REVIEW ARTICLE

Medycyna Ogólna i Nauki o Zdrowiu 2025, Tom 31, Nr 4, 211–218
www.monz.pl

Intranasal vaccination against influenza, pertussis, and COVID-19 – clinical efficacy, immunological challenges, and development of prevention of respiratory diseases

Szczepienia donosowe przeciwko grypie, krztuścowi i COVID-19: skuteczność kliniczna, wyzwania immunologiczne oraz rozwój metod prewencji chorób układu oddechowego

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Kiedrowska K, Pawlicka AA, Walczyńska J, Skonieczna-Żydecka K. Intranasal Vaccination Against Influenza, Pertussis, and COVID-19: Clinical Efficacy, Immunological Challenges, and Development of Prevention of Respiratory Diseases. Med Og Nauk Zdr. 2025;31(4):211–218. doi:10.26444/monz/213322

Abstract

Introduction and Objective. Respiratory diseases, such as influenza, COVID-19 and pertussis, pose a significant public health threat, especially among at-risk groups such as the elderly and chronically ill. The aim of the study is to assess the potential of intranasal vaccines as a modern, safe and promising alternative to classical immunization methods, especially in the context of preventing the spread of infectious diseases.
Review Methods. A literature review was performed based on PubMed and Google Scholar databases (up to 31 December 2024), with no restrictions on publication date. The search terms used were: 'pertussis vaccination', 'COVID-19 vaccination', and 'influenza vaccination'. Studies in animal and human models on the efficacy of intranasal vaccination were included.
Brief description of the state of knowledge. Intranasal preparations against influenza, pertussis and COVID-19 show great potential. The use of hydrogel substances can prolong the presence of the antigen in the nasal cavity, promoting the development of durable immunity. BPZE1 is a pertussis vaccine with good tolerability and efficacy in clinical trials. It has also been shown that the combination of intranasal and systemic vaccines can enhance the immune response.
Summary. Intranasal vaccines are effective in inducing mucosal and systemic immune responses against pertussis, influenza and SARS-CoV-2. The results obtained suggest that they may become an important tool for the prevention of respiratory diseases and further clinical trials are required.

Key words

influenza, pertussis, vaccinology, COVID-19, intranasal vaccines

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Received: 23.04.2025; accepted: 17.10.2025; first published:29.10.2025

Streszczenie

Wprowadzenie i cel pracy. Choroby układu oddechowego, takie jak grypa, COVID-19 czy krztusiec, stanowią istotne zagrożenie dla zdrowia publicznego, szczególnie dla osób z grup ryzyka, takich jak osoby starsze i przewlekle chore. Celem niniejszego artykułu jest ocena potencjału szczepionek donosowych jako nowoczesnej, bezpiecznej i obiecującej alternatywy dla klasycznych metod immunizacji, zwłaszcza w kontekście zapobiegania rozprzestrzenianiu się chorób zakaźnych.
Metody przeglądu. Przegląd literatury oparto na bazach PubMed i Google Scholar (do 31 grudnia 2024 roku), bez ograniczeń co do daty publikacji. Wykorzystano hasła: „szczepienie przeciwko krztuścowi", „szczepienie przeciwko COVID-19" oraz „szczepienie przeciwko grypie". Uwzględniono badania na modelach zwierzęcych i ludzkich dotyczące skuteczności szczepień donosowych.
Opis stanu wiedzy. Donosowe preparaty przeciwko grypie, krztuścowi i COVID-19 wykazują duży potencjał. Zawarte w nich substancje hydrożelowe mogą wydłużać obecność antygenu w jamie nosowej i wspomagać rozwój pamięci immunologicznej. BPZE1 to szczepionka przeciw krztuścowi, która cechuje się dobrą tolerancją i skutecznością. Wskazuje się również, że połączenie szczepień donosowych z ogólnoustrojowymi może nasilać odpowiedź odpornościową.
Podsumowanie. Szczepionki donosowe są skuteczne w indukowaniu śluzówkowej i ogólnoustrojowej odpowiedzi immunologicznej przeciwko krztuścowi, grypie i SARS-CoV-2. Obecne wyniki sugerują, że mogą one stać się ważnym narzędziem zapobiegania chorobom układu oddechowego i wymagają przeprowadzenia dalszych badań klinicznych.

Słowa kluczowe

grypa, wakcynologia, COVID-19, krztusiec, szczepionki donosowe

INTRODUCTION

The earliest documented attempts at variolation occurred in India and China in the 16th century, which involved the intranasal administration of smallpox pustular material or scabs. But it was not until the turn of the 18th and 19th centuries that vaccination against smallpox (Variola vera) first appeared. Mass vaccination activities in England included such personalities as Robert Sutton and the surgeon Edward Jenner (1749–1823), who performed the first experimental vaccination against smallpox [1]. Subsequently, attenuated vaccines were developed by researchers such as Louis Pasteur (1822–1895), Albert Calmette (1863–1933), and Camille Guérin (1872–1961), followed by inactivated vaccines, pioneered by Daniel Elmer Salmon (1850–1914) and Theobald Smith (1859–1934) in the United States [2]. The first inactivated viral vaccine was the influenza vaccine [3]. Currently, vaccination remains the most effective and long-term preventive strategy against severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) and other viral respiratory infections, which contribute to severe health complications and have the potential for rapid human-to-human transmission [4].

