Flu-based and PD1-based vaccines for SARS-CoV-2: abridged secondary publication

KY Yuen *, IFN Hung, H Chen, Z Chen

KEY MESSAGES

1. In study 1, the intranasally delivered DelNS1-nCoV-receptor-binding domain (RBD) live attenuated influenza virus (LAIV) vaccine for COVID-19 demonstrated safety and immunogenicity among healthy adults who had not previously received a COVID-19 vaccine.
2. In study 2, the PD1-RBD-DNA vaccine demonstrated safety and immunogenicity among healthy adults who had not previously received a COVID-19 vaccine.
3. In study 3, the intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine booster demonstrated safety among healthy individuals who had received two doses of the BNT162b2 vaccine, enhancing both pre-existing cellular and mucosal immune responses.

Hong Kong Med J 2025;31(Suppl 4):S9-13
HMRF project number: COVID190123

[1] KY Yuen, [2] IFN Hung, [1] H Chen, [1] Z Chen

[1] *Department of Microbiology, The University of Hong Kong, Hong Kong SAR, China*
[2] *Department of Medicine, The University of Hong Kong, Hong Kong SAR, China*

* Principal applicant and corresponding author: kyyuen@hku.hk

Introduction

As of 2022, 9.7 billion doses of COVID-19 vaccines have been administered,[1] and 10 vaccines have been approved for emergency use by the World Health Organization.[2] These vaccines demonstrated satisfactory efficacy in preventing severe disease and death. Emerging variants have increased viral transmission and reduced vaccine effectiveness.[3-7] During the Omicron outbreak, neutralising antibody levels decreased 40-fold in individuals who had received two doses of BNT162b2, highlighting the need for a third dose.[8-10] A nebulised viral vector vaccine has shown satisfactory immunogenicity.[11]

Intranasal vaccines can stimulate mucosal immunoglobulin (Ig). Nebulised vaccines pose risks of aerosol generation and unintended spread of vectors or antigens. We previously reported that the intranasally delivered DelNS1-live attenuated influenza virus (LAIV) vaccine conferred complete protection against homologous and heterologous influenza virus challenges in mice.[12] We developed an intranasally delivered DelNS1-nCoV-receptor-binding domain (RBD) LAIV vaccine[13] and evaluated its safety and immunogenicity in COVID-19 vaccine-naïve healthy adults (study 1). Additionally, we evaluated the safety and immunogenicity of PD1-RBD-DNA vaccine via intramuscular electroporation in COVID-19 vaccine-naïve healthy adults (study 2), and the immunogenicity of the DelNS1-2019-nCoV-RBD-OPT1 booster among individuals previously vaccinated with two doses of BNT162b2 (study 3).

Methods

Study 1

A phase 1, randomised, double-blinded, placebo-controlled, dose-escalation study was conducted to evaluate the safety and immunogenicity of the DelNS1-nCoV-RBD LAIV vaccine among healthy adults aged 18 to 55 years who had not received a COVID-19 vaccine. Participants were randomly assigned (in a 4:1 ratio) to receive two doses (4 weeks apart) of low- or high-dose DelNS1-nCoV-RBD LAIV vaccine or placebo.

Reactogenicity was assessed by recording solicited local and systemic events within 14 days of each dose, unsolicited adverse events (AEs) within 28 days, AEs of special interest, and serious AEs. Reactogenicity events were graded using the toxicity scale for healthy adult and adolescent participants in vaccine trials.[14] Unsolicited AEs were graded according to the Common Terminology Criteria for Adverse Events, version 5.0. Immunogenicity—including humoral, cellular, and mucosal responses—was evaluated. Serum samples were collected at multiple time points post-vaccination. RBD-specific IgG was measured using a chemiluminescent microparticle immunoassay. Neutralising antibody titres were assessed using a microneutralisation assay. T-cell responses to an RBD peptide pool were measured using an interferon-gamma ELISpot assay.[15] Influenza A antibody responses were evaluated using a haemagglutination inhibition assay. Saliva samples were used to assess mucosal

immunity; total Ig against SARS-CoV-2 RBD was measured using an in-house assay.[16]

Study 2

A phase 1, randomised, double-blinded, placebo-controlled, dose-escalation study was conducted to evaluate the safety and immunogenicity of the PD1-RBD-DNA vaccine among healthy adults aged 18 to 55 years without prior COVID-19 vaccination. Participants were randomly assigned to receive two doses (3 weeks apart) of high-dose (2 mg) or low-dose (1 mg) PD1-RBD-DNA vaccine or placebo via intramuscular electroporation. Safety (reactogenicity, unsolicited AEs, AEs of special interest, and serious AEs) and immunogenicity (cellular and humoral responses) of the vaccine were assessed.

