduces the risk of developing long COVID symptoms[8–10]. A retrospective cohort study suggested that the SARS-CoV-2 vaccine is associated with reduced risk of long COVID in children and adolescents, particularly against delta and omicron variants[11]. Within 12 months after vaccination, adjusted vaccine effectiveness (VE) was 35.4% (95% CI 24.5-44.7) against probable long COVID and 41.7% (95% CI 15.0-60.0) against diagnosed long COVID. All of the above have heightened attention among the public regarding the COVID-19 vaccination of children.

The dNS1-RBD (Pneucolin®) is an intranasal vaccine based on a live-attenuated influenza virus (LAIV) vector. Series of clinical trials and preclinical studies of dNS1-RBD have demonstrated excellent safety, multiple immune responses, and broad protection against SARS-CoV-2 in individuals aged 18 and older[12–16]. In December 2022, dNS1-RBD obtained emergency use authorization for adults in China. dNS1-RBD provides a new option for children, with the advantages of being needle-free and non-invasive. However, the characteristics of dNS1-RBD in children have not yet been described. This phase 1 clinical trial aimed to preliminarily evaluate the safety of dNS1-RBD in healthy children aged 3–17 years, and pave the way for future research.

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Results

Baseline demographic characteristics

A total of 93 volunteers were screened, among which 63 were eligible and enrolled in the trial, with 23 aged 12–17 years, 21 aged 6–11 years, and 19 aged 3–5 years, respectively. The participants were randomly assigned to the vaccine group (n = 15/15/11) or the placebo group (n = 8/6/8) at stages I, II, and III. One participant aged 12–17 years did not receive the second dose of vaccine, all the remaining 62 participants (98.4%, 62/63) received two doses according to the protocol (Fig. 1). The baseline characteristics of participants are described in Table 1. The mean age of participants was 9.3 years, with the vaccine and control groups having mean ages of 9.5 years and 8.8 years, respectively. There were 31 (49.2%) boys and 32 (50.8%) girls in this study. Among them, 52 had a history of SARS-CoV-2 vaccination, while 11 did not.

Safety

All the 63 enrolled participants were included in the SS-1. As shown in Table 2, the incidences of adverse events (AEs) in the vaccine and placebo groups were 43.9% (18/41) and 45.5% (10/22). The incidence for adverse reactions (ARs) was 39.0% (16/41) in the vaccine group, including 15 participants (36.6%) with local reactions and 7 (17.1%) with systemic reactions, while the incidence in the control group was 36.4% (8/22), with 1 participant (4.5%) experiencing local reactions and 8 (36.4%) experiencing systemic reactions. ARs were less frequent after the second vaccination than after the first vaccination (Table S1). According to Table S2, the three most common local adverse reactions among vaccine recipients primarily included influenza-like symptoms, such as rhinorrhea (22.0%, 9/41), sore throat (17.1%, 7/41), and nasal congestion (12.2%, 5/41). The three most common systemic reactions included cough (14.6%, 6/41), fever (9.8%, 4/41), and diarrhea (4.9%, 2/41). Most ARs (91.7%, 22/24) occurred within 7 days post-vaccination. Specifically, within 7 days of either dose, the incidence of ARs in the vaccine group was 36.6% (15/41), with 14 participants (34.1%) experiencing local reactions and 7 (17.1%) participants experiencing systemic reactions. In the control group, the incidence was 31.8% (7/22), 1 participant (4.5%) experienced local reactions and 6 (27.3%) experienced systemic reactions (Table S2). All ARs were mild or moderate and resolved in a short time. There were no ARs of grade 3 or above (Fig. 2). During the trial period, only one participant reported two SAEs. This participant was hospitalized for bronchopneumonia and mycoplasma infection 307 days after receiving the second dose of the vaccine. The investigators judged these events to be unrelated to the vaccination (Table S3).