In 2014, the World Health Organization (WHO) recorded 139,786 cases of pertussis [5,6], and in the United States, between 2011 and 2015, 15,942 patients were hospitalized for the disease [7]. *Bordetella pertussis* is a leading cause of vaccine-preventable death [8], particularly among children, the elderly, and immunocompromised individuals [3]. The first acellular pertussis vaccine was developed in 1981 [3], and efforts are being made to eradicate *Bordetella pertussis* from the human population [10]. In 2004, the live attenuated influenza vaccine, FluMist, was officially approved [12], while currently, two types of vaccines are in use – whole-cell vaccines (wPVs) and acellular vaccines (aPVs) [9]. However, there is still an urgent need for further vaccine development [10,11].

In 2009, the H1N1 influenza virus pandemic (A subtype) presented a significant challenge for scientists, and to achieve population-level immunity as quickly as possible, work on developing vaccines began almost immediately. In June, the WHO declared a pandemic and by September the first vaccine preparations were available in both Australia and the United States. However, access to these vaccines was primarily limited to residents of highly developed countries, and the number of immunized individuals significantly increased with the introduction of a single-dose vaccine [13]. Based on the experience with the H1N1 virus, there was the opportunity to develop effective vaccines for new strains of the influenza virus, and progress was made possible by the development of live and recombinant vaccines, as well as those derived from cell culture-based platforms [13,14].

Worldwide, Over 600 million people have been affected by Coronavirus Disease 2019 (COVID-19) [15], caused by the SARS-CoV-2, especially among high-risk groups, including the elderly and individuals with diabetes, severe asthma, and other underlying conditions. Additionally, higher mortality risks have been observed among Black individuals and those of South Asian descent [16]. The development of the first COVID-19 vaccine began on 10 January 2020, following the release of the SARS-CoV-2 genetic sequence by the Chinese CentRe for Disease Control and Prevention. Research on the BNT162b2 vaccine, the technical name for the Pfizer-BioNTech COVID-19 vaccine, developed using mRNA technology. The vaccine contained lipid nanoparticles encoding the SARS-CoV-2 spike protein, and when administered in a two-dose regimen demonstrated a 95% efficacy in individuals aged 16 and older, which consisted of the administration of the first dose (primary dose) followed by a second – booster – dose 21 days later to enhance the immune response [17].

Currently, vaccination remains the most effective and long-term method of prevention against SARS-CoV-2, which causes severe health complications and spreads rapidly within the population [4]. However, despite the high efficacy of mRNA-based systemic vaccines against COVID-19 in preventing severe cases of the disease, there is a need for research into intranasal vaccines to induce stronger mucosal immunity, and provide increasingly better protection against new viral variants, such as Omicron [18]. Intranasal vaccines represent an innovative prophylactic approach to respiratory diseases, offering several practical and immunological advantages. Their non-invasive, needle-free administration improves patient compliance, particularly among children and individuals with needle phobia, while also reducing the risks of needle-associated injuries and lowering the need for trained healthcare personnel. These formulations are associated with relatively low production costs, can be easily applied in large-scale immunization campaigns, and are considered to have a low risk of severe adverse events. Importantly, they stimulate both systemic and mucosal immunity, providing a first line of defence at the entry site of respiratory pathogens and limiting pathogen transmission. Nevertheless, their efficacy may be compromised by physiological barriers, including rapid clearance due to mucociliary transport, and the limited permeability of the nasal mucosa [19,20].

OBJECTIVE

The aim of this study was to review the current state of research on intranasal vaccines against pertussis, influenza, and COVID-19, as well as to assess their potential advantages and associated challenges.

MATERIALS AND METHOD

The literature review in this study was conducted in five main phases.

1) **Formulation of the research question** – 'What is known about intranasal vaccines against *Bordetella pertussis,* SARS-CoV-2, and influenza virus?' With a broad scope to capture various aspects of the topic.
2) **Identification of relevant studies.** English-language studies in the PubMed and Google Scholar databases were searched using key terms, such as: 'pertussis vaccination', 'COVID-19 vaccination', and 'influenza vaccination'. Additionally, the reference lists of relevant review and original research articles were reviewed. Studies were included without restrictions on the initial publication date, up to 31 December 2024.
3) **Study selection. B**oth animal and human studies were included, provided they contained data on the efficacy of intranasal vaccination against pertussis, influenza, or

COVID-19. The selection process was conducted by the first and senior authors over a three-week period.

