Draft Memo:

Intranasal SARS-Cov-2 boosters

Content

Executive Summary

In the battle against SARS-CoV-2, vaccines have proven to be the most effective public health intervention for protecting people against the virus. So far, 66.8% of the world population has received at least one dose of a COVID-19 vaccine, with 12.15 billion doses having been administered globally[1]. As a result, it has been estimated that almost 20 million COVID-19 deaths have been averted worldwide in their first year of use[2].

Nevertheless, the persisting emergence of highly transmissible variants has led to a reconsideration of the current vaccination strategy against SARS-CoV-2. The initial priority of vaccination programmes was to reduce disease severity and morbidity. However, this focus is now shifting towards fighting future variants, which could be supported by halting infection and transmission altogether.

Current SARS-CoV-2 vaccines (almost all[3] of which are administered as intramuscular injections) induce systemic immunity but insufficient mucosal immunity in the upper respiratory tract - the first point of entry for the virus. In light of this, vaccine developers are turning to intranasal vaccines, in the hope that they can serve as superior delivery models for boosters, with the potential to reduce infection and slow down transmission as variants emerge. At the point of writing, 73 intranasal vaccines are in development worldwide, with 13 of those in the middle of clinical trials.

This memo provides an overview of intranasal SARS-CoV-2 vaccines currently in development and evaluates their potential use as boosters for reducing infection and transmission. It identifies the most likely candidates to receive authorisation within the next two years as well as the potential barriers for authorisation.

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[1] Our World in Data: https://ourworldindata.org/coronavirus#explore-the-global-situation
[2] (Watson, 2022)
https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(22)00320-6/fulltext
[3] Two intranasal vaccines have received emergency authorisation in Iran and Russia.

1. Is an intranasal vaccine strategy likely to be a better booster than an intramuscular vaccine?

A. How likely is an intranasal booster to produce sterilizing immunity?

Sterilizing immunity is the immune system's ability to completely prevent infection of host cells[4]. For sterilizing immunity to be achieved, neutralizing antibodies (NAb) must be triggered, which block the attachment of virus particles to host cells, thus preventing replication. For respiratory viruses which are transmitted via respiratory droplets and aerosols, such as SARS-CoV-2, NAb are required at the mucosal surfaces of the upper and lower respiratory tracts to prevent viral replication and thus achieve sterilizing immunity[5]. Immunoglobulin A (IgA) (also referred to as sIgA in its secretory form) is the most abundant NAb at mucosal sites (such as the lining of the respiratory tract and digestive system) and is therefore primarily associated with mucosal immunity, while immunoglobulin G (IgG) is primarily located in serum and most tissues, and elicits systemic immunity.

Intramuscular vaccines provide strong systemic immunity, however due to a lack of mucosal immunity, they are poor at controlling viral replication and nasal shedding in the upper respiratory tract, leading to asymptomatic or milder symptomatic infection that can still transmit virus to others[6]. Natural infection on the other hand leads to both systemic and mucosal immunity[7]. This is suggested in a preprint study by Krammer et al.[8] which shows that intramuscular vaccines elicit stronger mucosal immunity in individuals who have been previously infected by SARS-CoV-2 than in uninfected individuals. Vaccination induced only a weak mucosal sIgA response in individuals without pre-existing mucosal antibody responses to SARS-CoV-2 while SIgA induction after vaccination was efficient in COVID-19 survivors.

Unlike conventional intramuscular SARS-CoV-2 vaccines, intranasal vaccines are likely to have a superior advantage for sterilizing immunity because the nasal mucosa is often the initial site of infection. Moreover, the dimeric[9] nature of mucosal IgA may be particularly significant for achieving sterilizing immunity, since dimeric Ig are generally more effective than monomeric Ig (such as IgG) in terms of binding to cell surfaces.

A recent study by Sheikh-Mohammed et al.[10] found that participants who experienced breakthrough infections with SARS-CoV-2 variants following two doses of an mRNA vaccine, had lower levels of vaccine-induced serum anti-Spike/RBD IgA at 2–4 weeks post-dose 2 compared to participants who did not experience an infection, whereas IgG levels were comparable between groups. These data suggest that SARS-CoV-2 vaccines that elicit a durable IgA response may have utility in preventing infection.

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[4] In viral challenge experiments, sterilizing immunity is inferred if viral load is undetectable in the days following the challenge.
[5] (Fröberg & Diavatopoulos, 2021) https://pubmed.ncbi.nlm.nih.gov/33899752/
[6] For SARS-CoV-2, there is evidence that infection and replication occurs in the nasal ciliated cells, a site that is not accessible to serum IgG unless there is inflammatory damage to the mucosal tissues that allows transudation of serum proteins to the site. Hence, current vaccines for COVID-19 prevent disease but not infection.
[7] (Krammer, 2022) https://cdn.who.int/media/docs/default-source/blue-print/platforms_florian-krammer_whoconsulation_covid19framework_23feb2022.pdf?sfvrsn=f5a1e435_7
[8] (Krammer, 2021) https://www.medrxiv.org/content/10.1101/2021.12.06.21267352v1.full-text
[9] Dimeric IgA antibodies are formed by the covalent linkage of two individual IgA monomers. They are predominantly found in mucosal tissues, including the upper respiratory tract where SARS-CoV-2 is first encountered. (Wang, 2020)
[10] (Sheikh-Mohammed, 2022) https://www.nature.com/articles/s41385-022-00511-0

Preclinical studies for intranasal vaccines support this hypothesis, demonstrating that single doses of intranasal vaccines have the potential to elicit high neutralizing antibody generation and mucosal IgA and T cell responses that avoid SARS-CoV-2 infection in both the upper and lower respiratory tract. Results from preclinical trials for intranasal *boosters* have similar results, showing that a heterologous schedule (intramuscular prime followed by an intranasal boost) offers superior sterilizing immunity to homologous intramuscular schedules.

Study	Vaccine type	Method	Preprint	Results
Single dose intranasal studies				
Hassan, 2020	Chimpanzee adenovirus-vectored vaccine	Challenge study mice	No	A single intranasal dose of ChAd-SARS-CoV-2-S induces high levels of neutralizing antibodies, promotes systemic and mucosal immunoglobulin A (IgA) and T cell responses, and almost entirely prevents SARS-CoV-2 infection in both the upper and lower respiratory tracts.
Du, 2021	RBD subunit vaccine	Challenge study in mice	No	The vaccine elicited a robust systemic humoral immunity with high titers of IgG antibodies and neutralizing antibodies as well as a significant mucosal immunity.
Intranasal booster studies				
Shamseldin, 2022	BcfA adjuvanted subunit vaccine	Prime and pull method in mice	Yes	Systemic priming followed by a mucosal booster with a BcfA adjuvanted subunit vaccine provides sterilizing immunity against wildtype SARS-CoV-2, and a variant of concern.
Sui, 2021*	Adjuvanted subunit vaccines with spike protein S1	A comparison of two vaccine strategies: an IM primed/booster vaccine and an IM primed/IN boost mucosal vaccine in rhesus macaques	No	Following SARS-CoV-2 challenge, both strategies demonstrated full protection against viral replication, with the mucosal vaccine appearing more efficient at rapidly clearing the input virus (gRNA) in the upper respiratory tract than the systemic counterpart, providing a potent strategy for achieving sterilizing immunity.
Sui, 2021*	Adjuvanted subunit vaccines with spike protein S1	A follow-up study using a beta variant of the mucosal booster in rhesus macaques	Yes	The one-year beta variant mucosal booster given intranasally elicited high quality immune responses and mediated protections against subsequent SARS-CoV-2 beta variant viral challenge in rhesus macaques. Notably, the protection in the upper respiratory tract was better than in the lower respiratory tract, showing nearly full protection against viral replication in the nasal cavity which is

Study	Vaccine type	Method	Preprint	Results
Single dose intranasal studies				
				especially encouraging for its ability to induce sterilizing immunity.
Lapuente, 2021	Adenovirus vectored vaccine	Intranasal vaccinations with adenovirus 5 and 19a vectored vaccines following a systemic plasmid DNA or mRNA priming in mice	Yes	The intranasal booster resulted in systemic and mucosal immunity. Their heterologous prime-boost strategy led to complete protection against a SARS-CoV-2 infection in mice, with an increased levels of mucosal IgA and lung-resident memory T cells in contrast with two intramuscular vaccines.

While these preclinical studies demonstrate promising results, sterilizing immunity is yet to be demonstrated in humans at the clinical trial stage.

Variance between results from preclinical and clinical studies has already been seen in SARS-CoV-2 intranasal vaccine studies. Preclinical data for AdImmune's AdCovid showed highly promising results for the vaccine's potential to produce sterilizing immunity[11]. However, following Phase I data, Altimmune discontinued development of its SARS-CoV-2 vaccine since the vaccine did not elicit a sufficient immunological response immunity[12].

This variance could be the result of greater divergence between modelling intranasal administration in animal models and humans, compared with modelling intramuscular administration. Whereas the administration of intramuscular vaccination for mice and humans is fairly analogous, this is not the case for intranasal vaccines. For example, a completely different nasal spray device would be used for mice and for humans, which could vary the dosage administered. In addition, the anatomy of a mouse's nose could be more favourable for intrasanal immunisation than humans (or vice versa), which has significant implications for our interpretation of preclinical data and inferences based on animal models.

A second consideration is the potentially wider 'error window' for administering intranasal vaccines compared to intramuscular vaccines and its impact on the production of sterilizing immunity. In order to reliably prevent infection, administering an accurate dose of the vaccine is likely to be important yet difficult intranasally, due to 'nasal clearing' as a result of the natural defences of nasal cilia, sneezing and the dense mucus lining the respiratory tract. Intramuscular administration is a more accurate dosing strategy that does not face such challenges. This consideration is particularly important if intranasal vaccines are to be administered by non-professionals, at home, which increases the 'error window' even further.

Furthermore, the viral challenges of SARS-CoV-2 administered to animals with a high dose of virus via the intranasal route do not reflect realistic human exposure. Although the natural

[11] (King, 2020) https://www.biorxiv.org/content/10.1101/2020.10.10.331348v1

[12] https://ir.altimmune.com/news-releases/news-release-details/altimmune-announces-update-adcovidtm-phase-1-clinical-trial

dose one might encounter is much lower than that of animals, the human is more permissive to SARS-CoV-2. Whether the intramuscular vaccination or mucosal vaccination would protect the upper and lower respiratory tracts in humans under the natural exposure remains to be determined in phase III trials.

Thirdly, we currently lack any long-term data for sterilizing immunity and its durability. Longitudinal studies following natural infection so far suggest that mucosal immunity is less durable than systemic immunity following SARS-CoV-2 infection. Wright et al.[13] measured the persistence of antibodies following SARS-CoV-2 infection showing that Nba responses to SARS-CoV-2 were detected in serum and respiratory samples for 96% and 54%, respectively, of infected participants.

Finally, it is worth being mindful that achieving sterilizing immunity has proven extremely difficult within vaccine programmes so far. This includes oral and mucosal vaccines in clinical use. The HPV vaccine is one of a few vaccines that are capable of preventing infection almost 100% of the time.

Despite promising preclinical results with intranasal vaccines producing high concentrations of mucosal sIgA in animals, the ability of intranasal vaccines to induce durable sterilizing immunity in humans remains in question until further data emerges from clinical trials.