Laboratory parameters

To further assess the safety of the dNS1-RBD, a total of 8 hematological parameters were analyzed for changes before and 2 days after the first and second vaccinations. Finally, a total of 125 pairs of pre- and post-vaccination blood samples from the 63 participants (one participant only received the first dose) were collected. Thus, the detection of 250 blood samples yielded three routine hematological indexes and five biochemical indexes per

Table 1 | Baseline demographic characteristics of the participants

	Vaccine group *n* (%)	Placebo group *n* (%)	Total *n* (%)
No. of participants	41	22	63
Mean age (SD)	9.5 (4.0)	8.8 (4.5)	9.3 (4.1)
Age group			
12–17 years	15 (36.6%)	8 (36.4%)	23 (36.5%)
6–11 years	15 (36.6%)	6 (27.3%)	21 (33.3%)
3–5 years	11 (26.8%)	8 (36.4%)	19 (30.2%)
Sex			
Male	22 (53.7%)	9 (40.9%)	31(49.2%)
Female	19 (46.3%)	13 (59.1%)	32 (50.8%)
SARS-CoV-2 vaccination history			
Yes*	35 (85.4%)	17 (77.3%)	52 (82.5%)
No	6 (14.6%)	5 (22.7%)	11 (17.5%)
SS-1	41 (100.0%)	22 (100.0%)	63 (100.0%)
SS-2	41 (100.0%)	22 (100.0%)	63 (100.0%)

SD: standard deviation.
SS-1: The safety set-1 included participants who received at least one dose and had at least one safety visit.
SS-2: The safety set-2 included participants who received at least one dose and had blood index test results before vaccination (0 day) and at 2 days post vaccination.
* Participants had received their last dose of COVID-19 vaccine more than 6 months prior to study enrollment.

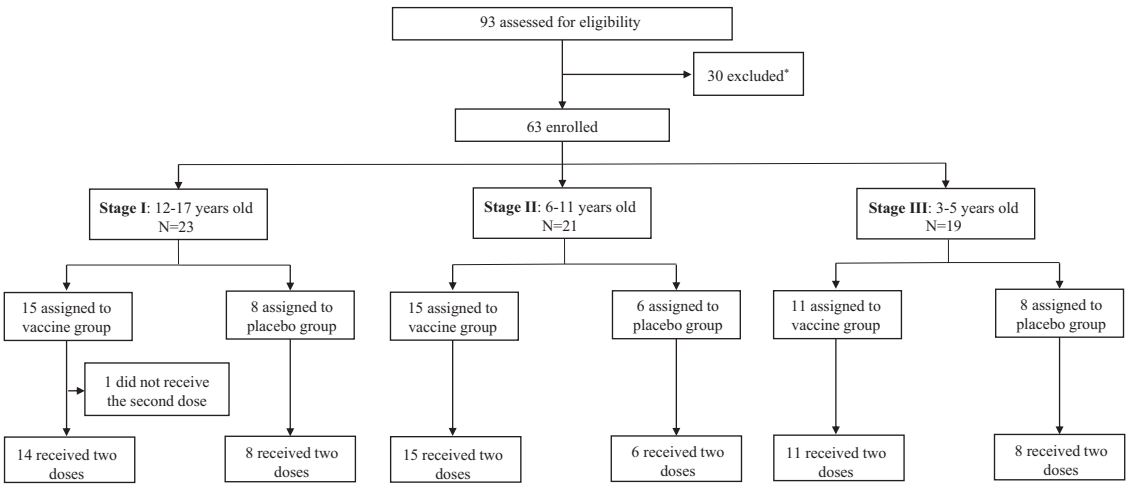

Fig. 1 | Trial profile. This age-escalation phase 1 study was carried out in three stages. Seven days after the first dose of vaccination in each stage, total adverse events were collected and analyzed under blind status. If the incidence of adverse events of grade 3 or above does not exceed 15% of the total vaccinated participants, the enrollment of participants for the next stage could be initiated. *2 could not comply with follow-up; 2 had axillary temperatures of more than 37°C before vaccination; 5 excluded for abnormal laboratory test results; 1 had history of fever (≥38.0°C) within 3 days or acute illness requiring systemic antibiotic or antiviral therapy within 5 days; 1 had received a subunit or inactivated vaccine within 14 days; 19 were identified by the researchers as having potential medical or other issues that might influence the conduct of clinical research.