4) **Data extraction.** Key study parameters were extracted, including disease type, intervention details, and primary outcomes.
5) **Synthesis, summarization, and reporting of results.** The collected data were thematically organized into predefined categories: 1) Intranasal pertussis vaccination, 2) Intranasal influenza vaccination, and 3) Intranasal COVID-19 vaccination. Additionally, a brief historical background of vaccination was included in the introduction to each subsection.

STATE OF KNOWLEDGE

Intranasal pertussis vaccine. *Bordetella pertussis* is a small, aerobic, Gram-negative bacillus responsible for causing pertussis (whooping cough) [5,14] which, despite high global vaccination coverage, remains a significant public health concern [5,21,22]. The rising number of reported cases is likely attributable to molecular adaptations of the pathogen, improved diagnostic capabilities, and reduced vaccine efficacy due to the declining vaccination uptake [5]. Moreover, *B. pertussis* colonization may be asymptomatic and can contribute to natural immunization [8]. The classical clinical course of pertussis occurs primarily in unvaccinated children and progresses through three distinct stages: catarrhal, paroxysmal, and convalescent [23,24]. The illness typically lasts 6–12 weeks [5]. Current treatment includes azithromycin, which eliminates bacterial colonization within 48 hours in 88% of cases [8]. Clarithromycin is also recommended due to comparable efficacy and improved tolerability compared with erythromycin [11], while cotrimoxazole may be used in patients with macrolide intolerance [25].

In many countries, the acellular pertussis (aP) vaccine is an integral part of immunization schedules. However, its efficacy is lower than that of the whole-cell pertussis (wP) vaccine, which was widely used between the 1940s – 1980s. As a result, infants, young children, and immunocompromised individuals remain at a significantly higher risk of infection [10]. The limitations of both acellular (aPV) and whole-cell (wPV) pertussis vaccines in preventing infection and transmission have created a need to investigate intranasal pertussis vaccines, such as BPZE1 – a live attenuated intranasal pertussis vaccine [9].

In 2008, the construction of the BPZE1 vaccine was described by Prof. Locht et al., as being created through genetic inactivation or deletion of three toxins: tracheal cytotoxin, dermonecrotic toxin, and pertussis toxin [9,22]. It was designed to prevent *B. pertussis* infections, [11,21,26,27,28,29] mimicking the natural route of infection [26], and providing protection against nasal colonization and bacterial transmission [10,28]. As a part of further studies Feunou et al. conducted a study evaluating the efficacy of BPZE1 in protecting against pertussis. The experiment was performed on three-week-old and eight-week-old female Balb/C mice which received 10^6 colony-forming units (CFU) of BPZE1 in 20 µl of phosphate-buffered saline (PBS), applied to the tip of the nostrils. Eight weeks post-immunization, the mice were challenged intranasally with *B. pertussis* and euthanized after seven days. Lung homogenates were subsequently plated on agar. In all vaccinated mice, *B. pertussis* viable cell counts were undetectable, whereas the control group (unvaccinated mice) exhibited *B. pertussis* loads of 7 log10 CFU. These findings demonstrated that a single dose of BPZE1 conferred complete protection against pertussis in mice. Genetic analysis of lung-derived bacterial material confirmed the genomic stability of BPZE1 throughout the study [22].

Ten years later Soland et al. investigated the immune mechanism induced by BPZE1 in eight mice, comparing it to acellular pertussis vaccines (aPVs). The study demonstrated long-term protection against nasal colonization following BPZE1 immunization, despite the rapid decline of *B. pertussis*-specific secretory IgA (SIgA) in the nasal cavity. This protection is likely attributed to the generation of $CD4^+$ tissue-resident memory T cells, which produce interleukin-17 (IL-17), a key cytokine involved in SIgA secretion [26].

The first clinical trial of a live attenuated intranasal *Bordetella pertussis* vaccine was conducted in 48 male volunteers by Thorstensson et al. in Sweden. This was a placebo-controlled, dose-escalation study of the BPZE1 vaccine efficacy. Twelve participants received either 10^3, or 10^5, or 10^7 colony-forming units (CFU) in droplet form, with half the dose administered into each nostril. *B. pertussis* colonization in the nasopharynx was observed in one participant in the low-dose group, one in the medium-dose group, and five in the high-dose group. All colonized individuals exhibited a significant immune response against pertussis antigens. Colonization frequency was significantly higher in the high-dose group compared to the low- and medium-dose groups ($p=0.029$). Additionally, a significant increase in IgG antibody levels against pertactin was observed both on day 28 ($p=0.018$) and at 5–6 months post-vaccination ($p=0.001$), as well as against fimbriae on day 28 ($p=0.015$) and at 5–6 months ($p=0.006$) [27].

