



## Mucosal delivery of RNA vaccines by Newcastle disease virus vectors

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### ABSTRACT

The rapid evolution of SARS-CoV-2 since its pandemic outbreak has underscored the need for improved SARS-CoV-2 vaccines that efficiently reduce not only hospitalizations and deaths, but also infections and transmission. This might be achieved by a new generation of intranasally administered SARS-CoV-2 vaccines to stimulate protective mucosal immunity. Among all different approaches, preclinical and clinical information using Newcastle Disease Virus (NDV)-vectors expressing S of SARS-CoV2 as a COVID-19 vaccine show the potential of this vaccine platform as an affordable, highly immunogenic, safe strategy to intranasally vaccinate humans against SARS-CoV-2 and other infectious diseases. These vaccine vectors consist on the use of a harmless avian negative strand RNA virus to deliver intranasally a self-replicating RNA expressing the vaccine antigen in the cells of the respiratory mucosa. The vector also incorporates the antigen in the virus particle used for RNA delivery, thus combining the properties of nanoparticle-based and RNA-based vaccines. Other advantages of NDV-based vectors include the worldwide availability of manufacturing facilities for their production and their stability at non-freezing temperatures. While phase 3 clinical studies to evaluate efficacy are still pending, phase 1 and 2 clinical studies have demonstrated the safety and immunogenicity of NDV-S vaccines against SARS-CoV-2.

### 1RNA vaccines and the COVID-19 pandemic

One of the greatest success in the history of vaccination has been the rapid generation and deployment of mRNA vaccines able to prevent hospitalization and severe disease by SARS-CoV-2, the causative agent of the COVID-19 pandemic (Polack et al., 2020; Baden et al., 2021). Even though several other COVID-19 vaccines have also been shown to be efficacious in preventing disease and have contributed to mitigate the consequences of the COVID-19 pandemic, mRNA vaccines represent one the newest technologies on vaccine platforms, and have been widely used in two slightly different formulations according to the primary manufacturer, Pfizer/BionTech or Moderna.

The principle of the mRNA vaccines consists in the intramuscular injection of an mRNA encoding a vaccine antigen, in the case of SARS-CoV-2, the S protein, leading to its translation into protein that is presented to the immune system, resulting in the stimulation of antigen-specific T and B cells, generating protective antibody and T cell responses. Despite the apparent simplicity of the mRNA vaccines, their success was only possible due to pre-pandemic research to optimize chemically the mRNA to prevent excessive innate responses and improve its half-life, and to identify appropriate formulations (nanoparticles) for the delivery of the mRNA into the cytoplasm of cells once

intra-muscularly injected. This made possible that the mRNA vaccine technology was mature enough to be rapidly deployed for clinical development of a SARS-CoV-2 vaccine through phase 1, 2 and 3 clinical trials to assess safety, immunogenicity and efficacy.

We know now that mRNA vaccines expressing SARS-CoV-2 S induce high levels of SARS-CoV-2 neutralizing antibodies in sera able to protect from SARS-CoV-2 replication in the lung, preventing severe disease (Corbett et al., 2021; Meschi et al., 2021). Other approved SARS-CoV-2 vaccine platforms, such as those based on adenovirus vectors (Sadoff et al., 2021; Logunov et al., 2021; Ramasamy et al., 2021) and on recombinant protein (Heath et al., 2021), also result in the induction of sera neutralizing antibodies and protection from disease. However, a combination of waning antibodies with time and of immune evasion in the form of new SARS-CoV-2 variants that are more resistant than the vaccine homologous variants to vaccine-induced antibody neutralization, make re-vaccinations necessary to maintain levels of protection. Moreover, the capacity of the existing intramuscular vaccines, including the mRNA vaccines, to generate protective immunity in the upper respiratory tract, as opposed to the lung, is limited, resulting in poor levels of protection against infection and transmission of the new variants. While current vaccines are still very useful in preventing hospitalizations, improved vaccines inducing protection of both the upper and

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lower respiratory tracts are desirable. Another disadvantage of mRNA vaccines is the need of a cold freezing chain for their distribution, encumbering their use in countries with limited resources. Thus, safe, stable without freezing and efficacious mucosal vaccines will be preferred over the existing SARS-CoV-2 vaccines (Fig. 1).