B. How likely is an intranasal booster to reduce overall viral load and therefore transmission?

Preventing viral infection and replication in the mucosa halts a host's potential to transmit the virus. Intramuscular SARS-CoV-2 vaccines generally provide limited protection against viral replication and shedding within the airway. This is because they are designed to produce a systemic IgG response (which reduces disease), and lack a local mucosal secretory IgA response which is required to prevent viral replication[14]. This suggests that intramuscularly vaccinated individuals could still become infected and transmit the virus, despite being asymptomatic[15]. Mucosal vaccines offer a promising avenue for reducing viral load and transmission, as Professor Akiko Iwasaki describes[16]:

> As SARS-CoV-2 primarily spreads by respiratory droplets and aerosols, viral load within the oropharynx is a key determinant of transmission risk. Vaccination approaches that specifically target the mucosa can elicit local neutralising IgA antibodies to reduce both the viral load to below the transmissibility threshold and symptomaticity. Mucosal vaccines could generate a more robust plasma cell response through generation of dimeric IgA, which is transported into the mucosal lumen, and produces a more potent response in comparison with its monomeric counterpart.

Preclinical and clinical studies have so far demonstrated that intranasal boosters have the potential to reduce viral load and consequent transmission against SARS-CoV-2.

Study	Vaccine type	Method	Preprint	Results

[13] (Wright, 2022) https://academic.oup.com/jid/advance-article/doi/10.1093/infdis/jiac065/6533756?login=false
[14] (Sterlin, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7857408/
[15] (Bleier, 2021) https://pubmed.ncbi.nlm.nih.gov/33320052/
[16] (Iwasaki, 2021) https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(21)00472-2/fulltext#seccestitle90

Preclinical studies				
Iwasaki, 2022	Unadjuvanted intranasal spike booster vaccine against sarbecoviruses	Prime and Spike method in mice	Yes	The study demonstrates that Prime (intramuscular) and Spike (intranasal) significantly reduced the viral load in the nasal cavity and the lung compared to injected vaccine alone, indicating the promise of Prime and Spike in reducing infection and transmission.
Ku, 2020	A single intraperitoneal injection of a lentivirus-vectored SARS-CoV-2 vaccine	A single dose followed by a booster in golden hamsters	No	The study shows that a single dose decreased viral replication in the lungs. Further intramuscular boosting failed to improve the protection rate, whereas an intranasal booster induced high levels of neutralizing IgG and IgA antibodies in the serum, resulting in >3 log10 decreases in lung viral loads.
van Dolerman, 2021	ChAdOx1 nCoV-19 (adenovirus-vectored vaccine)	The AstraZeneca SARS-CoV-2 vaccine currently in clinical use was administered intranasally in hamsters and rhesus macaques	No	In both models, intranasal vaccination reduced viral shedding after SARS-CoV-2 challenge relative to control animals. In hamsters, intranasal vaccination exhibited reduced viral shedding, indicating 100% transmission control. Phase I clinical trials are currently underway and are expected to conclude in May 2022.
Langel, 2022	Adenovirus type (Ad) 5-vectored SARS-CoV-2 vaccine	Two oral or intranasal doses	No	Oral and IN r-Ad-S vaccination accelerated SARS-CoV-2 viral RNA clearance and protected against disease in hamsters. They also limited SARS-CoV-2 transmission to unvaccinated, naïve hamsters leading to decreased clinical evidence of disease.[17]
Clinical trials				
Manufacturer	**Vaccine**	**Tested use**	**Participants**	**Research results**
Codagenix	**COVI-LIV** *Live attenuated vaccine*	1 dose of IN CoviLiv after previous course of Covid vax (2 IM doses of Moderna or Pfizer, or 1 IM dose of J&J), in 3 dosages.	48	Phase I interim results press release (without data):[18] CoviLiv induced a mucosal antibody response and blocked nasal replication, suggesting it may be the only vaccine candidate with the potential to reduce viral transmission. 40% of participants presented anti-Covid Immunoglobulin A (IgA) antibodies.

[17] Phase I data show that the oral vaccine elicited mucosal cross-reactive SARS-CoV-2-specific IgA responses

[18] Full Phase I data expected mid-2022

				Phase II / III results (conference abstract): Vaccination resulted in minimal viral shedding without sequence instability. Safety and shedding data supports continued development in a wider Phase 2/3 population.
Cansino Biologics	**Ad5-nCoV** (Convidecia)	2 IN doses of CanSino or IM or 1 IM of CanSino followed by 1 IN; high or low dosage.	149	Phase I results: Two doses of IN CanSino elicited neutralising antibody responses, similar to one IM dose. An IN booster at 28 days after first IM dose induced strong IgG and neutralising antibody responses.
		High or low dose single intranasal dose of CanSino after 2 doses of Sinovac's CoronaVac (inactivated vaccine); or additional dose of CoronaVac.	420	Phase I / II results: The low-dose and high-dose of IN CanSino boosters elicited titres of SARS-CoV-2 neutralising antibodies which were significantly higher than those at days 14 and days 28 elicited by homologous boosting with CoronaVac.
		Single dose of IN or IM CanSino or IM dose of inactivated (CoronaVac) or protein subunit vaccine (ZF2001) as a booster after 2 IM doses of CoronaVac.	904	Phase IV results: The CanSino booster induced potent neutralizing activity against the wild-type virus and Omicron variant, while IN CanSino generated the greatest neutralizing antibody responses against the Omicron variant at day 28 after booster vaccination, at 14.1-fold that of CoronaVac, 5.6-fold that of ZF2001 and 2.0-fold that of IM CanSino.
Razi Vaccine and Serum Research Institute	**Razi Cov Pars** *Protein subunit*	1 IN dose after 2 IM doses of Razi Cov Pars, or IM doses of Sinopharm Beijing vaccine.	41,128	Reported in the Tehran Times as reducing transmission by 90%. The vaccine is delivered in three doses: two injections and one nasal spray.

It is worth highlighting that CoviLiv's two dose intranasal schedule induced anti-Covid IgA antibodies in 40% of participants (there were c. 48 participants). IgA antibody titres were not measured as part of CoviLiv's preclinical studies[19][20] for comparison. Less than half of the participants producing IgA post vaccination is somewhat discouraging, especially as clinical trial participants are healthy adults and administration occurs within a clinical setting, therefore these results reflect the upper-bound for efficacy, which is very likely to decrease during real world application.

[19] https://www.tandfonline.com/doi/full/10.1080/22221751.2021.1971569
[20] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8307828/

Results from the clinical trials for Meissa's intranasal SARS-CoV-2 vaccine candidate[21] are yet to be published. However, preliminary clinical data for Meissa's respiratory syncytial virus vaccine (RSV), showed that RSV-specific mucosal IgA response were induced in the majority of healthy adult, seropositive recipients. These results are potentially promising for Meissa's SARS-CoV-2 candidate, as the vaccine was derived by modifying the company's RSV LAV candidate, replacing the RSV glycoproteins with a functioning SARS-CoV-2 Spike protein. In addition, Interim Phase I analysis[22] for Mexico's intranasal candidate, NDV-HXP-S (Patria), has been published, however an evaluation of the nasal mucosal humoral immunity post vaccination will feature in a future Phase I publication.

Preclinical and clinical trials have thus far demonstrated the potential of intranasal vaccines to significantly reduce viral load and transmission. However, their durability remains in question.

How likely is an intranasal booster to reduce disease severity?

The systemic immunity induced by intramuscular SARS-CoV-2 vaccines has been shown to effectively reduce disease severity. Potential contributing factors include sufficient IgG levels to protect the lower respiratory tract, non-neutralizing antibodies, T cells and memory B cells[23]. As with intramuscular vaccines, prior infection has been found to protect against severe disease during reinfection[24].

While the primary benefit of mucosal vaccines is their potential to prevent infection and transmission, in the event of a break-through infection, it is critical that intranasal regimes are capable of reducing disease severity.

The following table outlines preclinical and clinical results thus far:

Study	IN Vaccine type	Method	Preprint	Results
Preclinical studies				
Van Doremalen, 2021	ChAdOx1 nCoV-19	A side by side comparison of intramuscular and intranasal delivery of the AstraZeneca SARS-CoV-2 vaccine currently in clinical use in hamsters and rhesus macaques	No	Hamsters that had received the vaccine through the nose had higher levels of antibodies against SARS-CoV-2 in their blood than those who received it through the muscle.

[21] https://www.meissavaccines.com/post/meissa-vaccines-provides-a-pipeline-update-on-vaccine-candidates-for-covid-19-and-rsv

[22] https://www.medrxiv.org/content/10.1101/2022.02.08.22270676v1.full

[23] (Krammer, 2022) https://cdn.who.int/media/docs/default-source/blue-print/platforms_florian-krammer_whoconsulation_covid19framework_23feb2022.pdf?sfvrsn=f5a1e435_7

[24] (Mensah, 2022) https://www.journalofinfection.com/article/S0163-4453(22)00010-X/fulltext

Lapuente, 2021	Intranasal adenovirus 5 and 19a vectored vaccines following a systemic plasmid DNA or mRNA priming	IM prime and IN boost in mice	No	In contrast to two intramuscular applications of an mRNA vaccine, intranasal boosts induce high levels of mucosal IgA and lung-resident memory T cells (TRM); mucosal neutralization of virus variants of concern was also enhanced. The mRNA prime provokes a comprehensive T cell response consisting of circulating and lung TRM after the boost, while the plasmid DNA prime induces mostly mucosal T cells.
Afkhami, 2022	Two different trivalent adenoviral-vectored COVID-19 vaccines	Single-dose	No	Single-dose intranasal immunization, particularly with chimpanzee Ad-vectored vaccine, was shown to be superior to intramuscular immunization in the induction of the tripartite protective immunity consisting of local and systemic antibody responses, mucosal tissue-resident memory T cells and mucosal trained innate immunity. This was effective against the wild type and two VOC, B.1.1.7 and B.1.351.
Clinical trials				
Manufacturer	**Vaccine**	**Tested use**	**Participants**	**Research results**
Altimmune	**AdCovid** *Adenovirus type 5-vectored vaccine*	1 or 2 IN doses of AdCOVID (3 dosages); or placebo.	92 people planned, but only 2 of 3 age cohorts recruited.	Phase I results press release: Immunogenicity data of AdCOVID showed reduced immune responses than anticipated for all the immune parameters analysed. [DISCONTINUED]
Codagenix	**COVI-LIV** *Live attenuated vaccine*	1 dose of IN CoviLiv after previous course of Covid vax (2 IM doses of	48	Phase I interim results press release (without data): the regime induced strong cellular immune response in healthy adults against many conserved proteins in known

		Moderna or Pfizer, or 1 IM dose of J&J), in 3 dosages.		variants of SARS-CoV-2, in particular, a peptide pool >99% Omicron BA.2. Spike protein focused vaccines have shown lower protection against viral mutants.
Laboratorio Avi-Mex (Mexico)	**Patria** *Inactivated NDV-HXP-S intranasal vaccine*	2 intranasal doses of Patria, 1 intranasal dose followed by 1 injection of Patria, or 2 injections of Patria, in 3 dosages.	91	Phase I results: Only the high-dose vaccine regimen induced robust antibody and cellular immune responses when given via the IM-IM or IN-IM routes, comparable to those in convalescent individuals. Cellular immune responses were induced by the IN-IN route but systemic antibody responses were not as robust.