An additional immunological analysis of the same cohort, published later in 2014 by Jahnmatz et al., extended these observations by focusing on B-cell responses. The double-blind, randomized, placebo-controlled clinical trial was conducted to assess the safety, colonization, and immunogenicity of the BPZE1 vaccine. The study involved 48 participants who were previously unvaccinated against pertussis. Colonization with the live attenuated *B. pertussis* strain was observed in seven out of 36 individuals who received the BPZE1 vaccine, along with an increase in the B-cell response ($p<0.05$) to three tested antigens: pertussis toxin, filamentous hemagglutinin, and pertactin, between day 0 and day 28 [30].

In 2020, again in Sweden, building on these initial findings, a subsequent phase 1b trial was reported by Jahnmatz et al. The next double-blind, randomized, placebo-controlled clinical trial was conducted on 48 healthy individuals aged 18–32 years. Both BPZE1 and placebo were administered at a dose of 0.4 mL to each nostril, and nasal colonization was observed in 29 out of 36 vaccinated participants. A rise in IgA and IgG antibody titers against four *B. pertussis* antigens was observed from baseline to 12 months post-vaccination, with memory B-cell responses specific to pertussis decreasing between months five and six [11].

In a following study, Keech et al. conducted a randomized, double-blind clinical trial to compare the efficacy of the BPZE1 vaccine with the tetanus-diphtheria-acellular pertussis (Tdap) vaccine. A total of 300 healthy adults aged 18–50 were assigned to one of four groups: 1) BPZE1

vaccination followed by attenuated BPZE1 challenge, 2) BPZE1 vaccination followed by placebo challenge, 3) Tdap vaccination followed by attenuated BPZE1 challenge, and 4) Tdap vaccination followed by placebo challenge. Participants received 0.4 mL of lyophilized BPZE1, dissolved in sterile water, administered intranasally (0.2 mL per nostril), along with an intramuscular placebo (saline). Those who received the intramuscular Tdap vaccine were administered a lyophilized intranasal placebo solution. BPZE1 induced systemic anti-pertussis toxin, anti-filamentous haemagglutinin, and anti-pertactin IgA and IgG responses, as well as anti-whole-cell extract IgG responses, with a balanced IgA-to-IgG profile. Tdap vaccination generated higher IgG responses; however, antibody decay was slower following BPZE1 vaccination. By the end of the study, serum *B. pertussis*-specific antibody levels were comparable between participants who received Tdap and those who received BPZE1. That was proofed that BPZE1 are well-tolerated [21].

In the study by Thorstensson et al., the immediate adverse effects within six hours of vaccination were minor and transient, with no significant differences between the placebo group and the groups receiving different doses. The most common adverse effects were rhinitis, sneezing, and coughing [27]. In studies conducted by Jahnmatz et al. [11], Keech et al. [21], and Thorstensson et al. [27], no serious adverse effects, such as spasmodic cough, difficulty breathing, or vital sign abnormalities, were observed [11,21,27]. Adverse events reported after BPZE1 vaccination are summarized in Table 1.

Although these early-phase studies involved small cohorts, they are particularly informative as they provide the first human evidence of BPZE1 safety, colonization, and immunogenicity. These findings lay the groundwork for subsequent trials and remain crucial for understanding the potential of the vaccine in larger populations.

Building on the development of the live attenuated pertussis BPZE1 vaccine, researchers have engineered a modified version – BPZE1P, which lacks pertactin, a key protective antigen present in most pertussis vaccines. Belcher et al. demonstrated the efficacy of BPZE1P in preventing and treating allergic airway inflammation (AAI) induced by exposure to the house dust mite (HDM) in a murine model. BPZE1P was administered intranasally at a dose of 10^6 CFU either before or after HDM sensitization, followed by subsequent HDM exposure. Vaccination prior to exposure resulted in a reduction of eosinophil infiltration and pro-inflammatory cytokines (IL-1α, IL-1β, IL-33) in the lungs, as well as decreased production of HDM-specific IgG1 antibodies. BPZE1P provided protective effects even when administered after HDM exposure or between two exposure episodes [29].

Intranasal vaccines have also been tested in dogs in the context of *Bordetella bronchiseptica* infection. The results showed that puppies vaccinated intranasally experienced fewer adverse symptoms, such as sneezing, coughing, nasal discharge, and vomiting reflexes, compared to the control group of unvaccinated puppies [31].

Aibani et al. developed an acellular pertussis vaccine containing pertactin, pertussis toxin, fimbrial antigens 2/3, and a triple adjuvant system which enhances the immunogenicity of the vaccine. The antigens were incorporated according to two strategies: 1) bound to the lipid component of the adjuvants through electrostatic attraction, or 2) enclosed within a lipid nanoparticle. After intranasal administration of the vaccine with lipid nanoparticles, a Th1-type immune response was induced, with elevated IgG2a and IgA antibody titers in serum. Additionally, four weeks after a single vaccine dose, high SIgA antibody titers were observed in the nasal mucosa. Nanoparticles in strategy (1) resulted in higher changes in serum antibody levels [32].