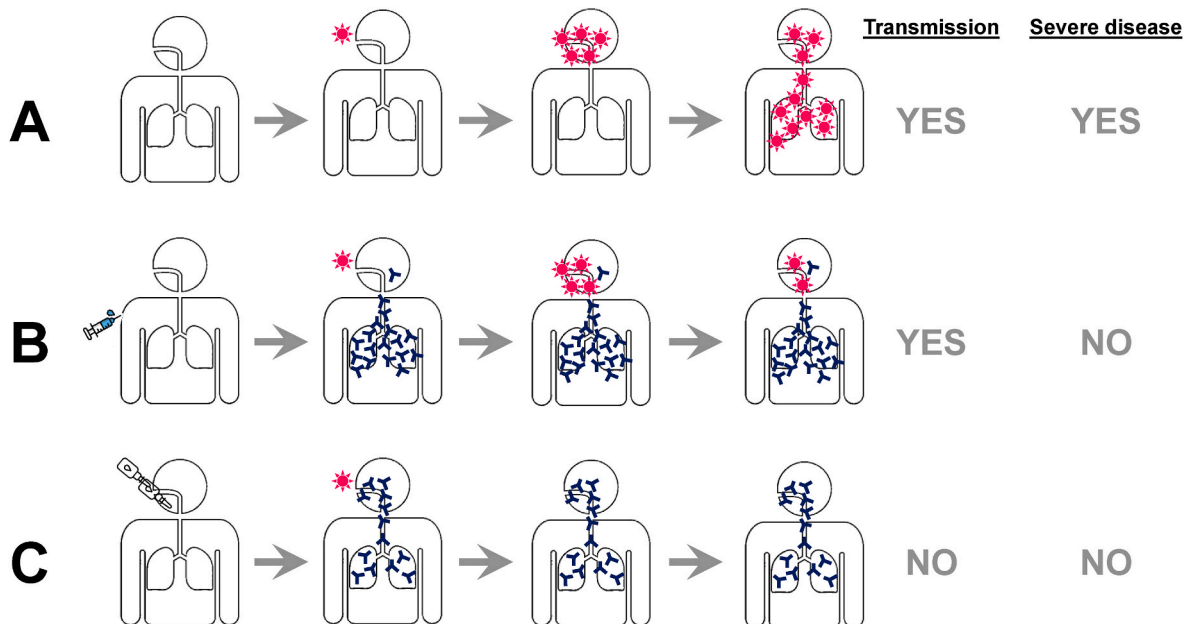
## 2 Mucosal respiratory vaccines

Influenza virus vaccines are the first respiratory virus vaccines that were developed. Prior to SARS-CoV-2, influenza virus represented the most important viral cause of respiratory disease in humans, responsible of approximately 500,000 deaths per year despite available vaccines and antivirals. In addition, influenza virus has caused four pandemics since 1900, including the 1918 flu pandemic with an estimated 40–60 millions of deaths worldwide. Most common current influenza virus vaccines are the so-called split vaccines and consist in preparations of partly purified hemagglutinin (HA), the functional equivalent to the S protein of SARS-CoV-2, generated from inactivated viruses previously cultured in embryonated eggs. These vaccines are administered intramuscularly, induce influenza virus neutralizing antibodies in sera that prevent severe disease, but need to be administered annually due to the short duration of protective immunity combined with the antigenic variation of circulating influenza viruses from year to year. In addition, the protection decreases in one of the most vulnerable risk groups, the elderly (Krammer et al., 2018). This somehow resembles to the situation with the current SARS-CoV-2 vaccines. Nowadays, there are also split influenza virus vaccines derived from tissue-culture, recombinant HA-based vaccines and vaccines specific for the elderly based on a higher dose of split vaccine or on the co-administration of the split vaccine with an adjuvant (Becker et al., 2021). Despite these improvements, all these vaccines are administered intramuscularly, leading to limited mucosal immunity, and still require annual revaccinations. There is a mucosal influenza virus vaccine that is administered intranasally based on an attenuated virus strain that can only grow at the lower temperature of the upper respiratory tract, but is only used for kids and young adults, as its efficacy is very limited in adults with increased levels of pre-existing

influenza virus immunity (Belshe et al., 2004). Before COVID-19, and with the exception of adenovirus vaccines used in the military, there have not been any other approved human vaccine for a respiratory virus, including respiratory syncytial virus, coronaviruses, paramyxoviruses, rhinoviruses and respiratory enteroviruses. Although effective mucosal respiratory vaccines might be the best solution to reduce the burden in humans of viral respiratory disease, there is a lack of understanding on what constitutes safe and effective intranasal vaccine formulations. In fact, an attempt on an intranasal inactivated influenza virus vaccine co-administered with a mucosal adjuvant needed to be stopped due to the incidence of Bell's palsy cases in a percentage of vaccinees (Mutsch et al., 2004).