An additional factor of mucosal immunity that warrants attention is that it can clear the virus at the site of transmission before it disseminates systemically, which could potentially prevent serious complications of COVID-19, such as blood clotting disorders and kidney, heart, liver and brain damage[25].

A potential risk with intranasal vaccines, due to a lack of long term data, is that they increase mucosal immunity but do not boost systemic immunity to such an extent as intramuscular vaccines and could consequently expose patients to severe disease.

An intramuscular prime and intranasal boost regime appears to be the most promising approach to establish mucosal immunity without compromising the systemic response. However, long-term data is required to measure the durability of systemic immunity following intranasal boosting.

C. How likely is an intranasal booster to reduce risk of long covid?

It remains uncertain how well vaccination protects against long covid following breakthrough infection. Research undertaken by the Office for National Statistics concluded that vaccination could reduce the risk of long covid following infection[26]. The study of 6,180 people found that receiving two doses of a SARS-CoV-2 vaccine at least two weeks before infection was associated with a 41% decrease in the odds of reporting long covid symptoms, in a comparison with people who weren't vaccinated. On the other hand, a study by Al-Aly et al.[27] suggests that vaccines modestly reduce but do not eliminate the risk of long covid following breakthrough infection. The study of more than 13 million people shows that vaccines (prior to infection) only reduce long covid risk by 15% with the largest risk reduction in blood clots and pulmonary sequelae but with less protection of other organ systems.

As expected at this stage in their development, there is limited research on the impact of intranasal vaccines on long covid in comparison to intramuscular vaccines. However, there

[25] (Sui, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262352/

[26] https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/selfreportedlongcovidaftertwodosesofacoronaviruscovid19vaccineintheuk/26january2022.

[27] (Al-Aly, 2022) https://www.nature.com/articles/s41591-022-01840-0

have been findings from long covid studies that suggest intranasal vaccines could be particularly effective in reducing the risk of long covid. Some recent studies[28] suggest the risk of brain complications as part of long covid is directly related to the amount of virus in the nose. Reducing the viral load in the nose is thought to be effective in reducing these long covid complications, since the virus is believed to travel from the nose to the brain via the olfactory bulb (which is linked with loss of sense of smell experienced by some individuals). Murdoch Children's Research Institute will be exploring this further as part of their intranasal Heparin treatment trials[29].

On the other hand, some studies give cause to believe that systemic immunity may be the primary driver in reducing the risk of long covid, as opposed to mucosal immunity. Researchers have identified an immunoglobulin "signature" that could be used to predict which patients are most at risk of developing long covid. In the study[30], patients who developed long covid were found to have lower levels of IgM and IgG3 antibodies than those who quickly recovered.

Currently, we lack sufficient data to make any meaningful estimation of the likelihood that intranasal boosters could reduce the risk of long covid. However, from initial research it appears that intranasal boosters could contribute to the reduction of the risk of long covid primarily by (a) minimising the viral load and potential transfer to the brain and (b) blocking the virus before it disseminates systematically and causes broader damages.

2. To what extent will intranasal vaccines be easier to deliver?

A. Are cold chain requirements/supply chain requirements/dosing regimens likely to be different/easier?

Cold chain requirements

Most nasal vaccine candidates under development for SARS-CoV-2 do not require cold storage as they are designed to be heat stable[31]. A clear advantage for the intranasal route is that liquids and dry powder formulations may be used. The latter could provide clear advantages on the transportation and wastage issues, as a cold-chain would not be required and a longer shelf life could be achieved.

Protein subunit vaccines only need ordinary fridges and vaccine distribution, and the technical capacity to manufacture them is widespread. This includes Razi Cov Pars, which has been authorised in Iran. Nguyen et al.[32] have also designed a subunit vaccine with enhanced thermostability to eliminate the need for an ultra-cold chain. During preclinical studies, the vaccine did not lose activity when stored at either room temperature (21-22°C) or 4°C for at least one month.

In addition to these protein subunit vaccines, the intranasal NDV-HXP-S (Patria)[33] developed in Mexico can be stored and distributed without the need for freezers and incorporates an advanced HexaPro antigen design compared to most other SARS-CoV-2 vaccines on the market. CoviLiv (by Codagenix) can be stored in a normal refrigerator[34], warranting its

[28] (Serrano, 2021) https://pubmed.ncbi.nlm.nih.gov/33619496/
[29] (Clinical Trial registration) https://clinicaltrials.gov/ct2/show/NCT05204550
[30] (Cervia, 2022) https://www.nature.com/articles/s41467-021-27797-1
[31] (Karr, 2022) https://onlinelibrary.wiley.com/doi/full/10.1002/iid3.604
[32] (Nguyen, 2022) https://www.frontiersin.org/articles/10.3389/fimmu.2022.858904/full
[33] (Krammer, 2022) https://www.medrxiv.org/content/10.1101/2022.01.25.22269808v1.full
[34] https://codagenix.com/pipeline/

inclusion within the World Health Organisation (WHO) global, placebo-controlled Phase 2/3 efficacy trial for COVID-19 (WHO-sponsored, Solidarity Trial Vaccines)[35]. CanSino's Ad5-nCoV vaccine only requires standard refrigeration temperatures so is easy to store and ship[36].

A major impediment to widespread, global vaccination is the lack of cold chain infrastructure and the technology required for vaccine storage, distribution, and transportation, particularly in rural areas. The potential of intranasal vaccines to reduce cold chain requirements, particularly ultra-cold chain requirements currently needed for mRNA intramuscular vaccines, could have a significant impact on the equitable distribution of vaccines and overall reduction of vaccine wastage.

Supply chain requirements

There appears to be a priority for developers of intranasal vaccines to ensure they can be economically scaled for mass manufacturing, particularly for lower middle-income countries. For instance, the intranasal NDV-HXP-S (Patria)[37] vaccine has been designed to aid vaccine equity since it can be economically produced in influenza vaccine manufacturing plants that are located in LMICs. The vaccine's development program also provides a vaccine platform and model that can be used for optimal pandemic preparedness and response in LMICs in the future. Peter Palese, who is also working on the nasal vaccines, was quoted in an article[38] as saying a nasal dose could be produced for about 30 cents compared to $30 for a Moderna or Pfizer dose.

A further advantage is that intranasal vaccines require smaller doses than intramuscular vaccines in order to be as effective. The intranasal variation of the AstraZeneca currently in clinical use only used 50% of the dose needed for intramuscular vaccination during clinical trials in order to achieve the same efficacy[39]. In the case of Ad5-nCoV, the dose needed in Phase I clinical trials was 20% (for low dose) or 40% (for high dose) of that needed with the intramuscular version, which could make supplies of the vaccine stretch further[40][41]. This has the potential for significant cost reduction and increased vaccine supply.

In contrast, the use of adjuvants for some intranasal vaccines could increase production costs as well as increase manufacturing complexity, burdening countries with less well-resourced manufacturing facilities. Vaccine developers should strive to use adjuvants and delivery systems that are simple in composition, easy for manufacturing, and low-cost.

In addition to its ingredients, a significant percentile of the cost of a vaccine is its primary packaging and delivery device. Intranasal vaccines could circumvent having to use costly needles and syringes, and could instead use multi-dose bottles.

Dosing regimens

[35] The selection of candidate vaccines for the STV involves evaluation of pre-defined criteria, which includes demonstration that a vaccine can be stored and transported easily under normal conditions: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-trial-of-covid-19-vaccines

[36] https://pharmaphorum.com/news/first-published-data-backs-cansinos-inhaled-covid-vaccine/

[37] (Krammer, 2022) https://www.medrxiv.org/content/10.1101/2022.01.25.22269808v1.full

[38] https://www.webmd.com/vaccines/covid-19-vaccine/news/20220509/nasal-sprays-for-covid-vaccine-being-developed

[39] (van Doremalen, 2021) https://www.science.org/doi/10.1126/scitranslmed.abh0755

[40] https://pharmaphorum.com/news/first-published-data-backs-cansinos-inhaled-covid-vaccine/

[41] (Wu, 2021) https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(21)00396-0/fulltext#seccestitle170

The administration of nasal vaccines does not require the presence of trained healthcare professionals since it is needle-free, in contrast to the intramuscular vaccination process. Needle-free administration reduces the need for trained personnel to administer vaccinations, which could lead to a larger population being immunised over a shorter period, significantly accelerating a vaccination programme, especially during case surges.

The advantages of intranasal SARS-CoV-2 vaccines might significantly extend the practical options for vaccine distribution, particularly in resource-limited settings where other preventive measures, such as social distancing, may be more difficult to maintain. In addition, intranasal vaccinations can be administered using simple devices, which eliminates the requirement for sterilised settings during vaccination, which is particularly beneficial for immunisation programs in developing nations.

These advantages address challenges that are experienced in particular by LMIC and LIC. However, their impact is entirely dependent on the exact dosing regimen used. Intranasal vaccines currently in development vary in their dosing regimens. These include:

1. A single intranasal dose
2. Two intranasal doses
3. One intramuscular dose (of a vaccine in clinical use) followed by an intranasal dose (sometimes referred to as ‘prime and spike’ or ‘prime and boost’)
4. Two intramuscular doses (of vaccines in clinical use) followed by an intranasal dose

For countries with low vaccination rates (LMIC and LIC) only a single or two- intranasal dose regime would provide these benefits. A dosing regime that required an initial intramuscular dose would do little to remove pre-existing obstacles. This is a key issue for the development of intranasal vaccines and the careful balance between ensuring the balance is struck between efficacy and ease of delivery. A potential route could be regulatory flexibility to allow intranasal vaccines to be used as a booster following intramuscular vaccination or as a homologous regime.

Is there data concerning vaccine hesitancy around shots vs. intranasal spray?

Fear of needles (otherwise referred to as blood injection injury (BII) type phobia) has been cited by both the general public and health professionals as a reason for vaccination refusal, including for influenza, tetanus, pneumococcal infections and most recently for SARS-CoV-2[42][43].

Daniel Freeman, a professor of clinical psychology at the University of Oxford, has led two pieces of research on needle phobia and vaccine hesitancy in the UK. One study found that it could be the cause of 10% of COVID-19 vaccine hesitancy, with needle phobia being more prevalent in younger adults and in Black and Asian ethnic groups[44]. Another study[45] shows

[42] (Johnson, 2008) https://pubmed.ncbi.nlm.nih.gov/18589065/
[43] (Yakub, 2014) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8220023/#ref43
[44] (Freeman, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8220023/
[45] (Freeman, 2021) https://www.cambridge.org/core/journals/psychological-medicine/article/injection-fears-and-covid19-vaccine-hesitancy/A70D5D859CC25804B7AC4FB3AD54F68D

that a quarter of the UK adult population screens positive for a potential injection phobia. These individuals were twice as likely to report that they were COVID-19 vaccine hesitant – they would put off getting vaccinated or never get the jab. In an article on this topic[46]. Dr Stephen Griffin, a virologist at Leeds University, said he was constantly asked by UK healthcare staff when there would be non-injectable formulations of Covid vaccines – not just for patients, “but because there are so many needle-phobic staff”.

In a US survey conducted in June 2020, 11.8% of those who were hesitant about COVID-19 vaccination reported that a reason was a dislike of needles and injections[47]. Another US study[48] of 9000 older adults conducted in November 2020, 1.7% were concerned about receiving a COVID-19 vaccine because of a fear of needles. These studies suggest that, although fear of injection is not the dominant reason for vaccine hesitancy, it may be a contributory factor.