A study by Galeas-Pena et al. demonstrated the efficacy of intranasal immunization with the aP-T-vant vaccine – comprising acellular pertussis antigens and the T-vant adjuvant derived from bacterial outer membrane vesicles – in clearing *B. pertussis* from both the lungs and nasopharynx. Nasopharyngeal immunity was IL-17 dependent, while protection in both the lungs and nasopharynx was associated with IFN-γ, IL-17, and $CD4^+$ T cells [10].

Table 1. Adverse events reported following BPZE1 vaccination

Number of participants (n)	Adverse Events	Country of study	Year of study	Authors
300	stuffy nose or congestion, runny nose, sneezing, headache, fatigue	USA	2019–2023	Keech et al. [21]
48	cough, sneezing, sore throat, rhinorrhoea, nasal congestion, headache, fatigue; common cold	Sweden	2015–2016	Jahnmatz et al. [11]
48	cough, nasal congestion, mild epistaxis, moderate rhinorrhoea, sneezing, mild ear and eye pain, mild dyspnoea, tiredness, headache, pyrexia	Sweden	2013	Thorstensson et al. [27]

Intranasal influenza vaccine. The influenza virus is one of the most pathogenic airborne viruses, responsible for respiratory infections and seasonal epidemics, causing up to five million severe cases worldwide each year. The high-risk group for infection, severe disease progression and complications, includes patients with chronic conditions, children under the age of five, individuals over 65, and pregnant women. It is estimated that approximately 650,000 cases, or about 10% of infections, result in death. Despite decades of research on this pathogen and the introduction of anti-viral drugs – such as neuraminidase inhibitors – for severe cases, influenza control remains a major challenge for scientists and healthcare systems worldwide. Vaccination programmes and the continuous development of new, effective immunogenic formulations play a crucial role in reducing morbidity [33]. According to WHO recommendations, annual influenza vaccination should primarily be administered to individuals at high risk of complications, as well as their household members and caregivers [34].

The first inactivated vaccines designed to protect humans from influenza were developed in the late 1930s. Initially, the virus was cultivated in mouse lungs and chicken embryos. Subsequently, research efforts shifted toward stimulating a stronger immune response and determining appropriate dosage levels using *in vitro* assays based on haemagglutination of chicken red blood cells. During the 1976 trials for 'swine flu' vaccines, testing methods were refined, leading to the adoption of the single radial immunodiffusion (SRID) assay by the WHO in 1978 as the most reproducible method. This

assay remained the standard for influenza vaccine potency testing until modern times [35].

In 2013, the quadrivalent intranasal influenza vaccine Fluenz Tetra was introduced to the European Union market, intended for children and adolescents aged 24 months to less than 18 years [36]. In Poland, it has been in use since 2019.

In the United Kingdom, Turner et al. conducted a post-registration study to evaluate the immunogenicity of a single dose of the Fluenz Tetra vaccine, a live attenuated influenza vaccine (LAIV) protecting against four viral strains. The study analyzed the local immunoglobulin A (IgA) response following vaccine administration in children aged 2–17 years. The results demonstrated a statistically significant increase in nasal IgA titers against the H3N2 virus and type B virus from the Brisbane genetic lineage (Phu line). However, no significant change was observed in response to the H1N1 virus. The findings, expressed as the mean fold increase in antigen-specific IgA titers as a percentage of total nasal IgA, were as follows: for H3N2 – 2.3, which was significantly higher than the fold increase for H1N1 – 1.0 ($p<0.001$) and for type B (Phu line) – 1.4 ($p=0.0048$). These results indicate that intranasal vaccines can induce an IgA response, but this response depends on the specific viral strains included in the formulation [20].

Cole et al. conducted a study in the United Kingdom on children aged 6–14 years, demonstrating that pre-existing immunity from prior influenza infection does not inhibit the immune response to LAIV vaccination. No correlation was found between baseline levels of influenza-specific immunoglobulin A (IgA) in nasal tissues and the increase in immunoglobulin G (IgG) levels specific to H1N1 and H3N2 viruses. Additionally, prior viral upper respiratory tract infections, which are common in children during vaccination periods, did not affect the immunogenicity of the live attenuated influenza vaccine. These findings support the use of LAIV as a recommended formulation for annual influenza vaccinations [37].

The efficacy of the live attenuated influenza vaccine (LAIV) Fluen Tetra was also investigated by Thwaites et al. The study included 40 adult participants aged 19–29 years, who underwent nasal swabs and blood sampling before and after vaccination to assess immune system activation. It should be noted that the relatively small number of participants limits the statistical weight of the findings. Participants received a quadrivalent vaccine containing attenuated influenza A and B viruses. Results showed that interferon lambda (IFN-λ) levels increased 72 hours post-vaccination compared to baseline, and remained elevated for up to 168 hours (both $p < 0.05$). Additionally, the IFN-induced chemokine CXCL10 and interleukin-6 were significantly elevated 72 hours post-vaccination (both $p<0.05$). However, the modest antibody response in blood samples did not fully reflect the protective effect of vaccination. It is likely that the desired immune response to LAIV, despite not being prominently detected in blood analyses, originated primarily from antibody production localized within the nasal mucosa [38].

