

Review

Mucosal COVID-19 vaccines in clinical development

Alina Tscherne^{a,*}, Weina Sun^b, Sean T.H. Liu^{b,c}, Florian Krammer^{a,b,d,e,*}^a Ignaz Semmelweis Institute, Interuniversity Institute for Infection Research, Medical University of Vienna, Vienna, Austria^b Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA^c Division of Infectious Diseases, Icahn School of Medicine at Mount Sinai, New York, NY, USA^d Center for Vaccine Research and Pandemic Preparedness (C-VarPP), Icahn School of Medicine at Mount Sinai, New York, NY, USA^e Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

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ABSTRACT

Mucosal vaccines are designed to elicit both a strong systemic and mucosal immune response gaining importance as the next generation of vaccines to combat the respiratory coronavirus disease 2019 (COVID-19). The ability of these vaccines to induce mucosal immune responses in the upper respiratory tract may allow efficient prevention of infection and transmission, which could potentially reduce virus circulation in the population. In addition, they have the advantage that they can be administered by non-medical personnel and without needles. Several preclinical studies in small animal models and non-human primates, but also early phase clinical studies confirmed the capability of mucosal COVID-19 candidate vaccines to induce long-lasting immunity and to provide protection against an infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In addition to five vaccines which are already approved/licensed, at least 28 mucosal COVID-19 vaccines, based on different vaccine platforms, are currently being tested in clinical trials. However, clinical data for most of the conducted and completed clinical trials are not publicly available yet. In addition, several initiated trials have been terminated or were withdrawn. In this review, we aim to discuss the advantages and disadvantages of mucosal COVID-19 vaccines and to summarize the current status of mucosal COVID-19 vaccines in clinical development, with an emphasis on the most advanced candidates and the key findings observed in preclinical animal models and clinical studies.

1. Introduction

The groundbreaking success of licensed, first-generation coronavirus disease 2019 (COVID-19) vaccines early in the pandemic led to reduced infection and mortality rates, saving millions of lives. However, these vaccines were not designed to induce robust immunity in the upper respiratory tract. The lack of a robust mucosal immune response to efficiently block viral replication, and the continuous virus evolution and immune escape, contributed to breakthrough infections in fully immunized individuals [1–3]. To counter virus evolution, vaccine formulations have to be adapted to new circulating variants regularly. However, the vaccine side effects, in addition to fear of needles, and general vaccine hesitancy, contributed to decreasing vaccination rates in the population [4,5]. Approved COVID-19 vaccines are mainly administered intramuscularly (IM) [6], which triggers a robust systemic cellular and humoral immune response. However, several studies demonstrated their inability to induce a robust mucosal immune

response in the upper respiratory tract [7–10] (Fig. 1). Thus, there is a need to develop innovative vaccines, which are capable to induce not only a systemic immune response, but also a strong upper and lower respiratory mucosal immune response, including tissue resident memory T cells (Trm) and secretory IgA (sIgA) antibodies. This is of importance for respiratory viruses, such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as the primary infection side includes tissues from both the lower and upper respiratory tract, depending on the viral entry side (e.g., intranasal, aerosol inhalation) [11,12].

sIgA is a polymeric form of IgA, consisting of two monomeric IgA, a joining (J) chain and a secretory component. The polymeric nature of sIgA can compensate for lower affinity binding through higher avidity and is therefore more crossreactive towards viral variants. Furthermore, polymeric immunoglobulin receptor (pIgR) recognizes and transports polymeric IgA across the mucosal epithelium [for review: [13]], where sIgA is thought to contribute to a faster clearance of respiratory viruses, such as SARS-CoV-2 [14]. Thus, the design and administration of

* Corresponding author at: Ignaz Semmelweis Institute, Interuniversity Institute for Infection Research, Medical University of Vienna, Vienna, Austria.

E-mail addresses: alina.tscherne@meduniwien.ac.at (A. Tscherne), florian.krammer@meduniwien.ac.at, florian.krammer@mssm.edu (F. Krammer).

mucosal COVID-19 vaccines may prevent infection and onward transmission, potentially reducing virus circulation. Mucosal vaccines have already been tested successfully against other mucosal pathogens, such as influenza virus, rotavirus, *Vibrio cholerae* or *Salmonella typhi* [15,16]. In addition, several COVID-19 vaccines administered via the mucosal route are currently being tested in preclinical and clinical trials. As of July 2025, five mucosal COVID-19 vaccines (inCOVACC®, Convidecia Air®, Sputnik V, Pneucolin®, RAZI-COV PARS) have already received approval for human use in China, Russia, India, Iran and Indonesia and are reviewed here [17].