Intranasal vaccination could offer an appealing alternative to individuals who experience needle phobia, reducing this percentile of vaccine hesitant individuals[49].

A Vaxart sponsored survey[50] found that 23% of respondents said they do not plan to get vaccinated but nearly a third of them said they would if the vaccine were available as a pill instead of by a needle injection. Although this survey focuses on oral vaccines as opposed to intranasal vaccines, it is possible that this increase in willingness to get vaccinated could translate to other needle-free options.

It has been argued that intranasal vaccines could ease vaccine administration for children, for whom vaccination is particularly unpleasant. That said, in an article[51] Kate O’Brien of the World Health Organisation said in her experience as a paediatrician, children are not necessarily much more willing to take the nasally administered flu shot, FluMist, than a jab. “It doesn’t solve the delivery issues,” said O’Brien, the agency’s director of immunization, vaccination, and biologics.

In addition, intranasal vaccination might not be sufficiently less uncomfortable than intramuscular vaccination to incentivise individuals. Nasal swab testing during the pandemic has widely been perceived as unpleasant or invasive, and may disincentivize individuals. There may also be a link with fears of invasive procedures associated with past trauma, which intranasal vaccines would not resolve.

Intranasal vaccines may have the biggest impact on individuals who experience needle phobia due to a fear of needle contamination or ‘needle stick’ - in particular HIV-infected patients, as well as multi-morbid patients, who are tired of injections[52].

3. Is an effective intranasal vaccine possible within the next 2 years?

A. How promising are pre-clinical results? How do these compare to pre-clinical results for other intranasal approaches for other diseases?

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[46] https://www.theguardian.com/world/2021/aug/02/research-into-non-injectable-covid-vaccines-brings-hope-for-needle-phobics

[47] (Ruiz & Bell, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7794597/

[48] (Nikolovski, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8143399/

[49] (Freeman, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8220023/

[50] https://investors.vaxart.com/news-releases/news-release-details/poll-oral-covid-19-vaccine-pill-offers-way-overcome-vaccine

[51] https://www.statnews.com/2021/08/10/covid-intranasal-vaccines/

[52] (Birkhoff, 2009) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2846493/

17 intranasal vaccines are currently in clinical trials (including the authorised Iranian vaccine, Razi Cov Pars, and the Russian vaccine, Sputnik V). 6 have reached efficacy trial stage (phase 3+); 3 have reached mid-stage trial (phase 2); 8 have reached first-in-human trial (phase 1).

The following table provides an overview of six vaccines which have reached the late stages of clinical trials:

Vaccine	Phase	Participants	Country/ies	Status
Ad5-nCoV (Convidecia) *(viral vector) Adenovirus* *Cansino (China)*	3	10,420	China	Intramuscular version authorised in multiple countries and included in the WHO's EUL[53].
	2	904 (booster tial for ZF2001 vax)	China	
BBV154 *Viral vector (adenovirus)* *Bharat Biotech (India)*	2 / 3 & 3	3,708		No authorisation
	3	875 (booster trial for Covaxin AZ/Covishield)		
CoviLiv *Live attenuated* *Codagenix (USA)*	2 / 3	*Not yet announced - joining WHO Solidarity Trial, a placebo-controlled trial. Dosing planned by mid-2022*[54]	Currently Colombia, Ghana, Philippines.	No authorisation
DelNS1-nCoV-RBD-OPT1 *Viral vector (influenza)*	3	45,400	Colombia, Ghana, Philippines, South Africa	No authorisation

53 https://www.who.int/news-room/feature-stories/detail/the--cansino-biologics-ad5-ncov-s--recombinant---covid-19-vaccine--what-you-need-to-know

54 https://codagenix.com/codagenix-intranasal-covid-19-vaccine-shows-potent-cellular-immune-response-against-conserved-viral-proteins-indicating-potential-for-immunogenicity-against-omicron-and-future-variants-in-phase-1-dat/?pag=1

Beijing Wantai Biological (China)				
GAM-COVID-VAC (Sputnik V) *Viral vector (adenovirus)* *Gamaleya Research Institute (Russia)*	2 / 3	1,320	Russia	Authorised for use in Russian in April 2022[55]. Intramuscular version authorised in multiple countries.
Razi Cov Pars *Protein subunit* *Razi Vaccine & Serum Research Institute (Iran)*	3	41,128	Iran	3-dose course (2 intramuscular & 1 intranasal) authorised for use in October 2021

As the above table demonstrates, two intranasal vaccines have received emergency use authorisation - Sputnik V in Russia and Razi Cov Pars in Iran. Unfortunately we lack any clinical trial data for either vaccine. The intranasal variation of Sputnik V is expected to be in circulation in Russia around July/August 2022[56]. As of 25 November 2021, 5 millions doses had been delivered to the Iranian Ministry of Health[57]. In addition, four countries have already started to manufacture Mexico's NDV-HXP-S(Patria) vaccine – Vietnam, Thailand, Brazil and Mexico – in anticipation of its authorisation[58].

Currently, there is no Phase 3 clinical trial data available for any of the candidate intranasal vaccines. This is the most meaningful phase for measuring the vaccines' effectiveness, as it involves a randomised control trial, comparing vaccinated individuals with a control group, as opposed to inferring a vaccine's efficacy from the immune responses of participants by measuring the antibody levels in serum samples, as is the case in Phases 1 and 2.

The most advanced results currently available are for CanSino's (Ad5-nCoV) Phase 2 trial[59], which involved 904 participants and evaluated the immune responses to booster vaccination

[55] Sputnik V is anticipated to be available for civil circulation in Russia within three to four months, according to, Alexander Gintsburg, the director of the Gamaleya Centre: https://www.ndtv.com/world-news/coronavirus-covid-vaccine-coronavirus-vaccine-world-gets-its-1st-nasal-covid-jab-as-russia-registers-sputnik-v-version-2858358

[56] https://www.indiatoday.in/world/story/russia-registers-nasal-version-of-sputnik-v-world-s-first-nasal-vaccine-against-covid-19-1932541-2022-04-01

[57] https://www.isna.ir/news/1400090402991/%DB%B5-%D9%85%DB%8C%D9%84%DB%8C%D9%88%D9%86-%D8%AF%D8%B2-%D9%88%D8%A7%DA%A9%D8%B3%D9%86-%D8%B1%D8%A7%D8%B2%DB%8C-%D8%A8%D9%87-%D9%88%D8%B2%D8%A7%D8%B1%D8%AA-%D8%A8%D9%87%D8%AF%D8%A7%D8%B4%D8%AA-%D8%AA%D8%AD%D9%88%DB%8C%D9%84-%D8%AF%D8%A7%D8%AF%D9%87-%D8%B4%D8%AF

[58] https://eu.usatoday.com/story/news/health/2022/05/08/nasal-vaccines-may-next-generation-protection-against-covid/9557964002/

[59] (Zhang, 2022) https://www.medrxiv.org/content/10.1101/2022.03.08.22271816v1.full-text

with intramuscular Ad5-nCoV, aerosolized Ad5-nCoV, a recombinant protein subunit vaccine (ZF2001) or homologous inactivated vaccine (CoronaVac) in those who received two doses of inactivated COVID-19 vaccines 6 months prior. The study found that the intranasal Ad5-nCoV generated the greatest neutralising antibody responses against the Omicron variant at day 28 after booster vaccination. It also generated the greatest IFNγ T-cell response at day 14 and the greatest spike-specific B cell response. The mucosal immune response was not measured, so the mucosal immune response at this time point is unclear.

CoviLiv (Codagenix Ltd.) is the only intranasal vaccine participating in the World Health Organisation's (WHO) global, placebo-controlled Phase 2/3 efficacy trial for COVID-19 (WHO-sponsored, Solidarity Trial Vaccines). The Solidarity Trial Vaccines[60] (STV) is an international, multi centre, multi vaccine, adaptive, shared placebo, event driven, individually randomised controlled clinical trial that aims to evaluate the efficacy and safety of promising new COVID-19 vaccines. The primary objective of STV is to evaluate the effect of each participant vaccine on reducing infection rates, regardless of severity. Vaccines are selected for inclusion by an independent panel of scientists, who review, select and prioritise vaccines that have been tested in phase 2 clinical trials. CoviLiv's participation in STV is particularly promising from a regulatory perspective. Phase II/III dosing is expected to take place in mid-2022.

Four of the intranasal vaccines currently in development/authorised are variations of injected vaccines already authorised for use in at least one country (AstraZeneca, CanSino, Medigen, and Sputnik V). Whereas mixing vaccines could have advantages[61], a homologous vaccination schedule, where the intranasal booster is the same/similar formulation to the first/second administered dose(s), could give these particular intranasal vaccines an advantage from a regulatory perspective.

With two intranasal vaccines already having received emergency use authorisation, one of which is already in clinical use in its intramuscular format in several countries[62], it is a promising landscape for future full authorisations of intramuscular vaccines. CoviLiv is a promising candidate for authorisation within the next two years, due to its participation in STV which will assist in expediting its Phase 2 and 3, however its live attenuated technology may prove challenging during the regulatory process. Moreover, if CanSino's Phase 3 trials offer promising results, it will be a likely candidate for authorisation within the next two years, seeing as it has a particular regulatory advantage due to its intramuscular format already being in clinical use.

B. <u>What are the biggest obstacles to intranasal administration</u>

The major challenge in developing intranasal vaccines is successfully delivering the antigens to antigen-presenting cells (APCs) in the respiratory tract while overcoming nasal clearing (otherwise referred to as mucociliary clearing)[63]. Mucus serves as a sticky solvent in the

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[60] https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-trial-of-covid-19-vaccines

[61] Trials from the UK and Spain found that mixed 2-dose schedules of AZ and Pfizer produced higher immune responses than 2 doses of AZ (Com-Cov, Com-Cov2, and CombiVacS). The Com-Cov trials found the same for combinations of AZ and Moderna or Novavax, and Pfizer with Moderna or Novavax.

[62] For a list of countries, see: https://en.wikipedia.org/wiki/List_of_COVID-19_vaccine_authorizations#Sputnik_V

[63] (Chavda, 2021) https://www.sciencedirect.com/science/article/pii/S1359644621003317#b0250

nasal mucosa, while cilia acts as a protective barrier, preventing infectious chemicals, bacteria, and debris from entering the lungs via inhaled breath. The absorption of an antigen is influenced by the duration it resides in the mucosa. If mucociliary clearance increases, antigen absorption decreases. Therefore, the antigen used in the vaccine must remain in the respiratory tract for an adequate amount of time to allow antigen absorption, in order to elicit a durable immune response. In addition, the antigen must be stable enough to withstand the enzymatic degradation in the mucosal lining[64].

Live attenuated and inactivated vaccines are less susceptible to mucociliary clearing and enzymatic degradation and have proved the most successful platforms for mucosal vaccine design to date[65]. Of the nine mucosal vaccines approved for use in humans — eight oral and one intranasal — all are either live attenuated or whole-cell inactivated vaccine formulations. Live attenuated vaccines tend to be potent enough to trigger an immune response, but not so potent to cause symptoms. They also have the advantage of mimicking natural infection by presenting antigens in their native conformation to the nasal mucosal tissue, without inducing symptoms. As a result, they create a strong and long-lasting immune response, and usually only require one or two doses. Nevertheless, live attenuated vaccines can also struggle to strike the right potency balance. Flumist - the only licensed intranasal vaccine in the US - had to be reformulated as it was no longer working effectively due to this reason. In addition to deciphering the correct viral potency for live attenuated vaccines, ensuring the delivery device supports a complete delivery of the vaccine also impacts the potency that ultimately reaches the nasal tract.