In mice, Jeong et al. described the effects of a nanoparticle-based vaccine (NanoVac) with photochemical immunomodulation, composed of a photoactivatable polymeric adjuvant and a high-molecular-weight antigen – influenza haemagglutinin protein. The nanoformulation was designed to extend the retention time of antigens in the oral cavity. Additionally, photochemical activation stimulated humoral immune responses, dendritic cell maturation, and immunoglobulin A and G production. Increased immune response levels were observed in mice administered the vaccine, with laser irradiation enhancing its effects compared to HA-free vaccines. Interleukin-2 (IL-2) levels were 2.2 times higher than in the group without influenza HA. Furthermore, cytokines CCL3 and CCL4 increased by 5% and 20%, respectively, in HA-NanoVac-treated animals subjected to laser irradiation, compared to the free HA group. The number of mature dendritic cells (DCs), essential for effective vaccination, increased up to nine-fold compared to the HA-NanoVac group without irradiation, and threefold compared to mice immunized without laser exposure.

These findings indicate that this vaccine strategy enhances DC maturation and effectively induces immune responses in mice. The study demonstrated that combining NanoVac with photochemical activation effectively protected mice from influenza infection and could serve as a promising defence against pathogenic viruses, including influenza and SARS-CoV-2 [39]. NanoVac has not been tested in human clinical trials. The current research is limited to preclinical studies in animal models. There is no publicly available information regarding plans for human trials or the anticipated timeline for such studies. Regarding safety, while the preclinical data suggest promising immunogenicity and efficacy, the safety profile in humans remains unknown until clinical trials are conducted.

One method to enhance the efficacy of intranasal vaccines is prolonging antigen retention in the nasal cavity, ensuring extended contact with epithelial tissues. To achieve this, Australian researchers incorporated chitosan, a natural polymer that forms covalent bonds with the mucosa and transforms into a hydrogel upon temperature increase, such as upon entering the body. Experiments in mice revealed that in those receiving the gel-forming vaccine, most antigens remained in the nasal tissue, whereas in the control group (receiving a non-gelling formulation), antigens primarily localized in lung tissue. Moreover, prolonged antigen retention in the nasal mucosa correlated with an increased number of influenza-specific tissue-resident memory CD8+ T cells, which provide protection against influenza infection. This formulation may be a crucial approach for enhancing localized immune responses [19]. Figure 1 presents a comparison of the mechanisms and efficacy of intranasal influenza vaccines.

Seasonal influenza vaccination, including intranasal formulations, must be administered annually due to the continuous antigenic drift of influenza viruses. The composition of each year's vaccine is determined by the WHO through its Global Influenza Surveillance and Response System (GISRS), which conducts biannual consultations and employs predictive modelling to indicate the strains most likely to circulate in the upcoming season [40].

Intranasal influenza vaccines, including live attenuated formulations such as Fluenz Tetra and novel nanoparticle-based approaches like NanoVac, demonstrate the potential to induce localized mucosal immune responses and systemic immunity. Intranasal vaccination represents a promising strategy to improve influenza protection, particularly in high-risk populations, but further clinical evaluation is needed to confirm safety and effectiveness.

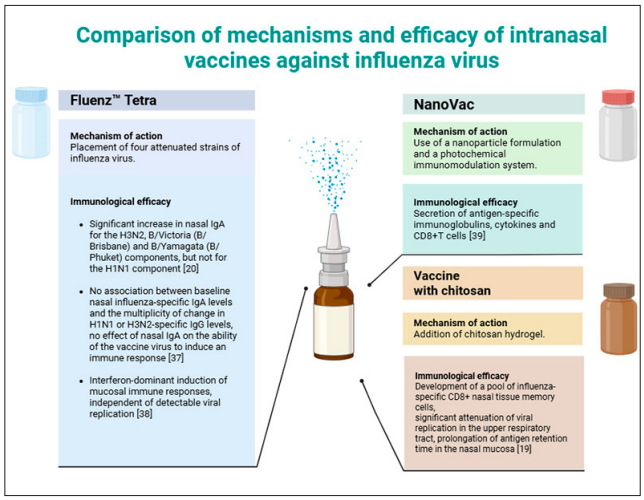

Figure 1. Comparison of mechanisms and efficacy of intranasal vaccines against the influenza virus

Intranasal COVID-19 vaccine. SARS-CoV-2 is an enveloped virus belonging to the *Coronaviridae* family, capable of infecting human cells through specific interactions with cellular receptors. The spike (S) protein plays a critical role in the infection process, enabling binding to angiotensin-converting enzyme 2 (ACE2) receptor and activation by serine protease. SARS-CoV-2 transmission primarily occurs via respiratory droplets containing viral particles, which are expelled through respiratory secretions. Infection can result from close contact with an infected individual or through viral transfer from contaminated surfaces and objects to the mucous membranes of the nose, mouth, or eyes [41]. SARS-CoV-2 poses a particular risk to elderly individuals and those with chronic illnesses. As of 3 October 2023, it has caused 676,609,955 infections and 6,881,955 deaths [15].