Mucosal vaccines use several vaccine platforms, including viral-vectored vaccines (replicating and non-replicating viruses), whole virus vaccines (inactivated and live attenuated viruses), protein subunit vaccines or nucleic acid-based vaccines (mRNA- and DNA-based), using mainly oral (O), intranasal (IN), or inhalation (IH) routes for application

(Fig. 2).

The public access to preclinical and clinical data is limited (summarized in Tables 1–3), as results for many of the conducted studies have not yet been published. Furthermore, several clinical studies were terminated due to poor enrollment or were withdrawn due to changes in the development plans. In addition, some candidates were discontinued because of poor immunogenicity, highlighting the difficulties encountered when developing mucosal vaccines. In this review, we aim to provide a comprehensive overview of the advantages and disadvantages of mucosal COVID-19 vaccines, the current status of mucosal COVID-19 vaccines in clinical development, highlighting in particular the efficacy and immunogenicity of the most advanced candidates obtained in both preclinical and clinical studies.

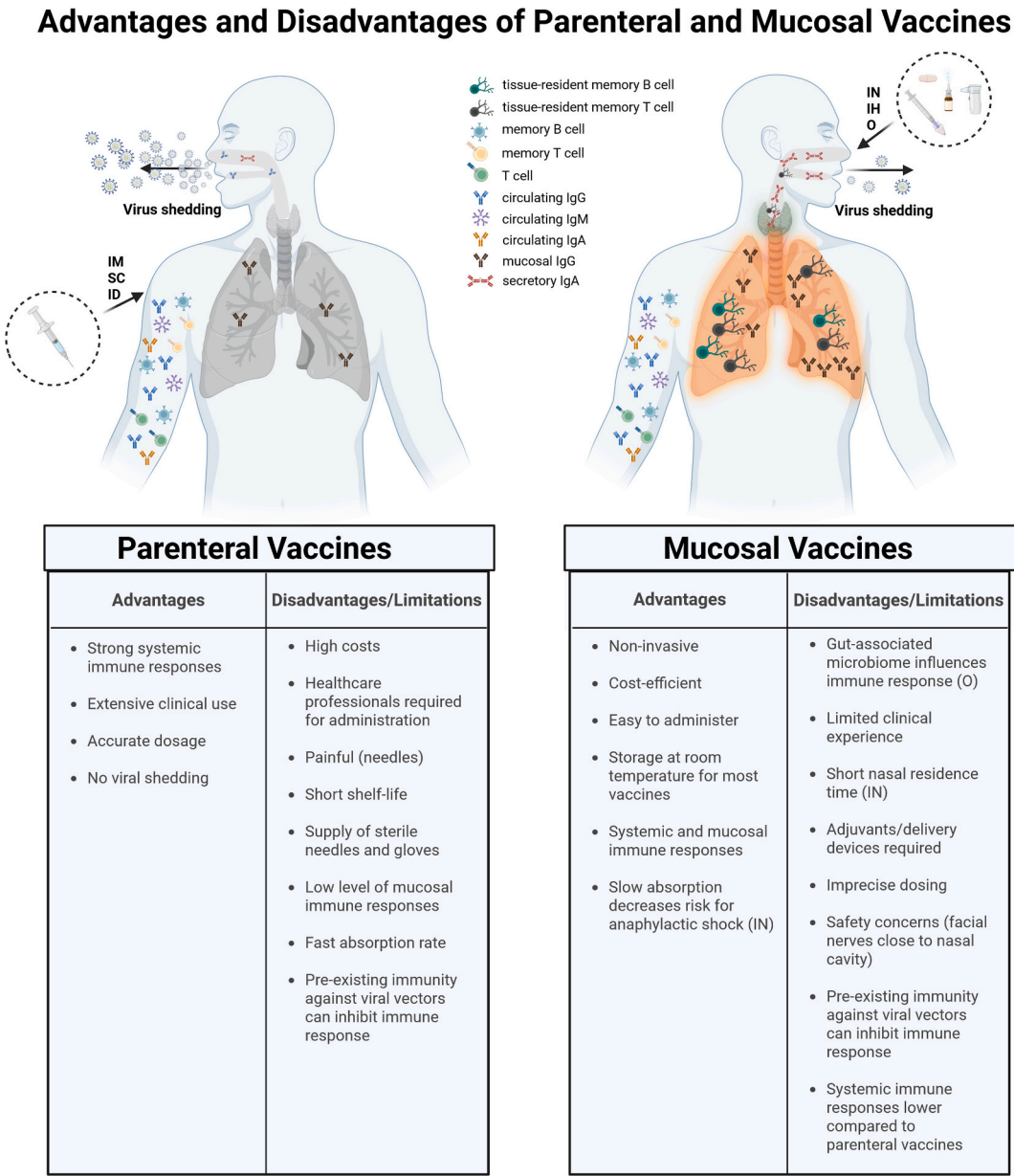


Fig. 1. Immune responses and advantages/disadvantages of parenteral and mucosal vaccines. Administration via the parenteral route results in the induction of a strong systemic immune response, involving circulating antibodies (IgG, IgM, IgA), antigen-specific T cells, and memory T and B cells. Contrarily, mucosal immunization induces a systemic and mucosal immune response. Tissue-resident memory T and B cells localize within the mucosal associated lymphoid tissues of the upper and lower respiratory tract, providing a rapid recall response upon antigen recognition, thus, viral shedding is reduced compared to parenteral administration. IN: intranasal; IH: inhalation; O: oral; IM: intramuscular; SC: subcutaneous; ID: intradermal. Adapted from [67]. Created in <https://BioRender.com/3gllkka2>.