Protein subunit vaccines[66], such as Razi Cov Pars (Iran), are particularly vulnerable to swift enzymatic degradation and a deficiency in stimulating APCs[67]. Even as intramuscular vaccines, subunit vaccines do not always create such a strong or long-lasting immune response as live attenuated vaccines. They usually require repeated doses initially and subsequent booster doses in subsequent years. One commonly accepted method for increasing their immunogenicity is the use of vaccine adjuvants[68] and delivery systems, to prolong the time in which the antigen remains in the mucosal lining while enhancing its stability. Razi Cov Pars uses an adjuvant[69] to this end, however the exact adjuvant used is unknown.

As with all types of vaccines, an essential consideration for intranasal vaccine development is safety. There are some concerns about whole pathogen-based vaccines (e.g. live attenuated vaccines[70]) due to the possibility of reversion into a replicating state. As a result, live attenuated vaccines, such as FluMist against seasonal influenza, cannot be administered to certain groups, such as immunocompromised individuals, individuals who are pregnant, anyone younger than 2 years or older than 49 years of age, or anyone aged 5 over with asthma[71]. These restrictions on the administration of a live attenuated vaccine warrants special attention, since CoviLiv (Codagenix) - one of the intranasal SARS-CoV-2 vaccine ‘front-runners’ and WHO solidarity trial participant - utilises this vaccine design.

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[64] (Alu, 2022) https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(22)00025-1/fulltext#bib0037

[65] The two authorised intranasal vaccines against seasonal influenza are the attenuated vaccines FluMist/Fluenz Tetra (MedImmune//Astra Zeneca) authorised in the US and Europe respectively, and Nasovac (Serum Insitute of India Ltd.) authorised for use in India.

[66] 14 intranasal protein subunit vaccines are currently in development/authorised.

[67] (Karch, 2016) https://www.sciencedirect.com/science/article/pii/S0006295216300740

[68] An adjuvant is an ingredient added to a vaccine that helps create a stronger immune response to vaccination.

[69] https://www.irct.ir/trial/52975

[70] CoviLiv (Codagenix) is an example of a Live attenuated vaccine, as well as the nasal influenza vaccine, FluMist.

[71] https://www.cdc.gov/vaccines/hcp/vis/vis-statements/flulive.html

As discussed above, adjuvants are essential for strong immune responses, especially for protein subunit vaccines. However, safety problems can also arise along with the addition of adjuvants owing to their immunomodulatory properties. After an inactivated intranasal influenza vaccine was introduced in Switzerland during the 2000–2001 influenza season, 46 cases of Bell's palsy were reported. A study was published in 2004 suggesting a strong association between the inactivated intranasal influenza vaccine used in Switzerland and Bell's palsy[72]. This vaccine is no longer in clinical use.

Iwasaki et al.'s 'prime and spike' method[73] attempts to address these safety issues, by taking advantage of the existing adaptive immunity and to use it as a natural adjuvant to boost immunity, thus avoiding the use of both live attenuated vaccines and adjuvants.

Another problem for intranasal vaccines is the possibility of viral transmission to the brain via olfactory nerves, which has been observed in live attenuated adenovirus[74]. In developing intranasal SARS-CoV-2 vaccines, increasing numbers of preclinical studies have proven their safety profiles. For instance, Sui et al.[75] reported no observation of vaccine-induced immune pathology even after 3 or 4 doses of their adjuvanted subunit intranasal vaccine in rhesus macaques. Seventeen intranasal vaccines having demonstrated sufficient safety profiles in animals to proceed to clinical trials, with six of those further proceeding to late-stage clinical trial phases after satisfying Phase I safety criteria, is also promising. Nevertheless, long-term observation and more clinical data, particularly Phase 3 data, are required to prove the safety of these intranasal vaccines.

C. What are the potential regulatory barriers for the authorisation of intranasal vaccines?

It is anticipated that the regulatory path for intranasal vaccines will be very challenging for intranasal developers, especially when applying with the FDA. This is primarily the result of a lack of precedent[76], safety concerns and limited resources.

We understand that a number of intranasal developers have already had difficulty acquiring non-human primates for preclinical purposes. There is a strong impetus from the FDA for intranasal developers to provide toxicological data in these animal models prior to advancing to clinical trials, however, there is a vast shortage of non-human primates, which have been monopolised by large pharmaceutical companies. Due to these shortages, when these animals become available, they are far too expensive for intranasal vaccines developed by smaller biotech or nonprofit companies, who lack the necessary funding. Multiple developers have also struggled to gain access to mRNA vaccines in clinical use for use as controls, due to legal barriers[77]. In order to enable intranasal developers to proceed beyond initial studies, there is a need for the FDA and other regulators to give prioritised access to resources for intranasal developers, in a market which is currently dominated by large pharmaceutical companies who are not focused on intranasal vaccines.

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[72] (Mutsch, 2004) https://www.nejm.org/doi/10.1056/NEJMoa030595?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200www.ncbi.nlm.nih.gov

[73] (Iwasaki, 2022) https://www.biorxiv.org/content/10.1101/2022.01.24.477597v1.full.pdf

[74] (Neutra, 2006) https://www.nature.com/articles/nri1777

[75] (Sui, 2021) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262352/

[76] Only one intranasal vaccine has been authorised by the FDA

[77] Patrick Collsion's writes in a recent blog post, following discussions with intranasal vaccine developerst: https://www.slowboring.com/p/we-could-have-universal-covid-vaccines?utm_source=substack&utm_medium=email

A further resource issue which may arise is the challenge of enrolling enough human volunteers to undertake intranasal clinical trials. Firstly - there may be less of an appetite to volunteer for COVID-19 vaccine trials in the current climate, where the pandemic seems to be less of a public threat and there are already vaccines in clinical use. Secondly - at this stage of the pandemic, a large proportion of the population have pre-existing immunity to SARS-CoV-2 in some form - either through natural infection, vaccination, or both. As a result, forming placebo control groups for measuring the efficacy of intranasal vaccines, will be especially challenging due to the decreased number of infection-naive patients avialble.

Another significant regulatory barrier for the authorisation of intranasal vaccines is providing robust safety data. All vaccines go through rigorous safety testing and clinical trials, but these processes are especially important for intranasal vaccines due to the fact that the nose is close to the brain. The viral tropism for SARS-CoV-2 in the brain and nervous tissue compared to the nose remains uncertain. The lack of precedent for intranasal vaccines, as well as historical links with Bell's Palsy (discussed further below), will also be at the forefront of regulators' minds. This is reflected by the FDA requiring numerous safety studies in non-human primates, which is not only expensive but incurs significant delays on the vaccine's pipeline[78]. An option for accelerating the regulatory process suggested by Andrew Kilianski would be to undertake a systematic study to measure how well the SARS-CoV-2 spike mediated vaccine can transport to the brain. These findings would apply to all vaccine modalities and could streamline safety data currently required for individual nasal vaccines. Moreover, a similar systematic approach could be undertaken for studying the safety of different adjuvants.

In a similar vein, providing appropriate and sufficient efficacy data for regulators to inform their decision during the approval process could also prove difficult for manufacturers. Approaches for measuring mucosal immunity lag behind those aimed at systemic immunity, making it more complicated to demonstrate the efficacy of nasal vaccines compared with more traditional injected versions.

One available guide at our disposal for anticipating potential regulatory barriers as well as the duration of the approval process is the precedent set by FluMist.The approval process for FluMist, from initial application to the FDA following clinical trials to the FDA's approval, took five years. This includes two application rejections by the FDA - the first on the grounds of insufficient data on manufacturing, validation and stability; the second due to safety concerns relating to asthmatic children.

The following table outlines the timeline for FluMist's approval process, beginning with clinical trials, and highlighting key issues along the way:

Date (if available)	Action
1975	Clinical trials begin in the US and Japan
1995	Aviron acquires FluMist through a Co-operative Research and Development Agreement (CRADA) with the US NIAID, and a licensing agreement with the University of Michigan, Ann Arbor, USA.
	Aviron completes phase II and III trials
July 1998	Aviron submits a Biologics Licence Application (BLA) to the FDA. FDA rejects this application on the grounds of a lack of data on manufacturing, validation and stability.

[78] Comments by Andrew Kiliansky

June 1999	Aviron announces that it has completed a bridging study on FluMist designed to provide some of the manufacturing data required by the FDA on FluMist prepared at one of two manufacturing sites.
July 2001	FDA advisory committee declines to recommend approval of the vaccine, citing concerns with safety. Aviron subsequently receives a Complete Response Letter from the FDA requesting additional clinical and manufacturing data.
Jan 2002	Aviron submits additional clinical and manufacturing data.
July 2002	A second Complete Response Letter is sent to MedImmune[79] from the FDA requesting clarification and additional data relating to previously submitted information.[80]
Aug 2002	MedImmune reports that it has completed the submission of information requested by the FDA.
Dec 2002	FDA's Vaccination and Related Biological Products Advisory Committee (VRBPAC) recommends that the FDA approve Flumist in healthy children, adolescents and adults (ages 5-49 years)
June 2003	FluMist first approved by the FDA

Timeline source: https://pubmed.ncbi.nlm.nih.gov/12952502/

3. What is the landscape for intranasal vaccines for other diseases (both in clinical use and in development)?

A. Existing mucosal-immunisation strategies for other diseases

Eight oral vaccines are currently licensed for use against cholera, salmonella, poliovirus and rotavirus. Live attenuated influenza vaccines remain the sole licenced intranasal vaccines. To date, live attenuated and inactivated vaccines have proved the most successful platforms for mucosal vaccine design. However, live attenuated vaccines are not usable in immunocompromised groups or pregnant individuals.

Adjuvanted inactivated vaccines have had safety concerns. After an inactivated intranasal influenza vaccine was introduced in Switzerland during the 2000–2001 influenza season, 46 cases of Bell's palsy were reported. A study[81] was published in 2004 suggesting a strong association between the inactivated intranasal influenza vaccine used in Switzerland and Bell's palsy. This vaccine is no longer in clinical use.

There are two approved intranasal vaccines against seasonal influenza currently in clinical use:

[79] MedImmune acquired Aviron in 2002, hence the name change.
[80] One of the most significant issues raised by the US FDA was the exacerbated rate of asthma and wheezing in 18-35-month-old patients using FluMist. MedImmune was required to consider two options to address this issue; to either exclude patients with asthma and wheezing from the label, or to exclude 18- to 30-month-old patients from the proposed indication.
[81] (Steffen, 2004) https://www.nejm.org/doi/10.1056/NEJMoa030595?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200www.ncbi.nlm.nih.gov

- **FluMist/Fluenz Tetra** (MedImmune//Astra Zeneca) authorised for use in the US and Europe, respectively.

- **Nasovac** (Serum Institute of India Ltd.) authorised for use in India.