Several vaccines against COVID-19 are currently in use, each employing distinct technologies to induce immunity. The first group comprises mRNA vaccines which deliver messenger RNA (mRNA) encoding the SARS-CoV-2 spike (S) protein. Once inside host cells, the mRNA is translated into the viral protein, subsequently degraded, and does not enter the nucleus or alter the human genome [42]. The two mRNA vaccines currently authorized and marketed in the European Union are Comirnaty (BNT162b2, Pfizer-BioNTech) [17] and Spikevax (mRNA-1273, Moderna) [42]. The second group consists of viral vector vaccines, which use replication-deficient adenoviruses to deliver the gene encoding the spike protein. Examples include Vaxzevria (AstraZeneca) [43] and COVID-19 Vaccine Janssen (Johnson & Johnson) [44]. The next group is represented by the protein subunit vaccine Nuvaxovid (NVX-CoV2373, Novavax), which contains recombinant spike protein formulated with the Matrix-M adjuvant. In May 2025, the vaccine received full FDA approval as the first protein-based COVID-19 vaccine in the USA [45].

Beyond conventional technologies, novel approaches are represented by the self-amplifying RNA (saRNA) vaccine Zapomeran (ARCT-154, Arcturus/CSL) [46] and by intranasal vaccines [18,47,48]. Zapomeran utilizes an alphavirus replicase to enhance antigen expression, thereby eliciting stronger immune responses at lower doses. Finally, intranasal vaccines are being actively investigated as a strategy to induce mucosal immunity. Their current development and future perspectives will be outlined in the subsequent section of the current review, which represents the central focus of this article. Although vaccines constitute the primary means of preventing COVID-19, effective treatments have also been critical in managing established infections. Remdesivir, a nucleotide analog that inhibits viral RNA polymerase – an essential enzyme for viral replication – has been utilized in the treatment of SARS-CoV-2 infections. Remdesivir was approved for use in adults and children aged 12 years and older, weighing at least 40 kg, in the European Economic Area (3 July 2020), Canada (27 July 2020), and the United States (22 October 2020) [49].

A study conducted by Tang et al. demonstrated that the intranasal Ad5-S vaccine (adenovirus type 5 encoding the S protein), in combination with a systemic mRNA vaccine, significantly enhances the mucosal immune response in the respiratory tract. Female C57BL/6 mice, aged 8–10 weeks, were immunized with the mRNA-S vaccine encoding the full-length SARS-CoV-2 spike protein at a dose of 1 μg, administered intramuscularly in either a single or two-dose regimen with a 21-day interval. After an additional 21 days, a booster dose was administered in different forms: an intranasal Ad5-S vaccine at 10^9 plaque-forming units (pfu) in a 30 μL volume, an additional intramuscular mRNA-S dose (1 μg), or a nasal trimeric S protein (3 μg) adjuvanted with 10 μg of 2'3'-cGAMP (a cyclic di-GMP-AMP immune system stimulator). Control groups consisted of mice receiving three doses of PBS. The mRNA plus Ad5-S vaccine combination increased IgA levels in bronchoalveolar lavage (BAL) fluid by nearly 1,000-fold ($p<0.001$), compared to the mRNA vaccine alone and improved neutralization of the Delta and Omicron BA.1.1 variants ($p<0.01$) [18]. The intranasal vaccine may also reduce the extent of lung damage in individuals infected with the Beta and Omicron variants ($p<0.01$).

A study by Chen et al. demonstrated a significant increase in the levels of IgA and IgG in bronchoalveolar lavage (BAL) in a group that received the dNS1-RBD vaccine (an intranasal vaccine based on a flu vector created by inserting the gene encoding the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2) compared to the viral vector CA04-dNS1 ($p=0.0022$). Moreover, the intranasal dNS1-RBD vaccine induced a 22-fold stronger local cellular response in the respiratory tract, compared to peripheral blood mononuclear cells (PBMCs). Immunization with dNS1-RBD within one day led to a statistically significant increase in the following pro-inflammatory cytokines in the lungs: IL-6 (interleukin 6) ($p=0.0017$), IL-1α (interleukin 1-alpha) ($p=0.002$), IFN-γ (interferon gamma) ($p<0.0001$), IFN-α (interferon alpha) ($p<0.0001$), MCP-1 (monocyte chemoattractant protein 1) ($p<0.0001$), IP-10 (interferon gamma-induced protein 10) ($p<0.0001$), MIP-1β (macrophage inflammatory protein 1 beta) ($p=0.0033$), GM-CSF (granulocyte-macrophage colony-stimulating factor) ($p<0.0001$), and TNF-α (tumor necrosis factor alpha) ($p<0.0001$), indicating rapid activation of innate immune mechanisms in the airways against SARS-CoV-2, which were suppressed on the third and fifth days, preventing further cytokine level rise and the occurrence of a cytokine storm [47].