Table 2 | Safety observations post-vaccination across different age groups

	12–17 years group (N = 23) n_1 (%) [n_2]		6–11 years group (N = 21) n_1 (%) [n_2]		3–5years group (N = 19) n_1 (%) [n_2]		Total (N = 63) n_1 (%) [n_2]	
	Vaccine group (n = 15)	Placebo group (n = 8)	Vaccine group (n = 15)	Placebo group (n = 6)	Vaccine group (n = 11)	Placebo group (n = 8)	Vaccine group (n = 41)	Placebo group (n = 22)
Total adverse events	8 (53.5%) [17]	2 (25.0%) [3]	4 (26.7%) [14]	2 (33.3%) [2]	6 (54.5%) [22]	6 (75.0%) [12]	18 (43.9%) [53]	10 (45.5%) [17]
Adverse reactions	6 (40.0%) [14]	2 (25.0%) [3]	4 (26.7%) [13]	1 (16.7%) [1]	6 (54.5%) [20]	5 (62.5%) [7]	16 (39.0%) [47]	8 (36.4%) [11]
Local adverse reactions	5 (33.3%) [11]	0 (0.0%) [0]	4 (26.7%) [8]	0 (0.0%) [0]	6 (54.5%) [10]	1 (12.5%) [2]	15 (36.6%) [29]	1 (4.5%) [2]
Systemic adverse reactions	1 (6.7%) [3]	2 (25.0%) [3]	2 (13.3%) [5]	1 (16.7%) [1]	4 (36.4%) [10]	5 (62.5%) [5]	7 (17.1%) [18]	8 (36.4%) [9]
Adverse events ≥ grade 3	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]
Serious adverse events	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	1 (9.1%) [2]	0 (0.0%) [0]	1 (2.4%) [2]	0 (0.0%) [0]
Discontinuation due to AE	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]	0 (0.0%) [0]

AE adverse event.
n_1 (%): the number and percentage of participants who reported experiencing at least one adverse event;
n_2: The number of reported adverse events.

sample, resulting in a total of 648 paired indexes in the vaccine group and 352 paired indexes in the placebo group. Parameters that were outside the normal range but did not reach grade 1 were classified as normal, while parameters of grade 1 or above were defined as abnormal. Table 3 summarizes the changes in blood index fluctuation before and after the vaccination. The results showed that most parameter pairs (98.5% in the vaccine group and 96.6% in the placebo group) remained normal before and after vaccination, and 1.1% and 2.0% of the pairs shifted from normal to abnormal, respectively. Some pairs changed from abnormal to normal (0.3% in the vaccine group and 0.6% in the placebo group) or remained abnormal after vaccination (0.2% in the vaccine group and 0.9% in the placebo group). According to the program, participants with laboratory parameters showing grade 3 or higher abnormalities (1.6%, 1/63), or those recommended for follow-up by clinicians based on a comprehensive assessment of indicators (17.5%, 11/63), were reexamined within 30 days. Overall, follow-up was suggested for 11 participants, with 6 in the vaccine group and 5 in the control group. All participants returned to normal or pre-immunization stable levels within 30 days post-vaccination (Table S4).

Shedding

Among the 250 nasal secretion samples collected post-vaccination using Polyvinyl Alcohol (PVA) medical sponges, three samples from participants in the 3–5 years group were tested positive for nuclear export protein (NEP) via Reverse Transcription-Polymerase Chain Reaction (RT-PCR), which were collected at 24 h and 48 h after the first vaccination, and at 24 h after the second vaccination, respectively. These NEP RT-PCR (+) samples were further cultured, and subsequently tested again for the NEP gene after cell passage, all of which were negative. Finally, no viral shedding was detected after vaccination of dNS1-RBD.

Discussion

To our knowledge, this is the first report presenting the safety findings of the intranasal SARS-CoV-2 vaccine (dNS1-RBD) in pediatric and adolescent populations. As expected, dNS1-RBD exhibited a favorable safety profile in participants aged 3–17 years following the administration of two doses spaced 14 days apart. The incidences of ARs were similar in the vaccine group compared to the placebo group (39.0% vs 36.4%), participants in the vaccine group experienced higher rates of local reactions (36.6% vs 4.5%) but lower rates of systemic reactions (17.1% vs 36.4%) compared to the placebo group. All observed ARs were mild and resolved quickly. Only one participant reported SAEs, which were deemed to be unrelated to dNS1-RBD by the investigators. The laboratory results also confirmed the safety of dNS1-RBD.