Both vaccines make use of a live attenuated influenza virus. The vaccines have been administered to several million patients so far without any reports of severe adverse events or vaccine failure[82] and are shown to produce a long-lasting, humoral and cellular immune response which closely resembles natural immunity. Further, nasal vaccination against influenza provides increased protection against virus drift variants and, especially, infants and children are better protected than with the inactivated, injectable influen[83]. However The US CDC (Centre for Disease Control) Advisory Committee on Immunization Practices (ACIP) voted that the Flumist nasal spray live attenuated influenza vaccine (LAIV) (sic), should not be used during the 2016–2017 flu season, based on “data showing poor or relatively lower effectiveness of LAIV from 2013 through 2016.”

B. Pre-clinical studies for mucosal-immunisation strategies for other diseases

Disease	Study	Vaccine approach	Results
Respiratory viruses			
Herpes simplex virus (HSV)	Bernstein, 2019	A prime and pull strategy using glycoprotein vaccines to boost HSV immunity and intravaginal/topical imiquimod to pull these immune cells into the vaginal tract.	This prime and pull strategy significantly reduced recurrent genital HSV lesions and the shedding of HSV-2 into the vaginal tract.
Influenza B virus	McMahon, 2019	An intranasal vaccine with a recombinant influenza B virus neuraminidase	Guinea pigs vaccinated with neuraminidase showed reduced virus titers; however, only vaccination via the intranasal route fully prevented virus transmission to naive animals.
Influenza virus	Oh, 2021	An intranasally administered protein-based vaccine	Intranasal administration of mice led to the establishment of IgA-secreting cells in the lung, but not when given intramuscularly or intraperitoneally. Local IgA secretion correlated with superior protection against secondary challenge with homologous and heterologous virus infection than circulating antibodies alone.

[82] (Dhere, 2011) https://pubmed.ncbi.nlm.nih.gov/21684421/
[83] (Belshe, 2007) https://pubmed.ncbi.nlm.nih.gov/17301299/

Sarbecoviruses	Mao, 2022	A prime and spike strategy using an unadjuvanted intranasal spike vaccine booster	Prime and Spike induces robust T resident memory cells, B resident memory cells and IgA at the respiratory mucosa, boosts systemic immunity, and completely protects mice with partial immunity from lethal SARS-CoV-2 infection.
Genital viruses			
Herpes simplex virus (HSV)	Bernstein, 2019	A prime and pull strategy using glycoprotein vaccines to boost HSV immunity and intravaginal/topical imiquimod to pull these immune cells into the vaginal tract.	This prime and pull strategy significantly reduced recurrent genital HSV lesions and the shedding of HSV-2 into the vaginal tract.

4. Miscellany

- How effective is the mucosal immunity engendered by natural infection?

There is a paucity of longitudinal studies focused on SARS-CoV-2 antibody levels in the mucosa following natural infection, with a greater attention being paid to systemic immunity via serum sampling[84][85]. However, there are some studies that offer insights to the durability of mucosal immunity.

A longitudinal study by Chan et al.[86] collected nasal epithelial lining fluid (NELF) samples from SARS-CoV-2 patients up to 6-months post diagnosis to record mucosal IgA titres. It shows that NELF IgA was still detectable in at least 50% of the COVID-19 patients after three months (day 83-to-99) of diagnosis.

A paper by Isho et al.[87] shows the results from a longitudinal analysis of anti-SARS-CoV-2 antibody responses in serum and saliva following natural infection. It reveals that anti-SARS-CoV-2 IgA and IgM antibodies rapidly decayed, while IgG antibodies remained relatively stable up to 105 days post-symptom onset in both serum and saliva, confirming that serum and saliva IgG antibodies to SARS-CoV-2 are maintained in the majority of COVID-19 patients for at least 3 months.

Perhaps the most insightful work on the effectiveness of natural mucosal immunity is by Krammer et al.[88], which compared natural immunity to SARS-CoV-2 infection with that induced by current intramuscular vaccines. Their preprint shows that intramuscular vaccines elicit stronger mucosal immunity in individuals who have been previously infected by SARS-CoV-2 than in uninfected individuals. Similar findings were made by Anichini et al.[89] whose study shows that higher antibody levels are obtained after a single mRNA vaccine (BNT162b2) dose in persons with a previous natural SARS-CoV-2 infection than after two vaccine doses in previously uninfected subjects.

[84] (Dan, 2020) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7919858/
[85] (Seow, 2020) https://pubmed.ncbi.nlm.nih.gov/33106674/
[86] (Chan, 2022) https://www.mdpi.com/2076-0817/11/4/397/htm
[87] (Isho, 2020) https://www.science.org/doi/10.1126/sciimmunol.abe5511
[88] (Krammer, 2021) https://www.medrxiv.org/content/10.1101/2021.12.06.21267352v1.full-text
[89] (Anichini, 2021) https://www.nejm.org/doi/full/10.1056/NEJMc2103825#:~:text=These%20findings%20provide%20evidence%20that,have%20received%20a%20second%20dose.

There is insufficient data to confidently assess the effectiveness of mucosal immunity post infection, especially in light of emerging variants. From the two studies referenced above, it appears that mucosal immunity post vaccination is unreliable and short-lived. However, it appears likely that natural infection provides enough immune memory to be recalled by future systemic vaccination in order to provide sufficient levels of protection. Therefore, it could be the case that mucosal immunity from natural infection is inefficient in isolation, but could function as an efficient mucosal booster with systemic vaccination.

- Iran vaccine study – how durable is the sterilizing immunity reported after the IN boost?

Razi Vaccine and Serum Research Institute have developed a recombinant S protein vaccine (Razi Cov Pars), which has completed phase I (IRCT20201214049709N1) and phase Ⅱ (IRCT20201214049709N2) clinical trials and is now under phase III study (IRCT20210206050259N3) in Iran. It was granted Emergency Use Authorisation in Iran on 31 October 2021[90]. It is a protein subunit vaccine and is delivered in three doses: two injections and one nasal spray.

The Tehran Times reported[91] on 12 October 2021 that a nasal dose of the vaccine reduces the transmission of the virus by as much as 90 percent.

No data for any of the trials has been published.

- Re: Codagenix, how positive were the mucosal results?

Interim results for the Phase I clinical trial of Codagenix's CoviLiv intranasal vaccine were published as a press release in March 2022. Full Phase I data is expected mid-2022. Interim results showed that two intranasal doses of CoviLiv generated a robust serum (IgG) antibody response as well as induced mucosal immunity in the nose, with 40% of participants (there were c. 48 participants) presenting anti-Covid Immunoglobulin A (IgA) antibodies. As already discussed, IgA plays a critical role in mucosal immunity. Less than half of the participants producing IgA post vaccination is somewhat discouraging, especially as clinical trial participants are healthy adults and administration occurs within a clinical setting, therefore these results reflect the upper-bound for efficacy, which is very likely to decrease during real world application.

IgA antibody titres were not measured as part of CoviLiv's preclinical studies[92][93] for comparison.

- What's going on with pHOXWELL? How promising is it?

pHOXWELL, a nasal spray developed by pHOXBIO Ltd. is a novel prophylactic nasal spray[94]. A Phase II/III study assessed the efficacy and safety of pHOXWELL nasal spray in the prevention of SARS-CoV-2 infection in high-risk healthcare professionals in India. The trial was carried out during the peak surge of the highly infectious Delta variant in India in April to July 2021.

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90 https://www.tehrantimes.com/news/466570/Two-homegrown-vaccines-receive-emergency-use-license

91 https://www.tehrantimes.com/news/465937/Nasal-dose-of-COV-Pars-reduces-virus-transmission-by-90

92 https://www.tandfonline.com/doi/full/10.1080/22221751.2021.1971569

93 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8307828/

94 https://www.qmul.ac.uk/media/news/2021/smd/queen-mary-researchers-help-develop-nasal-spray-that-prevents-covid-19.html

Phase II/III results[95] showed 13.1% of those receiving the spray tested positive for SARS-CoV-2, compared to 34.5% of those who received the placebo treatment.

In its accompanying press-release, pHOXBIO said that its self-administered prophylactic nasal spray is designed to offer a robust, variant-agnostic mechanism of action and that it provides six to eight hours of protection with two sprays per nostril.

- To what extent is mRNA intranasal vaccination possible in a medium time-frame (approval in next 2-5 years)?

It is not yet clear whether the mRNA vaccines that have been critical for the vaccination campaign thus far could be effectively reformulated to be delivered intranasally. Only two intranasal vaccines currently in development involve mRNA, and neither of these are in clinical trials, nor are they intranasal variations of mRNA vaccines currently in clinical use.

In a recent article[96], Barney Graham, who led the team at the National Institute of Allergy and Infectious Diseases that designed the Moderna vaccine, noted of intranasal mRNA vaccines - “It may be possible but would take a lot of work and may require some new innovations. Delivery to the respiratory tract is complicated by the mucociliary blanket that covers the [upper] airways. There are other groups working on it.” In a similar vein, Florian Krammer, Endowed Professor of Vaccinology at the Icahn School of Medicine at Mount Sinai, said that intranasally administered mRNA vaccines aren’t likely to emerge soon, suggesting it might be a “next, next, next-gen vaccine”.

One of the most promising intranasal reformulations of mRNA currently in development is Xanadu bio’s ‘Prime and PACE’[97]. The company obtained a licence agreement from Yale University for the next generation polymeric nanoparticle delivery platform known as PACE. Additionally, the company has executed options with Yale University to intranasal delivery of PACE/Spike mRNA and Spike recombinant proteins.

In a preprint, Iwasaki et al. showed intranasal delivery of mRNA polyplexes mediates mucosal boosting in mice. To assess the safety and efficacy of PACE encapsulating mRNA encoding spike protein, mRNA was extracted from Comirnaty and encapsulated in PACE polyplexes. For vaccination, mice were injected with 1 µg intramuscular Prime (mRNA-LNP), and 14 days later received 1 µg of mRNA encapsulated in PACE and administered intranasally (PACE-Spike). Additional control groups included PACE-Spike only and intramuscular Prime + extracted mRNA without PACE encapsulation (naked mRNA). Similar with Prime and Spike, Prime and PACE-Spike induced antigen specific CD8+ TRM (IV-Tetramer+) expressing canonical tissue residency markers (CD69+ and CD103+). Additionally, PACE-Spike boosted mice developed high levels of bronchoalveolar lavage fluid (BALF) anti-SARS-CoV-2 IgA; levels of BALF IgG and serum IgA and IgG were similar to IM Prime only mice. Intramuscular Prime followed by intranasal naked mRNA was unable to induce mucosal or systemic immune responses above that of intramuscular prime alone indicating that mRNA encapsulation by PACE was required for mucosal boosting. Additionally, a single dose of intranasal PACE-Spike alone was insufficient to elicit any detectable mucosal or systemic antibody response at this dose.

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[95] https://phoxbio.com/#/addendum (No prepint/paper is currently available)
[96] https://www.statnews.com/2021/08/10/covid-intranasal-vaccines/
[97] https://www.biospace.com/article/releases/xanadu-bio-obtains-licenses-and-options-to-novel-platform-technologies-from-yale-university-to-develop-an-intranasal-sars-cov-2-mrna-vaccine-booster-aimed-at-inducing-local-mucosal-immunity-to-prevent-sars-cov-2-infection-and-virus-transmission/

A preclinical study led by Li et al.[98] explored the feasibility of using Venezuelan equine encephalitis virus (VEEV) replicon, a replicating mRNA, to prevent SARS-CoV-2 respiratory infection through nasal route. The vaccination platform aims to express neutralizing antibodies in the lung with replicating mRNA basing on alphavirus replicon particle (VRP) delivery system, to prevent SARS-CoV-2 infections. Results showed that the lung target delivery with the help of VRP system resulted in efficiently block SARS-CoV-2 infection with reducing viral titer and less tissue damage in the lung of mice.