Platforms for Mucosal COVID-19 Vaccines

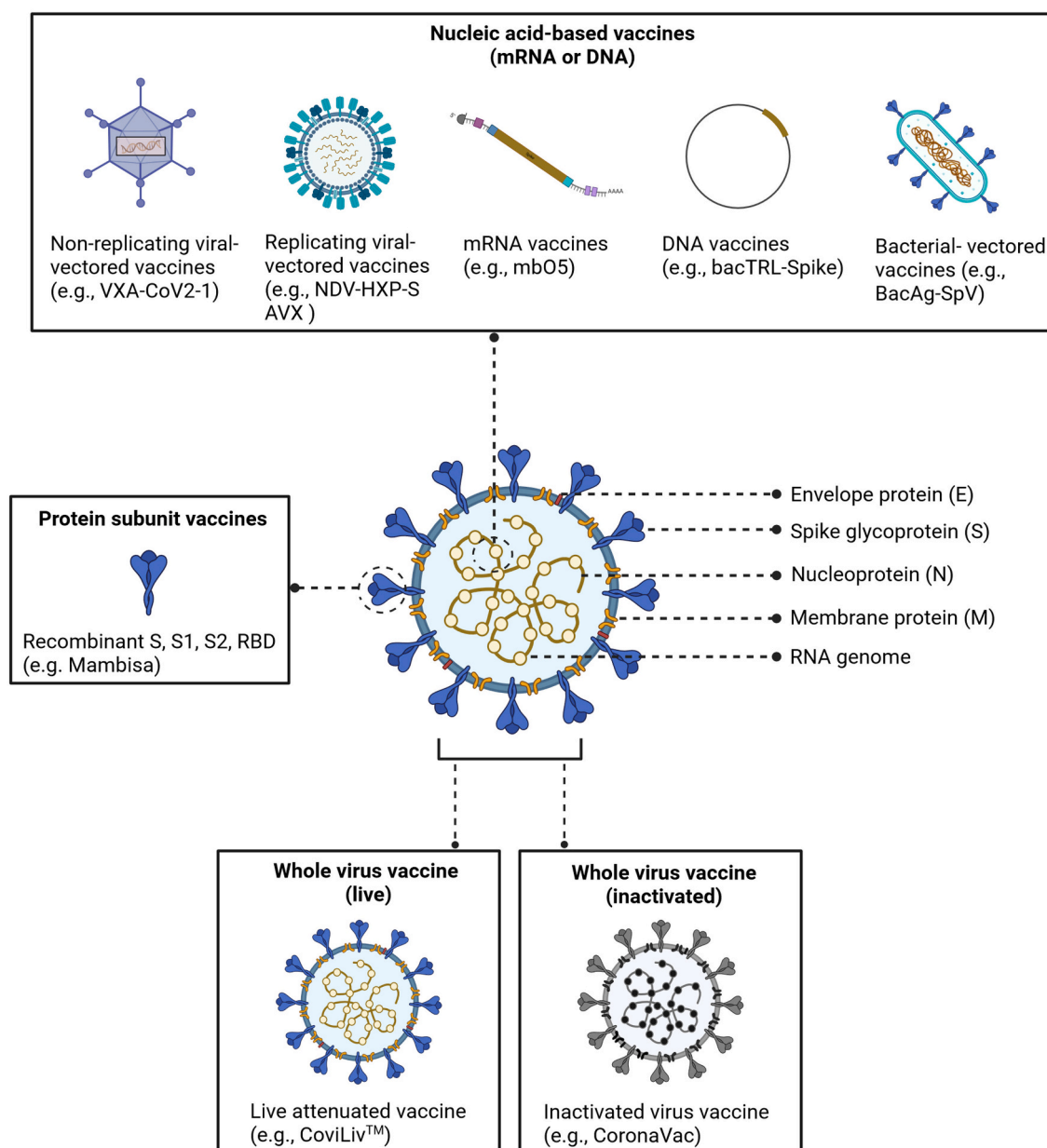


Fig. 2. Platforms for the development of mucosal COVID-19 vaccines. Different types of mucosal vaccines are approved for human use or are currently under investigation in clinical studies, including viral-vectored vaccines, whole virus vaccines, and protein subunit vaccines. Created in <https://BioRender.com/snemaxn>.

2. Mucosal COVID-19 vaccines in clinical trials

To identify mucosal COVID-19 vaccines in clinical trials, we performed an initial screening of commonly used data bases for clinical trials (e.g., ClinicalTrials.gov) searching for “COVID-19”, “SARS-CoV-2”, and “vaccines”. The search hits were further screened for the keyword “mucosal”, “intranasal”, “oral”, and “inhalation”. We excluded clinical studies on COVID-19 vaccines which are applied via other than the mucosal route (e.g., intramuscularly, intravenously). In addition, we only included preclinical data from those vaccines in which the vaccines were tested solely via the mucosal route or tested via the mucosal route in direct comparison to the intramuscular route. By doing so, we could identify at least 28 mucosal COVID-19 vaccines (July 2025) which are currently under clinical evaluation (Tables 1–3). At least 9 of 28 mucosal

COVID-19 candidate vaccines have been tested successfully in early phase clinical trials and data for both preclinical (Table 1) and clinical trials (Table 2) are publicly available. From at least 19 of 28 mucosal COVID-19 vaccines which are listed as candidates in clinical trials, results from human studies are pending (Table 3).

3. Mucosal COVID-19 vaccines with records of preclinical and clinical data

3.1. AZD1222/ ChAdOx1 nCoV-19

AZD1222 has a replication deficient chimpanzee adenovirus (ChAdOx1) viral backbone, which expresses the full-length ancestral SARS-CoV-2 spike (S) in its native form. A single IN or IM vaccine dose

Table 1

Preclinical data of the most advanced mucosal COVID-19 vaccines in clinical trials.

Route of administration (animals)	Name of vaccine	Vaccine platform	Animal models (immunogenicity)	URT (protective efficacy)	LRT (protective efficacy)	Preclinical data