The favorable safety profile of dNS1-RBD in adults has been proved in the phase 3 clinical trial among 30,990 adults aged 18 years and older[13]. The incidences of AEs and ARs within 30 days post-vaccination were almost the same across the vaccine and placebo groups (AEs: 15.6% vs 15.6%; ARs: 12.4% vs 12.4%). Overall, a majority of local and systemic ARs were mild, with more than 96% of ARs being grade 1 or 2. dNS1-RBD was also well tolerated in the elderly population (aged 60 years and above) and in individuals with underlying medical conditions, including hypertension, diabetes, heart disease, asthma, and so on. In the 3–17 age group, dNS1-RBD showed a similarly favorable safety profile, with the incidence rates of AEs and ARs in the vaccine group comparable to those in the placebo group. A previous study suggested inflammation-related adverse reactions following vaccination potentially indicate a stronger immune response[17]. In our study, local ARs were more frequent within 7 or 30 days of either dose in the vaccine group than in the placebo group. Conversely, systemic ARs were less common in the vaccine group compared to the placebo group. The three most common local reactions included rhinorrhea, sore throat, and nasal congestion; the three most common systemic reactions were cough and fever. All the ARs were mild and resolved in a short time. It's worth noting that the trial enrollment period, from February to April 2023, coincided with the peak season for respiratory infectious diseases in southeast China[18]. The flu-like symptoms (e.g., fever, rhinorrhea, and nasal congestion), that are

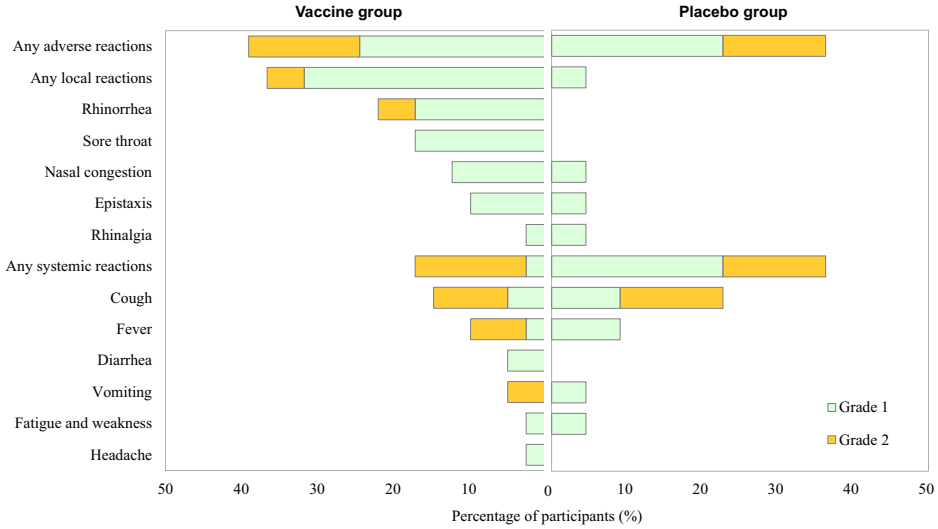

Fig. 2 | Incidence of adverse reactions occurring within 30 days after vaccinations. The severity of adverse reactions was classified into grade 1, grade 2, grade 3, or grade 4 based on the scale issued by the China National Medical Products Administration and the U.S. Department of Health and Human Services.

Table 3 | Laboratory parameters evaluated pre- and post-vaccination

Pre-vaccination (number of pairs, %)	Post-vaccination (number of pairs, %)					
	Vaccine		*P*-value*	Placebo		*P*-value*
	Normal	Abnormal		Normal	Abnormal	
Any indices						
Normal	638 (98.5%)	7 (1.1%)	0.18	340 (96.6%)	7 (2.0%)	0.18
Abnormal	2 (0.3%)	1 (0.2%)		2 (0.6%)	3 (0.9%)	
Blood routine indices						
Normal	237 (97.5%)	5 (2.1%)	0.06	128 (97.0%)	2 (1.5%)	0.50
Abnormal	0 (0.0%)	1 (0.4%)		0 (0.0%)	2 (1.5%)	
Serum biochemical indices						
Normal	401 (99.0%)	2 (0.5%)	1.00	212 (96.4%)	5 (2.3%)	0.45
Abnormal	2 (0.5%)	0 (0.0%)		2 (0.9%)	1 (0.5%)	

**P*-value by McNemar Test.

induced by these respiratory infections, could potentially confound the safety assessment in this trial. And the small sample size in this Phase I clinical trial also might introduce bias. Hence, further validation and exploration in larger cohorts are essential.