The safety of this platform was also assessed in BALB/c mice and ferrets, with doses of 50 and 500 μL of dNS1-RBD or CA04-WT, a wild-type A/California/04/2009 strain. Factors such as weight loss, clinical observations, and body temperature were assessed. The results indicated that the

dNS1-RBD vaccine did not cause significant side-effects, such as weight loss, fever, or visible clinical signs of infection in either BALB/c mice or ferrets. The thermosensitive properties of the vaccine limited its replication to 33°C and significantly reduced its replication efficiency at temperatures of 37°C and 39°C, thus decreasing the risk of lung damage. Histopathological studies showed no visible changes in the lungs of animals vaccinated with dNS1-RBD compared to control groups receiving the wild-type strain CA04-WT. These findings suggest that the vaccine exhibits a high safety profile, which is crucial for its application in clinical practice. In contrast to these findings, a clinical trial of the intranasal dNS1-RBD vaccine demonstrated a weaker cellular and mucosal immune response. In phase one, 63 participants, divided into two age groups (18–59 and ≥60), received an initial and booster dose on days 0 and 14, respectively. The primary objective of phase one was to assess local and systemic adverse effects over a 30-day period. In phase two, 724 participants were vaccinated on days 0 and 21. The study showed an increase in T-cell activity responsible for viral clearance in 46% of participants in phase two and 40% in the extended phase, compared to the placebo group ($p<0.0001$). Furthermore, the vaccine was confirmed to be safe, with no life-threatening adverse events reported. An increase in IgG antibody levels was observed in 10% of participants, while an elevation in s-IgA antibodies, indicating a mucosal immune response, was noted in 12% of participants [48].

Additionally, Tang et al. reported that the activation of B lymphocytes and CD8+ and CD4+ T cells, measured based on cytokine production such as interferon gamma in bronchoalveolar lavage fluid, was significantly stronger after using the mRNA vaccine in combination with the intranasal Ad5-S vaccine (three log units higher) compared to the mRNA vaccine alone ($p<0.001$) [18]. These results indicate that the use of the intranasal vaccine in combination with the systemic vaccine significantly improves the immune response at the mucosal and pulmonary levels in both animal and human models [18,47]. However, due to the small sample size in the study, no definitive conclusions can be drawn about the combined use of the intranasal and systemic vaccines, suggesting the need for further research.

Beyond viral vector–based vaccines, other intranasal agents have also been investigated. Similarly to the intranasal vaccines Ad5-S and dNS1-RBD, astodrimer sodium – an intranasal agent whose mechanism is based on the direct blockade of viruses rather than activation of the immune response – has proven effective in reducing viral load in the respiratory tract. The action of this preparation involves electrostatic binding of the viral spike proteins, preventing the attachment of virions to ACE2 receptors in epithelial cells. Clinical studies showed that using a 1% nasal spray four times a day in a dose of 100 µL per nostril effectively reduced SARS-CoV-2 replication in the nasal mucosa without detectable systemic absorption ($p<0.01$) [50].

In parallel, novel viral vectors are being explored. Compared to intranasal Ad5-S and dNS1-RBD vaccines, a vaccine based on MPV (mouse pneumonia virus) used as a vector for delivering the SARS-CoV-2 spike (S) protein gene may effectively enhance the mucosal immune response, making it a promising candidate in the fight against SARS-CoV-2. Rhesus macaques (three groups of four individuals each) were administered vaccines at a dose of 6.3 log10 PFU for each of the tested viral vectors (MPV, MPV/S, and MPV/S-2P). The vaccines were delivered both intranasally (IN) and intratracheally (IT). The control group received an empty MPV vector without SARS-CoV-2 protein expression [27].

Building on these preclinical findings, translation into clinical research is underway. In August 2024, the United States National Institutes of Health (NIH) announced the initiation of enrollment of healthy participants for a Phase I clinical trial evaluating the safety of the intranasal MPV/S-2P vaccine in humans. The trial aims to enroll 60 adult participants between 18–64 years of age who have previously received at least three doses of an mRNA-based COVID-19 vaccine [51].

CONCLUSIONS

Current studies indicate the immunogenic success of intranasal vaccines in humans. These preparations seem promising in terms of their ability to induce a strong immune response, not only at the mucosal level, but also systemically. This raises hope for better protection against respiratory diseases. Research on intranasally administered vaccines against Bordetella pertussis, influenza virus and SARS-CoV-2, highlights the impact of the vaccine components and technologies used on the immune system. Progress in this area, coupled with the potential advantages of intranasal vaccines, underscore the need to further evaluate their clinical utility.

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