IN	AZD1222/ ChAdOx1 nCoV-19	Replication- deficient ChAd-vector expressing full-length ancestral SARS-CoV-2 S in its native form	Syrian hamsters Ferrets Rhesus macaques	●Reduced amount of viral genomes detectable ●Reduced amount of infectious virus detectable ●Reduced amount of viral genomes detectable ●Reduced amount of viral genomes detectable ●Reduced amount of infectious virus detectable	●No viral genomes detectable ●No infectious virus detectable ●No lung pathology observed n/a ●Reduced amount of viral genomes detectable ●No lung pathology observed	[18–20]
IN	3R-NC + KFDi.n.	Triple RBD (one Delta, two Gamma) scaffold protein (3R-NC) adjuvanted with flagellin protein KFD	BALB/c mice	●Reduced amount of viral genomes detectable	●Reduced amount of viral genomes detectable ●Reduced lung pathology observed	[24]
IN	Mambisa/ CIGB-669	Protein subunit vaccine based on ancestral SARS-CoV-2 RBD and HbcAg	hACE2 mice n/a	n/a n/a	n/a n/a	n/a
IN, O	VXA-CoV2–1/ VXA-CoV2–1.1-S/ VXA-CoV2–3.1	Replication-deficient hAd5 vector expressing full-length ancestral SARS-CoV-2 S + N or S	Syrian hamsters	●Reduced amount of viral genomes detectable ●Reduced amount of infectious virus detectable	●Reduced amount of viral genomes detectable ●Reduced lung pathology observed	[27,28]
IN	NDV-HXP-S AVX/COVID-12- HEXAPRO (Patria)	Recombinant NDV expressing ancestral SARS-CoV-2 S in a pre-fusion stabilized form	Syrian hamsters	●Reduced amount of viral genomes detectable ●Reduced amount of infectious virus detectable	●No infectious virus detectable	[29–31]
IN	Ad5-S-Omicron BA.1 (NB2155)	Replication-deficient hAd5 expressing SARS-CoV-2 Omicron BA.1 S	Pigs BALB/c mice hACE2 mice	n/a n/a n/a	n/a n/a ●Reduced amount of viral genomes detectable	[34]
IH, IN	ChAd-triCoV/Mac (AeroVax)	Replication deficient ChAd68 expressing SARS-CoV-2 S1, N and truncated nsp12	BALB/c mice	n/a	●Reduced lung pathology observed ●Reduced amount of viral genomes detectable	[37]
IN	CVXGA1	Recombinant PIV5 expressing full-length SARS-CoV-2 S in its native form	K18-hACE2 mice BALB/c mice K18-hACE2 mice	n/a n/a n/a	●No viral genomes detectable n/a ●No infectious virus detectable ●Reduced lung pathology	[39]
O	S spores	<i>Bacillus subtilis</i> displaying ancestral SARS-CoV-2 RBD on spore coat	Ferrets BALB/c mice	●No viral genomes detectable n/a	●No viral genomes detectable n/a	[41]