In comparison to traditional intramuscular vaccines, dNS1-RBD represents an innovative approach to pediatric immunization. This intranasal vaccine offers a painless, needle-free alternative to conventional injections, thereby mitigating the common pain and anxiety associated with needle administration. This method is particularly appealing to children and adolescents who fear needles, reducing instances of distress such as crying or avoidance during vaccination. Consequently, it can boost their willingness to be vaccinated and improve overall compliance. Our results indicate that the most common local and systemic ARs were rhinorrhea (9/41, 22.0%), and cough (6/41, 14.6%), respectively, which are the same as the common symptoms observed in adults[12,13]. The observed incidence of AEs of dNS1-RBD was similar to the recombinant COVID-19 vaccine (ZF2001), but dramatically lower than those reported for other SARS-CoV-2 mRNA vaccines[19–24]. A survey involving 10,452 subjects found high satisfaction with the dNS1-RBD vaccine: 92.6% reported no discomfort during inoculation, and 99.8% reported a satisfactory vaccination process. Preferences for administration mode varied, with 58.8% favoring the intranasal spray, 8.4% opting for intramuscular injection, and 32.9% expressing no preference[25]. Most importantly, the success of dNS1-RBD emphasized the promising potential of the LAIV vector in a wide variety of future applications especially for pediatric and elderly vaccines. The LAIV vector has proven to be a promising vaccine platform, especially in combating respiratory infectious diseases. It provides an important needle-free tool for active intranasal immunization, reducing vaccine hesitancy, and improving the accessibility and ease of vaccination.

In the Phase I clinical trial of adults[12], it was found that out of 63 participants, the vaccine strain was detected in only one nasopharyngeal swab taken 24 h after the first dose, and even then, the RNA concentration was low. Similarly, in our study, no viral shedding was detected in the PVA medical sponge within 2 days post vaccination. These findings indicate that, whether in adults or children, the likelihood of transmitting the vaccine strain via close contact with someone vaccinated is very low. Others research on attenuated live influenza vaccines have shown shedding was more common among young children and less frequent among adolescents and adults[26–31]. For instance, in a study conducted in China on the intranasal influenza vaccine ("Ganwu", Changchun BCHT Biotechnology Co., Ltd., Jilin, China), the incidence of viral shedding was 3.33% in the ≥18 years old group (1/30) and 16.67% in the 3–17 years old group (5/30)[30]. Additionally, Stan L. Block's study demonstrated that among subjects aged 5–8 years, 9–17 years, and 18–49 years, 44%, 27%, and 17%, respectively, shed the vaccine virus after receiving the LAIV (FluMist, MedImmune, Gaithersburg, MD) vaccination[31]. The reduced shedding rate of the dNS1-RBD might enhance public confidence in the vaccine safety, particularly in terms of its use among various age groups. Furthermore, a low viral shedding rate might raise concerns about the vaccine immunogenicity, which is likely attributable to the characteristics of our vaccine. Our intranasal vaccine was

developed based on the NS1-deleted H1N1 vector carrying the gene encoding SARS-CoV-2-RBD (dNS1-RBD), that is, the virus vector of our vaccine is knocked out of the NS1 gene of the influenza virus, in addition to the cold-adapted technology. In case of NS1 deletion, the capacity of the viral replication of the vaccine strain is further limited, underscoring a reassuring safety profile[14]. Moreover, a series of clinical trials in adults have demonstrated that the dNS1-RBD vaccine induces multi-dimensional immune responses and provides good broad-spectrum efficacy against Omicron symptomatic infection[12,13]. Additionally, there is currently no evidence to suggest that pre-existing influenza antibodies have a negative effect on the immune response to dNS1-RBD. In the phases 1 and 2 clinical trials of the dNS1-RBD in adults[12], all participants were found to have detectable baseline anti-H1N1 IgG antibodies, but no differences in RBD-specific T-cell responses were observed between vaccine recipients in the high-titer (≥1:6400) and low-titer (<1:6400) groups. Unfortunately, our study did not measure the baseline anti-H1N1 antibodies in children, and the effect of pre-existing immunity against the influenza virus on vaccine immunogenicity in children requires further verification in the future.