In March 2020, eTheRNA immunotherapies NV (Niel, Belgium) announced[99] in a press release that a consortium had been formed with North American and European partners to develop a novel mRNA vaccine against SARS-CoV-2 to be administered intranasally and preclinical development had started. Their initial target was to commence clinical testing in early 2021. However, there have been no updates regarding the consortium's progress since the press release.

In comparison to other types of intranasal vaccines currently in development, research into intranasal mRNA vaccines is significantly scarcer. Despite the promising preclinical data for the Prime and PACE approach, no clinical data is yet available to make any meaningful interpretation of its performance in humans. Therefore, it appears to be unlikely that an intranasal mRNA will be approved in the next 2 to 5 years.

- How effective are viral vector-based vaccines compared to other intranasal platforms?

More than half of the SARS-CoV-2 intranasal vaccines in preclinical/clinical trials internationally are viral vector-based vaccines. This preference is likely the result of their incredibly immunogenic properties, versatility and capability for intracellular delivery[100]. As opposed to containing antigens like most vaccines, viral vector-based vaccines use the body's own cells to produce antigens. Viral vector vaccines infect the body's cells with a modified virus (the vector) that instructs the cells to produce antigens which triggers an immune response. In the case of SARS-CoV-2, the vector is the spike protein found on the surface of the virus, which delivers genetic code to the body's cells which then produce the required antigen. This process imitates natural infection, which is particularly advantageous since it triggers a strong cellular immune response via T cells and the production of antibodies via B cells.

Intramuscular viral vector vaccines currently in clinical use are AZ, J&J and Sputnik V. Whereas these three vaccines are all based on adenovirus, a number of viral vector intranasal candidates are based on influenza or parainfluenza viruses. Intramuscular viral vector vaccines have thus far been relatively easy to store and administer compared to other platforms., as well being incredibly immunogenic, even without the presence of adjuvants[101]. Whether viral vectors prove as immunogenic as an intranasal vaccine is yet to be fully demonstrated. However, it is particularly promising that four out of the six intranasal vaccines reaching the latest stages of clinical trials are viral vector vaccines.

- How effectively do intranasal vaccines produce an immune response in the lower respiratory tract?

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[98] (Li, 2021) https://www.nature.com/articles/s41392-021-00783-1

[99] https://epivax.com/news/press-release-etherna-consortium-cov-2-mrna-vaccine-for-high-risk-populations

[100] (Lavelle, 2022) https://www.nature.com/articles/s41577-021-00583-2#ref-CR55

[101] (Chang, 2022) https://pubmed.ncbi.nlm.nih.gov/35215973/

Preclinical studies have thus far demonstrated the potential of intranasal vaccines to elicit significant immune responses in the lower respiratory tract as well as the upper respiratory tract[102] of small animals:

Study	Vaccine Name	Results
Single intranasal dose		
Wu, 2020	Ad5-nCoV	A single dose administered intranasally was shown to protect mice completely against mouse-adapted SARS-CoV-2 infection in both the upper and lower respiratory tracts. No virus was detected in the lungs of all the intranasally vaccinated groups at 3 and 5 days post infection.
van Doremalen, 2021	ChAdOx1 nCoV-19	Intranasal vaccination of rhesus macaques resulted in reduced virus concentrations in nasal swabs and a reduction in viral loads in bronchoalveolar lavage and lower respiratory tract tissue.
Hassan, 2020 (mice) Bricker, 2021 (hamsters) Hassan, 2021 (rhesus macaques)	ChAd-SARS-CoV-2-S (Bharat version referred to as BBV154)	**Mice:** At day +7, in ChAd-SARSCoV-2-S-immunized animals, lower, if any, viral RNA was detected in the lung tissue compared to those receiving ChAd-Control. **Hamsters:** Both intranasal and intramuscular immunization reduced infectious virus titers and viral RNA levels in the lungs and nasal swabs of hamsters, albeit the effect was greater following intranasal immunization. No SARS-CoV-2-positive cells were detected in lung tissues of sacrificed hamsters following intranasal immunization. **Rhesus Macaques**: Intranasal delivery demonstrated remarkable protective efficacy as judged by an absence of infectious virus in the lungs and almost no measurable viral RNA in the lung.
Du, 2021	RBD subunit vaccine	In addition to the potent humoral immunity, the intranasal RBD vaccine in our study also induced significant mucosal immunity on the mucosal surfaces of the nasal cavity, lung, genital tract, and intestine. The sIgA secreted by the mucosal B cells in the nasal cavity (upper respiratory tract) and lung (lower respiratory tract) might have formed the first-line defense against viruses that enter through the respiratory tract, thereby preventing them from invading the cells.
Intranasal booster		
Iwasaki, 2022	Prime and Spike - Protein subunit & PACE encapsulating mRNA	Results demonstrated that Prime and Spike significantly reduced the viral load in the nasal cavity and the lung of mice compared to parenteral vaccine alone. Further, Prime and Spike led to significant protection from lung pathology with only 1 of 6 mice developing limited mononuclear infiltrates at 5 days post infection, while the remaining mice were completely protected with lung architecture similar to that seen in uninfected mice.
Shamseldin, 2022	Prime and Spike (BcfA)	Immunization with this heterologous vaccine prevented weight loss following challenge with mouse-adapted SARS-CoV-2 and reduced viral replication in the nose and lungs.

[102] The upper respiratory tract includes the nasal cavity, pharynx and larynx, whereas the lower respiratory tract contains the trachea, primary bronchi and lungs.

As this table demonstrates, it appears that significant immunity can be induced in the lower respiratory tract for both single-intranasal doses and intranasal boosters following intramuscular vaccines. This implies that intranasal vaccination has the potential to reduce viral loads in the lower respiratory tract independently, without relying on a priming intramuscular dose. However, this was not the case in Iwasaki *et al*.'s Prime and Spike study, which indicated that a single-dose unadjuvanted intranasal spike alone is not immunogenic, and that induction of a potent mucosal and systemic antibody response by unadjuvanted spike requires prior systemic priming, in this case by mRNA-LNP.

Furthermore, intranasal dosing may confer superior protection for the lower respiratory tract compared to intramuscular dosing. Hassan *et al.* compared intramuscular dosing of ChAd-SARS-CoV-2-S with intranasal dosing. With intramuscular dosing, despite the induction of high levels of neutralizing antibodies, neither dosing regimen (one or two intramuscular doses) completely protected against SARS-CoV-2 infection, as substantial levels of viral RNA were still detected in the lung. In comparison, a single intranasal dose of ChAd-SARS-CoV-2-S induced high levels of neutralizing antibody and anti-SARS-CoV-2 immunoglobulin A (IgA) and conferred virtually complete protection against infection in both the upper and lower respiratory tracts in mice. Similarly, van Doremalen *et al*. note that, at five days post infection, viral load and infectious virus titers were high in lung tissue of control animals, whereas they were unable to detect viral RNA or infectious virus in lung tissue from intranasally-vaccinated animals. Two animals in the intramuscular group were weakly positive for genomic RNA but not for subgenomic RNA or infectious virus.

Promisingly, these results appear to be replicable in transmission models, where animals are not inoculated with SARS-CoV-2 via intranasal administration, but rather via transmission from infected animals. This route offers a more realistic reflection of human exposure, particularly when measuring immunity in the lower respiratory tract, as virus particles can be inhaled directly into the lung as opposed to via the intranasal route. Hassan *et al*. investigated the vaccine efficacy of Ad5-nCoV in a transmission model and noted that intranasal vaccination resulted in a reduction in virus detection in swabs from sentinel hamsters compared to control animals and provided complete protection of the lower respiratory tract.

Despite promising results in animal models, it is uncertain whether this is replicable in humans. This limitation is largely due to there being no way of directly quantifying viral load in the lungs of humans during clinical trials. For preclinical trials, animals are euthanized in order to measure the viral RNA levels in the lung post infection. Bronchoalveolar lavage is also undertaken for animal models to collect samples from the lung for testing.

Annex A: Intranasal vaccines with clinical results and/or for vaccines that are already rolled out
(All records) in the first column below links to records on all forms of these vaccines.

Vaccine, type, manufacturer	Tested use	Participants	Location(s)	Research phase
Ad5-nCoV (Convidecia) Viral vector (adenovirus) CanSino (China)	2 doses intranasal CanSino or injection or 1 injection of CanSino followed by 1 intranasal; high or low dosage.	149 people.	China.	Phase 1. Results (for 130 people).

(All records)				
	High or low dose single intranasal dose of CanSino after 2 doses of Sinovac's CoronaVac (inactivated vaccine); or additional dose of CoronaVac.	420 people.	China.	Phase 1/2. Results.
	Single dose intranasal CanSino (low dosage) after 2 previous injections of inactivated vaccine (CoronaVac or Sinopharm's Beijing or Wuhan); or 3rd injection of inactivated vaccine.	10,420 people.	China.	Phase 3.
	Single dose of intranasal or intramuscular CanSino or injection of inactivated (CoronaVac) or protein subunit vaccine (ZF2001) as a booster after 2 injections of CoronaVac.	904 people.	China.	Phase 4. Results.
	Low dose intranasal CanSino followed by high dose intranasal.	10 non-human primates (macaques).	China.	Preclinical. Results.
AdCOVID Viral vector (adenovirus) AltImmune (USA) (All records)	1 or 2 intranasal doses of AdCOVID (3 dosages); or placebo.	92 people planned, but only 2 of 3 age cohorts recruited.	USA.	Phase 1. Results – press release only (vax discontinued).
	1 intranasal dose of AdCOVID	15+ mice (number unclear).	USA.	Preclinical. Results.
ChAdOx1 nCOV-19 Viral vector (adenovirus) AstraZeneca (AZ) (UK) or Serum Institute of India (as Covishield) (All records)	1 low, intermediate, or high dose intranasal AZ after previous course of Covid vax (2 injections of Moderna or Pfizer vax, or 1 injection of J&J).	15 people.	UK.	Phase 1.