CIGB: Center for Genetic Engineering and Biotechnology; ChAd: Chimpanzee adenovirus; ChAd68: Chimpanzee adenovirus serotype 68; (h)Ad5: (human) adenovirus serotype 5; HbcAg: hepatitis B core antigen; IH: inhalation; IN: intranasal; LRT: lower respiratory tract; N: nucleocapsid; n/a: not applicable; NDV: Newcastle disease virus; O: oral; RBD: receptor-binding domain; S: spike; URT: upper respiratory tract; vps: viral particles.

induced similar levels of systemic anti-S specific IgG antibodies in the serum of immunized Syrian hamsters [18]; however, IN immunization induced higher SARS-CoV-2 specific neutralizing antibodies (nAbs) in the serum. Both IN and IM immunizations partially protected hamsters against SARS-CoV-2 replication in the upper and lower respiratory tract. In oropharyngeal swabs, lower amounts of viral RNA were observed after IN or IM immunization compared to the control group. However, only after IN immunization was a significant reduction in infectious virus observed. In both IN and IM immunized hamsters, no infectious virus was detectable in lung tissues at 5 days post infection.

Furthermore, low levels of genomic viral RNA were detectable in lung tissues of IM, but not IN, immunized hamsters. IN and IM immunizations prevented lung pathology, as neither lesions, interstitial pneumonia, edema, nor perivascular leukocyte infiltrations were observed. In a follow-up experiment, unvaccinated and SARS-CoV-2 infected hamsters were co-housed with IN or IM immunized hamsters. Viral loads and infectious virus in oropharyngeal swabs of IN immunized hamsters were reduced compared to IM immunized hamsters. Neither viral RNA nor infectious virus were found in lung tissue samples of IN immunized hamsters. In a different study, Port and colleagues [19] investigated the

Table 2

Clinical data of the most advanced mucosal COVID-19 vaccines in clinical trials.