A limitation of our study is the lack of evaluation for immune responses. Data from the preclinical studies of dNS1-RBD showed that lung-resident memory RBD-specific CD4+ and CD8 + T cells could be induced by vaccination, with the T-cell immune response in lung tissue being ~26 times stronger than in peripheral blood mononuclear cells (PBMCs) in mice immunized with a single dose. Nevertheless, it is difficult to observe the immune response in the lungs in clinical trials when human lung sampling is impractical. Therefore, IFN-γ ELISpot responses in peripheral blood samples were selected as an assessment indicator in the adult phase I/II study. ELISpot assays revealed that 44% of vaccine recipients exhibited a detectable SARS-CoV-2-specific cellular immune response in their peripheral blood samples 1 month following the second vaccine dose, with this figure dropping to 35% at 6 months post-vaccination. Detecting IFN-γ ELISpot responses requires a large volume of blood samples. Considering the fact that exploring immunogenicity necessitates collecting more blood samples, complicating pediatric clinical trials, this phase 1 study did not concentrate on measuring immunogenicity. In addition, the small sample size limits a comprehensive evaluation of immunogenicity. Further studies are needed for a more in-depth evaluation.

In conclusion, data from this phase I clinical trial preliminarily demonstrated the safety of the dNS1-RBD vaccine in children aged 3–17 years, and these findings warrant further studies on its safety, immunogenicity and efficacy in this population.

Methods

Study design and participants

This study was a randomized, double-blind, placebo-controlled, age de-escalation phase 1 clinical trial conducted between February 2023 and April 2024 in Dongtai, Jiangsu, China. Briefly, eligible participants were children aged 3–17 years, who did not receive any COVID-19 vaccines within the 6 months before enrollment, were free from SARS-CoV-2 infection at the time of enrollment, had no acute or severe chronic medical conditions, and had no history of severe anaphylactic reactions. Informed consent was obtained from all participants prior to enrollment (children aged 3–7 years were signed by their guardians, and children aged 8–17 years were signed by both their guardians and themselves). This age de-escalation clinical trial consisted of three stages, sequentially enrolling participants in three age subgroups: 12–17, 6–11, and 3–5-years group. The criteria for initiating the next stage were as follows: within 7 days after vaccination, the incidence of grade 3 or above adverse reactions did not exceed 15% of the total vaccinated participants. An independent data monitoring committee supervised the process for pausing and advancing vaccinations.

This study was approved by the Institutional Ethics Committee (IEC) of the Jiangsu Provincial Centre for Disease Control and Prevention (JSJK2022-A052-01) and registered at www.chictr.org.cn (ChiCTR2300068044). The study was undertaken in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP).

Vaccines

The investigational vaccine including the dNS1-RBD and placebo used in this clinical trial were manufactured by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd. (Beijing, China). dNS1-RBD has been well-characterized in the previous publications. In short, dNS1-RBD is manufactured with a cold-adapted influenza strain (CA4) without the non-structural protein 1 (NS1) as the genetic backbone, into which receptor-binding domain (RBD) gene from ancestral SARS-CoV-2 is inserted by gene reassortment. This vaccine is a liquid preparation (0.2 mL per vial, 2×10^5 plaque-forming units (PFUs) of CA4-dNS1-nCoV-RBD) and stored at -15°C or below. The placebo, consisting of a diluent without vaccine virus components, had identical packaging and volume to that of dNS1-RBD. All the participants were administered by intranasal spray (0.1 mL per nasal cavity) with a sprayer (NEST Biotechnology, Wuxi, Jiangsu, China) for two doses with 14 days interval.

Randomization and masking

In our phase 1 trial, which included separate age-based enrollment phases, we utilized simple randomization. An independent statistician (from Nanjing CR Medicon Technology in Nanjing, Jiangsu, China) generated the randomization codes using computer software before the trial commenced. All participants, investigators, and laboratory staff were masked to treatment allocation. Participants were enrolled in three sequential phases by age (12–17, 6–11, and 3–5 years), with each phase randomly assigning participants in a 2:1 ratio to receive either the vaccine or a placebo. The randomization code was assigned sequentially to each participant in order of enrollment, and participants received investigational products labeled with the corresponding code. The vaccine and placebo were identical in appearance. Participants went to designated rooms for vaccination.

Procedures

Three age groups (12–17, 6–11, and 3–5 years) of participants were sequentially enrolled with a pre-scheduled sample size of around 21 participants in each group. Eligible participants were randomly assigned in a 2:1 ratio to either the vaccine or placebo group. Subsequently, they were correspondingly assigned to one of three vaccination and safety observation rooms (two rooms for the vaccine group and one room for the control group), the assigned room number was only visible within the randomization system to the designated investigator for a limited time. All the participants and investigators were blinded to the vaccine allocation. The study procedures were depicted in Fig. S1.