## 3 SARS-CoV-2 intranasal vaccines

Because of the previously discussed limitations of the current intramuscular SARS-CoV-2 vaccines, there are several approaches under preclinical or clinical investigation towards an intranasal SARS-CoV-2 vaccine with increased efficacy in preventing infection and transmission. These include adenovirus, influenza virus, Newcastle disease virus (NDV), paramyxovirus, bacterial vector vaccines, recombinant protein vaccines, and genetic vaccines, including RNA replicons and mRNA vaccines. However, there are several challenges that need to be surmounted for the generation of an effective SARS-CoV-2 intranasal vaccine. For those that are vector-based, one challenge is pre-existing immunity. This has been illustrated by the limited use of the live attenuated influenza virus vaccine due to influenza pre-existing immunity that limits the replication and antigen expression of the vaccine vector. A second challenge is safety. The olfactory bulb represents a possible access to the CNS, and due to its proximity to the upper respiratory epithelia, needs to be avoided by any means of intranasal vaccination. In addition, reactogenicity in the respiratory mucosa is less likely to be tolerated than that induced by intramuscular vaccines. New formulations are then likely needed for recombinant protein and mRNA vaccines to avoid excessive side effects associated with their intramuscular formulations while preserving their immunogenicity. In this



**Fig. 1.** Impact of vaccination in respiratory virus transmission and disease. **A.** Exposure of unvaccinated people with no pre-existing immunity to SARS-CoV-2 results in virus replication in the upper respiratory tract (associated with virus transmission) and subsequent invasion of the lungs, where virus replication can lead to excessive inflammation, systemic dissemination and severe disease. **B.** Intramuscular vaccination results in a systemic immune response characterized by neutralizing antibodies in blood. Serum antibodies reach the lung, preventing virus replication in the lung and severe disease. Serum antibodies are less likely to reach the upper respiratory tract, leading to breakthrough infections upon SARS-CoV-2 exposures and transmission. **C.** Intranasal vaccination results in mucosal antibodies in the upper respiratory tract that protect from infection, blocking virus transmission, virus invasion of the lung and severe disease.

article, I will focus on the potential use of NDV vectors as intranasal vaccines based on their safety profile and lack of vector pre-existing immunity, as well as in preclinical and clinical studies.