Study 3

A phase 2, randomised, double-blinded, placebo-controlled study was conducted to assess the immunogenicity of the intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine as a booster among healthy adults aged 18 to 75 years who were SARS-CoV-2-naïve and had received two doses of BNT162b2. Participants were randomly assigned (in a 1:1 ratio) to receive two doses (3 weeks apart) of the vaccine or placebo. Safety and immunogenicity of the vaccine were evaluated.

Results

Study 1

In total, 29 participants (median age, 26 years; median body mass index, 22.1 kg/m^2) received either low-dose (n=11) or high-dose (n=12) DelNS1-nCoV-RBD LAIV vaccine or placebo (n=6). No participant discontinued vaccination due to AEs. No serious AEs or AEs of special interest occurred within 56 days. The three groups had similar rates of reactogenicity (p=0.595) and unsolicited AEs (p=0.620). One high-dose participant reported self-limiting grade 3 abdominal pain and diarrhoea within 14 days of the first dose. Serum anti-RBD IgG and neutralising antibody titres were undetectable. Median serum T-cell responses were slightly higher in the high-dose group than in the placebo group on day 14 (15 vs 0 spot-forming units [SFU]/10^6 peripheral blood mononuclear cells [PBMCs], p=0.17) and day 42 (14 days after the second vaccination) [12.5 vs 5 SFU/10^6 PBMCs, p=0.18]. They were also higher in the high-dose group than in the low-dose group on day 14 (15 vs 0 SFU/10^6 PBMCs, p=0.09) and day 42 (12.5 vs 0 SFU/10^6 PBMCs, p=0.09). Saliva total Ig against SARS-CoV-2 RBD was higher in the high-dose group than in the placebo group on day 31 (3 days after the second vaccination) [0.24 vs 0.21, p=0.046] and in both vaccine groups than in the placebo group on day 56 (28 days after the second vaccination) [0.31 vs 0.31 vs 0.15, p=0.45]. The haemagglutination inhibition titre on day 28 was also higher in the high-dose group (640 vs 320 vs 240, p=0.29 and p=0.21, respectively).

Study 2

In total, 11 participants (five men) received either high-dose (n=1; median age, 43 years) or low-dose (n=8; median age, 43 years) PD1-RBD-DNA vaccine or placebo (n=2; median age, 46 years). Three low-dose participants discontinued, including two with confirmed COVID-19. No serious AEs or AEs of special interest occurred within 50 days. Pain was reported only in the high-dose group. Most reactogenicities were grade 1 or 2; one low-dose participant experienced grade 3 malaise. Neutralising antibodies were undetectable in all groups. Anti-RBD antibody was detected in both vaccine groups after two doses. Compared with the low-dose group, the high-dose group had higher geometric mean titres (GMTs) of anti-RBD antibody (246.0 vs 38.8) and higher seroconversion rates (100% vs 25%) on day 36 and day 50 (146.5 vs 70.3 and 100% vs 50%, respectively). In the low-dose group, the GMT of anti-RBD antibody significantly increased from day 1 to day 50 (70.3 vs 25.0, p=0.0057), whereas no such antibody was detected in the placebo group. On day 22, median T-cell responses increased from 0.0 to 28.0 SFU/10^6 PBMCs in the high-dose group and from 12.5 to 48.8 SFU/10^6 PBMCs in the low-dose group; differences between groups were not significant. No increase was observed in the placebo group. By day 50, the median T-cell response significantly increased in the low-dose group from 12.5 (day 1) to 77.5 SFU/10^6 PBMCs (p=0.0018), relative to 580.0 SFU/10^6 PBMCs in the high-dose group and 182.8 SFU/10^6 PBMCs in the placebo group.