In the study site, all participants were monitored for 30 min after each dose for immediate AEs and were trained to record the AEs occurring within 30 days after vaccination in the paper diary cards, including local AEs (eg, rhinorrhea, itchy nose, nasal congestion) or systemic AEs (eg, fever, headache, cough) together with their guardians. Participants were requested to report all SAEs and pregnancy outcomes throughout the trial period. All AEs were judged as definitely related, probably related, possibly related, possibly unrelated and unrelated to the vaccinations by investigators. ARs were defined as adverse events which were definitely, probably, or possibly causally related to vaccination.

Blood samples of each participant were collected before and 2 days after each vaccination to assess the fluctuation of blood index, a total of 8 laboratory indexes were measured including: (1) three routine blood test parameters: white blood cell count (WBC), hemoglobin (HGB), and platelets (PLT); (2) five serum biochemical indexes: total bilirubin (TBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea (UREA), and creatinine (CREA). The detection of laboratory indexes was performed by Dongtai People's Hospital, Jiangsu, China. The severity of AEs and abnormal laboratory indices were graded as grade 1, grade 2, grade 3, or grade 4 according to the guidelines issued by the National Medical Products Administration (NMPA) and the U.S. Department of Health and Human Services.

Nasal secretion samples were collected using the PVA medical sponge (Beijing Yingjia Medic Medical Materials Co., Ltd.) before vaccination, as

well as 1 and 2 days post-vaccination to investigate the shedding of the vaccine strain virus. The samples were stored and transported at -80°C or below, and detected by the National Institutes for Food and Drug Control using the RT-PCR. After nucleic acid extraction, specific primer probes designed for the NEP gene were used to quantitatively detect the presence of influenza virus. The NEP RT-PCR positive samples were cultured and tested again after cell passage. If the cell passaged samples were still positive for NEP gene, the presence of RBD gene and deficiency of NS1 gene would be confirmed by RT-PCR and sequencing to judge the presence of virus strains.

Outcomes

This study aimed to evaluate the safety of dNS1-RBD in children aged 3–17 years. The primary outcomes included solicited AEs/ARs within 7 days after each vaccination, unsolicited AEs/ARs within 30 days after each vaccination, and SAEs throughout the entire study period. The secondary outcomes included the changes in the blood index before and 2 days after vaccination, and the shedding of the vaccine strain virus at 1, and 2 days after vaccination.

Statistical analysis

The sample size for the trial was based on clinical and practical considerations rather than a formal statistical power calculation. The safety analysis set-1 (SS-1) included participants who received at least one dose of vaccine or placebo and had at least one safety visit. The safety analysis set-2 (SS-2) included participants who received at least one dose of vaccine or placebo and had blood index test results before and 2 days after vaccination. The viral shedding analysis cohort consisted of all participants who had received at least one dose of vaccine or placebo and had RT-PCR results for pre- and post-vaccination PVA medical sponge samples. All AEs/ARs were summarized as frequencies and percentages by each group. McNemar's test was used to compare the changes in laboratory indices before and 2 days after vaccination. Statistical analyses were performed using the SAS software (version 9.4). All reported tests were 2-sided and a P-value of <0.05 was considered significant.

Data availability

We will share individual participant data that underlie the results reported in this article beginning from 6 months post the major findings from the final analysis of the study were published, ending 2 years later. Proposals should be directed to huangshoujie@xmu.edu.cn. To gain access, data requestors will need to sign a data access agreement.

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Author contributions

H.-X.P., S.-J.H., T.W., J.Z. and N.-S.X. contributed to the study design. K.C., H.-X.P., X.Z., H.-M.J. and D.-L.L. contributed to sample collection and participant follow-up at the site. J.-L.Q., X.-H.L., Q.C., C.-L.Z. and J.-L.H. contributed to data interpretation. J.-L.Q., X.-H.L., Q.C., S.-J.H., and T.W. were the core team for data analysis and manuscript preparation. S.-J.H., T.W., X.-F.C., J.-L.H., X.-Z.Y., J.Z., and N.-S.X. monitored the trial. All authors critically reviewed the manuscript and approved the final version.

Competing interests

X.-F.C. was an employee of Beijing Wantai Biological Pharmacy Enterprise during the conduct of the study. J.-L.H. and X.-Z.Y. are employees of and have stock options in Beijing Wantai Biological Pharmacy Enterprise. All other authors declare no competing interests.

Additional information

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