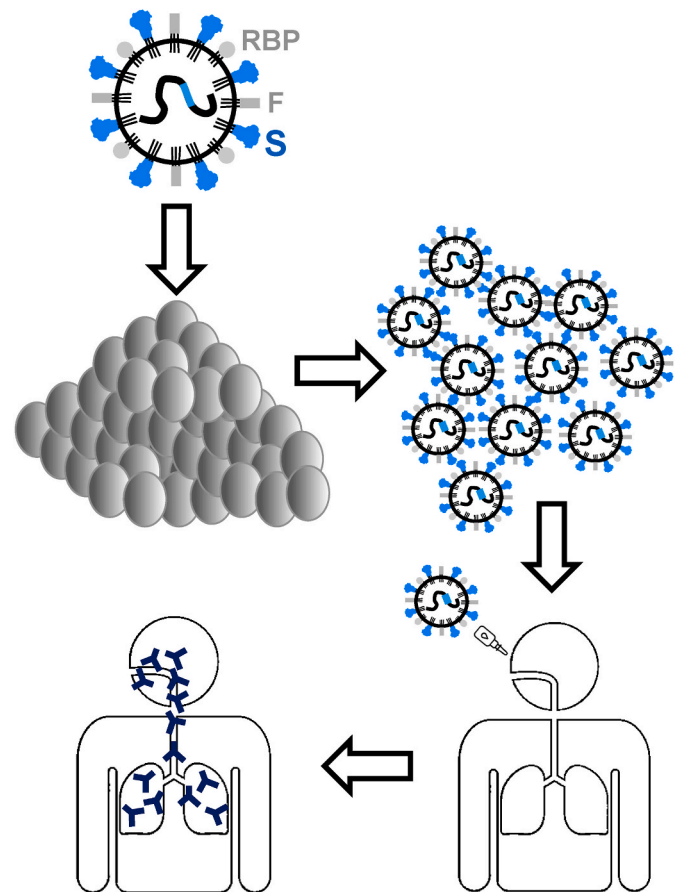
#### 4NDV-based SARS-CoV-2 vaccines

NDV is an avian paramyxovirus. Different strains of NDV cause different degrees of disease in poultry, with the more virulent ones causing a fulminant severe disease, and the less virulent leading to asymptomatic infections. Poultry is vaccinated against Newcastle disease with live attenuated NDV vaccines consisting of naturally circulating avirulent strains of NDV. NDV vaccines are produced in embryonated eggs and administered to chicken orally, ocularly or in the form of aerosols. The genetic material of NDV consists of a single molecule of RNA that is being transcribed in infected cells into six mRNAs by the viral RNA dependent RNA polymerase. Among the encoded viral proteins, the F and HN proteins are incorporated into the viral envelope and are involved in entry by binding to sialic acids and fusing the viral membrane with the plasma membrane of the infected cell. Because the receptor (sialic acids) is present in all vertebrates, the virus is able to enter both avian and mammalian cells, however, replication of the virus in mammalian cells appears to be limited to a few rounds of infection, as the virus is quickly stopped by innate immune responses. By contrast, in avian species, the virus is able to down modulate avian innate responses (Park et al., 2003). This explains why NDV does not cause disease in mammals and why there is no pre-existing immunity against NDV in mammals, including humans. These two characteristics are ideal for a viral vaccine vector.

In fact, NDV vaccine vectors, in which the genome of NDV has been engineered to express a novel antigen that confers protection against a disease, such as the S protein of SARS-CoV-2, might be considered as a different formulation of an RNA vaccine, in which instead of a nanoparticle, the RNA is being delivered by a viral vector. As NDV has self-adjuncting properties and is safe for intranasal vaccinations in humans, the use of intranasal NDV vaccines might overcome the challenge of finding a safe formulation of an intranasal mRNA vaccine. Preclinical studies in animals have shown SARS-CoV-2 S-expressing NDV vectors to be highly immunogenic and protect both the nose and the lung from infection with SARS-CoV-2 (Sun et al., 2020a, 2020b, 2021). Moreover, clinical studies in humans have confirmed the immunogenicity, safety and lack of reactogenicity of both live and inactivated NDV vaccines (Pitisuttithum et al., 2022; Duc Dang et al., 2022; Ponce-de-Leon et al., 2022).

Modifications that stabilize the S antigen have been key to the high immunogenicity of NDV vectors expressing SARS-CoV-2 S. These include i) its stabilization by introducing key proline residues (Hsieh et al., 2020) and by eliminating the furin cleavage site of S required for its membrane fusogenic functions, and ii) its incorporation into the NDV viral membrane by virtue of substituting the cytoplasmic and transmembrane domains of S by those of the F NDV protein, allowing the incorporation of S into the envelope of NDV, together with its own F and HN proteins. This is in contrast to the adenovirus vectors, that lack membrane and therefore only express S after delivering its genetic material inside cells. Phase 1 and 2 studies conducted in Thailand and Vietnam with intramuscular inactivated NDV have shown high immunogenicity and safety (Pitisuttithum et al., 2022; Duc Dang et al., 2022), and such studies are now also being conducted in humans with a live intranasal formulation (Fig. 2) in Mexico and US. As intranasal vaccines have been shown to induce great levels of mucosal immunity against SARS-CoV-2 in animal models primed with an intramuscular vaccine (Mao et al., 2022), intranasal NDV-S vaccines might optimally boost mucosal and systemic immunity in humans previously immunized with intramuscular S vaccines, including mRNA and adenovirus-based vaccines.