Study 3

In total, 106 participants received either the DelNS1-2019-nCoV-RBD-OPT1 vaccine (n=53) or placebo (n=53). No serious AEs or AEs of special interest were reported within 14 days of each dose. In the vaccine group, one participant reported grade 3 nasal irritation and congestion, and another reported grade 3 malaise. One participant experienced grade 3 reactogenicities. By day 50, saliva total Ig levels in the vaccine group had increased against Delta (1.2-fold), Omicron BA.1 (1.4-fold), and BA.2 (1.4-fold) variants; no change occurred in the placebo group. On day 50, neutralising antibody titres (viral microneutralisation GMTs) in the vaccine group remained stable against the ancestral strain (1.1-fold), Delta variant (0.9-fold), and Omicron BA.1 variant (1.0-fold). Median anti-RBD IgG levels slightly declined in both groups, but the decline was

significant in the placebo group (p=0.012). T-cell responses against the spike protein on day 50 were 1.2-fold in the vaccine group and 0.8-fold in the placebo group. T-cell activity increased from 46.1 to 54.7 SFU/2×10^5 PBMCs in the vaccine group but decreased from 43.2 to 31.8 SFU/2×10^5 PBMCs in the placebo group. Four vaccine recipients and three placebo recipients developed SARS-CoV-2 infection.

Discussion

Study 1

The intranasally delivered DelNS1-nCoV-RBD LAIV vaccine showed safety and immunogenicity among healthy adults. Common reactogenicities—malaise, myalgia, and sneezing—were mild and self-limiting, consistent with other LAIV vaccines.[17] Although no humoral immune response was detected, the vaccine elicited sustained cellular and mucosal responses, as evidenced by persistent increases in T-cell activity and saliva total Ig against SARS-CoV-2 in the high-dose group. Mucosal immunity is critical for preventing SARS-CoV-2 infection and transmission, particularly regarding the Omicron variant, which predominantly affects the upper respiratory tract. Local mucosal Ig protects the nasal and upper airway mucosa by blocking viral attachment, thus reducing replication and spread. Current injectable COVID-19 vaccines do not induce mucosal immunity and fail to prevent nasal infection or asymptomatic transmission.[18,19] However, intranasal administration of single-dose chimpanzee adenovirus-vectored vaccine,[20] helper-dependent adenoviral vector vaccine,[21] and RBD nanoparticles in animal models has elicited strong mucosal and systemic immunity against SARS-CoV-2. These approaches have demonstrated virus-specific $CD8^+$ T-cell responses and increased numbers of interferon-gamma-producing cells and IgA-secreting B cells in the nasal mucosa, trachea, lungs, and spleen, thus protecting both upper and lower respiratory tracts. Previous mouse model studies demonstrated that intranasal influenza-based vaccines (LAIV-CA4-RBD and LAIV-HK68-RBD) and the intramuscular PD1-RBD-DNA vaccine induced robust mucosal and systemic immunity. Bronchoalveolar lavage IgA/IgG levels and polyfunctional memory CD8+ T cells in the lungs provided effective protection against SARS-CoV-2 infection in upper and lower respiratory tracts; they also cross-neutralised variants of concern.[13] Accordingly, the intranasal vaccine may serve as a booster for individuals previously vaccinated with injectable COVID-19 vaccines or those who have recovered from infection.

Omicron variant infections are characterised by reduced replication efficiency and fusion activity, milder symptoms, and higher transmissibility. Neutralising antibodies generated after vaccination wane within 6 months. Winter surges of SARS-CoV-2 in the northern hemisphere may requires annual vaccination. The DelNS1-nCoV-RBD LAIV could serve as a combined COVID-19 and influenza vaccine, potentially improving uptake, particularly among children. Additionally, it can be administered at home and provided in a prefilled syringe. In contrast, aerosolised vaccines require 30 to 60 seconds of administration and a specialised nebuliser.[11]

Study 2

The PD1-RBD-DNA vaccine demonstrated safety; no participant withdrew due to AEs. Although neutralising antibodies were not detected after full vaccination, anti-RBD antibody levels had significantly increased by day 50 in the low-dose group. The vaccine also enhanced T-cell responses in both vaccine groups by day 50.