	1 low, intermediate, or high dose intranasal AZ in previously unvaxed; or 1 low, intermediate, or high dose intranasal AZ after previous 2 injections of AZ or no booster; or 1 high dose intranasal AZ after previous 2 injections of Pfizer vax.	54 people.	UK.	Phase 1.
	1 or 2 doses of intranasal AZ or injection.	20 ferrets.	UK.	Preclinical. Results.
	1 dose of intranasal AZ or injection or control.	30 hamsters; 8 non-human primates (macaques).	UK.	Preclinical. Results.
CoviLiv Live attenuated Codagenix (USA) (All records)	1 dose of intranasal COVI-VAC after previous course of Covid vax (2 injections of Moderna or Pfizer vax, or 1 injection of J&J), in 3 dosages.	48 people.	UK.	Phase 1. Press release stating successful (without data) and progressing to phase 2/3. Results (conference abstract).
	Not yet announced	To be powered for 150 cases of Covid-19.	WHO Solidarity Trial – currently Colombia, Mali, Philippines.	Announcement of joining Solidarity Trial. (Protocol.)
	1 intranasal dose of COVI-VAC (plus controls).	39 hamsters.	USA.	Preclinical. Results.
GAM-COVID-VAC (Sputnik V) Viral vector (adenovirus) Gamaleya Research Institute (Russia) (All records – Sputnik V, Sputnik Light)	1 intranasal dose of either rAd26 (Sputnik Light) or rAd5, both (Sputnik V), or placebo.	400 people. (500 in phase 2 according to Russia's trials registry, 400 in Moscow + 260 elsewhere in Russia	Russia.	Phase 1/2.

		according to media report).		
	Compares intranasal Sputnik with injected (no further details).	1,320 people (according to Russia's trials registry).	Russia.	Phase 3 (or phase 2/3 – not clear).
Mambisa Protein subunit Centre for Genetic Engineering & Biotechnology (CIGB) (Cuba) (All records)	3 intranasal doses of Mambisa (drops) or 1 injection of Mambisa followed by 2 intranasal doses, each in 2 dosages & 2 different intervals.	88 people.	Cuba.	Phase 1/2.
	1 intranasal dose of Mambisa drops in 2 dosages or 1 dose of intranasal spray Mambisa, or 1 injection of CIGB's Abdala vaccine.	120 people who had recovered from Covid-19.	Cuba.	Phase 1/2. Results (press release only).
	1 intranasal dose of Mambisa or 1 injection of Abdala vaccine, after a previous 3-dose course of Abdala.	1,500 – 5,000 people.	Cuba.	Phase 2.
MVC-COV1901 Protein subunit Medigen (Taiwan) (All records)	1 intranasal dose of core component of MVC-COV1901 with nanoemulsion adjuvant after 2 previous injections of MVC-COV1901.	46 hamsters.	Taiwan.	Preclinical. Results. (See also results for the nanoemulsion adjuvant.)
Patria (NDV-HXP-S/AVX-COVID-12-HEXAPRO) Viral vector (Newcastle Disease Virus) Laboratorio Avi-Mex (Mexico) (All records on Patria, early development of NDV-HXP-S)	2 intranasal doses of Patria, 1 intranasal dose followed by 1 injection of Patria, or 2 injections of Patria, in 3 dosages.	91 people.	Mexico.	Phase 1. Results.

	1 intranasal or 1 intramuscular dose of Patria after previous course of Covid vax (AZ, CanSino, CoronaVac, J&J, Moderna, Pfizer, Sinopharm Beijing, or Sputnik V).	396 people.	Mexico.	Phase 2.
Razi Cov Pars Protein subunit Razi Vaccine & Serum Research Institute (Iran) (All records)	1 intranasal dose after 2 injections of Razi Cov Pars in 3 dosages, or adjuvant only.	133 people.	Iran.	Phase 1.
	1 intranasal dose after 2 injections of Razi Cov Pars, or adjuvant only.	500 people.	Iran.	Phase 2.
	1 intranasal dose after 2 injections of Razi Cov Pars, or injections of Sinopharm Beijing vaccine.	41,128 people.	Iran.	Phase 3. (Press report of results, in the first 24,000 participants.)
	1 intranasal dose after 2 injections of Razi Cov Pars.	210 people (adolescents).	Iran.	In 12-17 year-olds.

Annex B: Other intranasal vaccines – with preclinical results and/or in clinical trial

Vaccine name	Manufacturer/developer	Vaccine type	Research phase
Ad5-N	Beijing Institute of Basic Medical Sciences (China)	Viral vector (adenovirus)	Preclinical results
Ad5.SARS-CoV-2-S1	University of Pittsburgh (USA)	Viral vector (adenovirus)	Preclinical results
Ad5-S-nb2	Guangzhou Institutes of Biomedicine & Health & GuangzhounBiomed (China)	Viral vector (adenovirus)	Preclinical results
AdC7-RBD-tr2	Chengdu Kanghua Biological Products (China)	Viral vector (adenovirus)	Preclinical results
AdCoV2-S	National Health Research Institutes (Taiwan)	Viral vector (adenovirus)	Preclinical results

BBV154	Bharat Biotech (India)	Viral vector (adenovirus)	Phase 1 Phase 2 Phase 2/3 Phase 3 Phase 3 (See ChAd-SARS-CoV-2-S below for preclinical)
BCPIV/S-2PM	BioComo (Japan)	Viral vector (parainfluenza)	Preclinical results
BReC-CoV-2	West Virginia University (USA)	Toxoid vector (diphtheria)	Preclinical results
ChAd-SARS-CoV-2-S	Precision Virologics with Washington University in St Louis & NIAID (USA)	Viral vector (adenovirus)	Preclinical results (macaques) Preclinical results (hamsters) Preclinical results (mice) (See BBV154 above for clinical trials of Bharat version)
CNUHV03-CA22	Chungnam National University (South Korea)	Live attenuated	Preclinical results
COH04S1	City of Hope & NCI (USA)	Viral vector (Modified Vaccinia Ankara)	Preclinical results (See records for intramuscular version of this vaccine)
CuMVTT-RBD	Saiba Biotech (Switzerland)	Virus-like particles	Preclinical results
CVXGA1	CyanVac and University of Georgia (USA)	Viral vector (parainfluenza)	Phase 1 Preclinical results
GLS-5310	GeneOne Life Science (South Korea)	DNA	Phase 1 (Trials of other versions of this vaccine)
DelNS1-nCoV-RBD-OPT1	Beijing Wantai Biological (China)	Viral vector (influenza)	Phase 1 (Hong Kong) Phase 2 (Hong Kong) Phase 3 (40,000 in Colombia, Philippines, South Africa) Phase 3 (5,400 in Ghana)
ΔNA(RBD)-Flu	Fred Hutchinson Cancer Research	Viral vector (influenza)	Preclinical results

	Center & University of Washington (USA)		
hA5-Covid-19	ImmunityBio (USA)	Viral vector (adenovirus)	Preclinical results (See records for intramuscular version of this vaccine)
LP18:RBD	Chinese Academy of Agricultural Sciences Changchun (China)	Bacterium vector (Lactobacillus plantarum)	Preclinical results
MVA-SARS-2S	Institute of Immunology Hannover (Germany)	Viral vector (Modified Vaccinia Ankara)	Preclinical results
NDV-FLS	University of Guelph (Canada)	Viral vector (Newcastle Disease Virus)	Preclinical results
NDV-HXP-S	Icahn School of Medicine Mt Sinai (USA)	Viral vector (Newcastle Disease Virus)	Phase 1 Preclinical results (See records for intramuscular form of this vaccine – see also Butanvac & Patria in this post, as well as versions in Thailand and Vietnam)
NDV-HXP-S – Butanvac	Butantan Institute (Brazil)	Viral vector (Newcastle Disease Virus)	Preclinical results (See note above for the other versions of NDV-HXP-S)
NYVAC-KC-pfsSpike	Arizona State University & University of Iowa (USA)	Viral vector (NYVAC-KC)	Preclinical results
pQAC-CoV	University of Wisconsin-Madison & Pan Genome Systems (USA)	DNA	Preclinical results
pSpike/PP-sNp	Third Military Medical University, Chongqing (China)	DNA	Preclinical results
rNDV-S	Lancaster University & Texas Biomedical Research Institute (USA)	Viral vector (Newcastle Disease Virus)	Preclinical results (See also results for other form)
rVSVSARS-CoV-2	Chinese Academy of Sciences & Shenzhen Kangtai Biotechnology (China)	Viral vector (VSV)	Preclinical results
SC-Ad6-1	Mayo Clinic & Tetherex Pharmaceuticals (USA)	Viral vector (adenovirus)	Phase 1 Preclinical results

scPR8-RBD-M2	BIOTEC, NSTDA & Chulalongkorn University (Thailand)	Viral vector (influenza)	Preclinical results
(Unnamed)	Abera Bioscience (Sweden), Vrije Universiteit Amsterdam (Netherlands) & Johns Hopkins University (USA)	Extracellular vesicles	Preclinical results
(Unnamed)	Academy of Military Sciences & Chinese Academy of Sciences (China)	Live attenuated	Preclinical results
(Unnamed)	ACM Biolabs (Singapore)	Protein subunit	Preclinical results
(Unnamed)	BioTechMed-Graz & University of Graz (Austria)	Extracellular vesicles (OMV)	Preclinical results
(Unnamed)	FARVET, Cayetano Heredia University (Peru)	Viral vector (Newcastle Disease Virus)	Preclinical results
(Unnamed)	Chungbuk National University (South Korea)	Protein subunit	Preclinical results
(Unnamed)	Georgia Institute of Technology & Emory University (USA)	Protein subunit	Preclinical results
(Unnamed)	ID Pharma & National Institute of Infectious Diseases (Japan)	Viral vector (F-deleted Sendai virus)	Preclinical results
(Unnamed)	Intravacc (Netherlands)	Extracellular vesicles (OMV)	Preclinical results
(Unnamed)	Mahidol University & Institute of Biological Products (Thailand)	Protein subunit	Preclinical results
(Unnamed)	McMaster University (Canada)	Viral vector (adenovirus)	Preclinical results
(Unnamed)	National Cancer Institute NIH (USA)	Protein subunit	Preclinical results
(Unnamed)	National Institute of Allergy & Infectious Diseases (NIAID) NIH (USA)	Viral vector (parainfluenza)	Preclinical results
(Unnamed)	Oragenics & National Research Council (Canada)	Protein subunit	Preclinical results
(Unnamed)	Shanghai Bovax Biotech & GeneSail Biotech (China)	Viral vector (adenovirus)	Preclinical results
(Unnamed)	Sichuan University (China)	Protein subunit	Preclinical results
(Unnamed)	Stanford University (USA)	DNA	Preclinical results

(Unnamed)	State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, Xiamen (China)	Viral vector (influenza)	Preclinical results
(Unnamed)	Sun Yat-Sen University & Chinese Academy of Sciences (China)	Protein subunit	Preclinical results
(Unnamed)	Texas Biomedical Research Institute (USA)	Live attenuated	Preclinical results
(Unnamed)	TheraVectys & Pasteur Institute (France)	Viral vector (Lentivirus)	Preclinical results (mice) Preclinical results (mice) Preclinical results (mice, hamsters)
(Unnamed)	University of California Los Angeles (UCLA) (USA)	Bacterium vector (ΔcapB)	Preclinical results
(Unnamed)	University of Hong Kong (Hong Kong)	Protein subunit	Preclinical results
(Unnamed)	University of Hong Kong (Hong Kong)	Live attenuated	Preclinical results
(Unnamed)	University of Houston & BD Biosciences	Protein subunit	Preclinical results
(Unnamed)	University of Manitoba (Canada)	Viral vector (recombinant VSV)	Preclinical results
(Unnamed X 2)	Yale University & Xanadu Bio (USA)	Protein subunit & PACE encapsulating mRNA	Preclinical results
(Unnamed)	Yunnan Key Laboratory & IMBCAMS (China)	Peptide	Preclinical results
VSV-SARS2-EBOV	National Institute of Allergy and Infectious Diseases (NIAID) NIH & University of California Irvine (USA)	Viral vector (VSV)	Preclinical results (hamsters) Preclinical results (macaques)

NYVAC-KC = a vaccinia virus; LVS ΔcapB = a live vaccine strain with a deletion in capB, from a vaccine against tularemia; OMV = outer membrane vesicles; PACE = Poly(amine-coester)s; VSV = vesicular stomatitis virus.

Source:
https://absolutelymaybe.plos.org/2022/03/30/the-race-to-reduce-covid-19-transmission-an-update-on-66-intranasal-6-oral-vaccines/