One caveat of live viral vector vaccines is their limitations if additional vaccinations are required. One expects pre-existing immunity



**Fig. 2.** NDV-based SARS-CoV-2 vaccines. An NDV vaccine vector has been engineered containing one additional transcriptional unit in its RNA genome encoding a stabilized form of the S of SARS-CoV-2. The vector incorporates the S protein into virus particles together with the two other glycoproteins of NDV, the NDV Receptor Biding Protein (RBP, previously designated as HN) and the NDV Fusion (F) protein. This “live” vector also delivers its RNA genome when in contact with cells, resulting in S protein expression. The NDV vaccine vector is manufactured in embryonated eggs and we are investigating whether intranasal administration of this vector in humans is safe and tolerated and induces protective antibody and T cell responses against the S protein in the respiratory mucosa, blocking transmission and preventing COVID-19 disease.

against the vaccine vector to prevent their efficient use in subsequent vaccinations. This limitation can be at least mitigated in the case of NDV vectors by changing the vaccine strain to another one based on an antigenically different avian paramyxovirus (APMV). There are more than 20 antigenically diverse APMVs (Liu et al., 2022), NDV being APMV-1, allowing the overcoming of pre-existing immunity, if needed, by replacement of the vaccine strain with a different serotype.

NDV vaccine vectors can be produced in tissue culture or in embryonated eggs. As such, existing influenza virus vaccine manufacturers can be used to generate doses of NDV-based vaccines. As many countries, including those with low resources, have influenza virus vaccine manufacturing capability, they can repurpose their facilities to generate enough NDV vaccine doses for their citizens. Moreover, the virus is highly stable at 4°, avoiding the need of congelation and facilitating distribution to costumers. Multivalent NDV-vaccines are also possible, and we recently demonstrated in an animal model enhanced protection against omicron variants of SARS-CoV-2 of multivalent NDV-S vaccines based on multiple SARS-CoV-2 variants previous to the omicron one (Gonzalez-Dominguez et al., 2022). While both the preclinical and clinical data generated up until now appears highly promising, efficacy human clinical trials will be needed to demonstrate the levels of protection achieved by NDV-S vaccination.



## 5 Additional NDV-based vaccines

In addition to SARS-CoV-2, NDV vaccine vectors have also been investigated as vaccines against other pathogens. NDV vaccine vectors expressing avian influenza virus HAs have been developed as dual vaccines against Newcastle disease and avian influenza in poultry (Nagy et al., 2016; Ma et al., 2017; Liu et al., 2015) and being used as veterinary vaccines in Mexico. NDVs-expressing F of RSV have been shown to be protective in mice and cotton rats against RSV challenge (Martinez-Sobrido et al., 2006; Grieves et al., 2018). Other NDV-based vaccines with encouraging preclinical data include those expressing GP from Ebola virus (DiNapoli et al., 2010) and Gn/Gc from Rift valley virus (Kortekaas et al., 2010). Finally, due to its immunomodulatory properties, NDV has also been shown to cure tumors in mice when used intratumorally by increasing inflammation in the tumor, and improving T cell elimination of tumors (Zamarin et al., 2009). Future clinical studies will be needed to see whether the potential of an NDV/APMV-based vaccine platform for infectious diseases and cancer is fulfilled.

## Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The A.G.-S. laboratory has received research support from Pfizer, Senhwa Biosciences, Kenall Manufacturing, Blade Therapeutics, Avimex, Johnson & Johnson, Dynavax, 7Hills Pharma, Pharmamar, ImmunityBio, Accurius, Nanocomposix, Hexamer, N-fold LLC, Model Medicines, Atea Pharma, Applied Biological Laboratories and Merck. A. G.-S. has consulting agreements for the following companies involving cash and/or stock: Vivaldi Biosciences, Contrafact, 7Hills Pharma, Avimex, Vaxalto, Pagoda, Accurius, Esperovax, Farmak, Applied Biological Laboratories, Pharmamar, Paratus, CureLab Oncology, CureLab Veterinary, Synairgen and Pfizer. A.G.-S. has been an invited speaker in meeting events organized by Seqirus, Janssen, Abbott and Astrazeneca. A.G.-S. is inventor on patents and patent applications on the use of antivirals and vaccines for the treatment and prevention of virus infections and cancer, owned by the Icahn School of Medicine at Mount Sinai, New York.

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