Dendritic cells activate both adaptive and innate immunity.[12] The PD1-RBD-DNA vaccine leverages PD1-ligand interactions to improve antigen uptake by dendritic cells, leading to antigen-specific immune activation.[8] In mice, two doses of the vaccine induced anti-RBD and neutralising antibodies, as well as cellular and mucosal immune responses by day 30.[9] Similar humoral and cellular responses were observed in vaccinated adults. This antigen-PD1 fusion strategy has also been applied to an HIV vaccine (ICVAX), which combines soluble PD1 with Gap-P41.[8]

The RBD of the spike protein is a key SARS-CoV-2 antigen that induces neutralising antibodies. After PD1-RBD-DNA vaccination, only anti-RBD antibodies were detected, whereas in an animal study, neutralising activity was observed after two doses. This discrepancy may be due to differences in assay methods: the animal study used a pseudovirus-based neutralisation assay, whereas we used a live virus microneutralisation assay. Furthermore, the animal study used a much higher relative dose (2.5 vs 0.02 mg/kg).

Study 3

The intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine demonstrated safety as a booster in healthy adults previously vaccinated with two doses of BNT162b2; no serious AEs were reported. In the vaccine group, saliva total Ig levels against Delta, BA.1, and BA.2 variants increased by day 50. The vaccine group also maintained pre-existing neutralising antibodies—induced by BNT162b2—against the ancestral strain, Delta, and BA.1 variants. The T-cell response on day 50 was 1.2-fold in the vaccine group, compared with 0.8-fold in the placebo group.

In an animal study, mice receiving one BNT162b2 dose followed by one DelNS1-2019-nCoV-RBD-OPT1 dose exhibited higher levels of bronchoalveolar lavage fluid IgA, serum neutralising antibodies, and T-cell responses than those given two BNT162b2 doses.[13] These results suggest that the DelNS1-2019-nCoV-RBD-OPT1 vaccine enhances immune responses primed by the BNT162b2 vaccine. Vaccine-induced immunity against SARS-CoV-2, particularly against the Omicron variant, begins to wane within 6 months.[22-25] As SARS-CoV-2 continues to evolve and co-circulate with influenza, annual COVID-19 and influenza vaccination may be necessary.

Repeated homologous mRNA vaccination may diminish immunogenicity.[26-28] mRNA vaccines rely on cellular uptake and intracellular antigen expression. Modified mRNA can be recognised by immune cells, potentially triggering an anti-mRNA immune response.[26,27] In animal models, such immunity substantially reduced antigen expression after booster doses.[28] Heterologous vaccination may help overcome this limitation. The intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine could serve as a booster for those who have received three or more doses of mRNA vaccines.

Intranasal vaccines can induce mucosal immune responses, which are essential for preventing viral infection and transmission. Animal studies have demonstrated that intranasal vaccines—including RBD nanoparticles, LAIV-CA4-RBD, adenoviral vectors, and spike-based vaccines—elicit strong mucosal immunity against SARS-CoV-2.[13,21,29,30] However, the DelNS1-2019-nCoV-RBD-OPT1 vaccine showed only moderate mucosal responses to Delta and Omicron variants. This difference may be due to the use of saliva samples in our study versus the use of bronchoalveolar lavage fluid in the animal study.[13] Among individuals given one intramuscular mRNA vaccine dose followed by an intranasal adenovirus-vectored vaccine dose, mucosal antibodies were detectable in saliva.[31]

Conclusions

Both the intranasally delivered DelNS1-nCoV-RBD LAIV vaccine and the PD1-RBD-DNA vaccine showed safety and immunogenicity among healthy adults. The intranasally delivered DelNS1-2019-nCoV-RBD-OPT1 vaccine showed safety as a booster dose and enhanced pre-existing mucosal and cellular immune responses among healthy adults who had received two doses of an mRNA vaccine.

Funding

This study was supported by the Health and Medical Research Fund Commissioned Research on the Novel Coronavirus Disease, Health Bureau, Hong Kong SAR Government (#COVID190123). The full report is available from the Health and Medical Research Fund website (https://rfs1.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Zhang R, Chan KH, Wang P, et al. A phase 1, randomized, double-blinded, placebo-controlled and dose-escalation study to evaluate the safety and immunogenicity of the intranasal DelNS1-nCoV-RBD LAIV for COVID-19 in healthy adults. Vaccines (Basel) 2023;11:723.

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