Route of administration (human)	Name of vaccine	Developer/manufacturer/sponsor	Clinical phase	Applied dose/application	Status	Trial registries, public reports	Clinical data
IN, IH	AZD1222/ChAdOx1 nCoV-19	University of Oxford (UK)	I	1 × 10 ⁹ vps 5 × 10 ⁹ vps 1 × 10 ¹⁰ vps 5 × 10 ¹⁰ vps 2 × 10 ¹⁰ vps	Completed Active, not recruiting	NCT04816019 (Phase I) NCT05007275 (Phase I)	[21]
IN	3R-NC + KFDi.n.	Shanghai Public Health Clinical Center (China)	I	25 µg (3R-NC) + 5 µg (KFD) 50 µg (3R-NC) + 10 µg (KFD) 80 µg (3R-NC) + 20 µg (KFD) 160 µg (3R-NC) + 40 µg (KFD)	Completed	ChiCTR2400081625 (Phase I)	[24]
IN	Mambisa/ CIGB-669	Center for Genetic Engineering and Biotechnology (CIGB) (Cuba)	I/II	50 µg (RBD) 40 µg (N)	Unknown Unknown	RPCEC00000345 (Phase I/II) RPCEC00000382 (Phase I/II)	[26]
O	VXA-CoV2-1/ VXA-CoV2-1.1-S/ VXA-CoV2-3.1	Vaxart (California, U.S.)	II	1 × 10 ¹¹ IU 1 × 10 ¹⁰ IU 5 × 10 ¹⁰ IU	Completed Terminated Recruiting	NCT04563702 (Phase I) NCT05067933 (Phase II) NCT06672055 (n/a)	[28]
IH	ChAd-triCoV/Mac (AeroVax)	McMaster University (Canada)	II	1 × 10 ⁵ TCID ₅₀ 1 × 10 ⁶ TCID ₅₀ 1 × 10 ⁷ TCID ₅₀ 3 × 10 ⁷ TCID ₅₀ 6 × 10 ⁷ TCID ₅₀ 1 × 10 ⁸ TCID ₅₀	Completed Completed Recruiting	NCT05094609 (Phase I) NCT06381739 (Phase II)	[38]
IN	CVXGA	CyanVac LLC (Georgia, U.S.)	IIb	10 ⁶ PFU 10 ⁷ PFU	Completed Completed Recruiting	NCT04954287 (Phase I) NCT05736835 (Phase II) NCT06742281 (Phase IIb)	[40]
IN	NDV-HXP-S AVX/COVID-12- HEXAPRO (Patria)	Icahn School of Medicine at Mount Sinai (New York, U.S.), CastleVax (US), Avimex (Mexico)	II	10 ^{7-7.49} EID ₅₀ 10 ^{7-5-7.99} EID ₅₀ 10 ^{8-8.49} EID ₅₀ 3.3 × 10 ⁸ EID ₅₀ 1 × 10 ⁹ EID ₅₀	Completed Completed Completed	NCT04871737 (Phase I) NCT05181709 (Phase I/II) NCT05205746 (Phase II)	[32,33]
IN	Ad5-S-Omicron BA.1 (NB2155)	Guangzhou nBiomed Ltd. (China)	n/a	n/a	Completed	ChiCTR2300070346 (n/a)	[35,36]
O	S spores	DreamTec Research Limited (China)	n/a	1 × 10 ¹⁰ CFU 1 × 10 ¹¹ CFU 5 × 10 ¹⁰ CFU	Completed Completed	NCT05057923 (n/a) NCT05158855 (n/a)	[44]

CFU: colony-forming units; CIGB: Center for Genetic Engineering and Biotechnology; ChAd: Chimpanzee adenovirus; (h)Ad5: (human) adenovirus serotype 5; HBcAg: hepatitis B core antigen; IU: infectious units; N: nucleocapsid; n/a: not applicable; NDV: Newcastle disease virus; O: oral; RBD: receptor-binding domain; S: spike; vps: viral particles.

impact of pre-existing immunity on SARS-CoV-2 infection and disease progression. Syrian hamsters were either vaccinated with ADZ1222 (IN or IM) or infected (abbreviated with PI) with the SARS-CoV-2 Delta variant to establish a pre-existing immunity. Following a subsequent infection with SARS-CoV-2, the level of viral genomes was reduced in nasal turbinates of IN and IM immunized hamsters and in the PI group. Viral genomes were completely absent in the lung tissues of IM vaccinated hamsters and the PI group, and only 1 of 6 IN immunized hamsters showed viral genomes in the lungs. Interestingly, viral respiratory shedding, measured in oral swabs, was significantly reduced in the PI group and only marginally or moderately reduced in IM or IN immunized hamsters, respectively. When performing an airborne chain transmission experiment, IM immunized hamsters showed a reduction in transmission over three chains by 60 % in comparison to the control animals, whereas hamster from the PI group and IN immunized hamster showed reductions of up to 80 %.

In an additional study, ferrets [20] were IN or IM immunized via a prime or prime-boost (0–28 days) immunization schedule and challenged with SARS-CoV-2 28 days after the last immunization. IM prime

immunization induced SARS-CoV-2 specific nAbs in the serum, which increased after challenge. However, after IN prime immunization, low levels of SARS-CoV-2-specific nAbs were detectable, which slightly increased after challenge and were detectable in 75 % of the animals. In contrast, IN or IM prime-boost immunizations induced high levels of nAbs in the serum, which declined before challenge infection. Following SARS-CoV-2 infection, no viral genomes were detectable in rectal swabs of IN or IM prime-boost immunized animals and in only one IN prime (day 5) and one IM prime (day 9) immunized ferret. At day 3 post challenge infection, IN prime-boost immunized ferrets showed reduced viral genomes in oral swabs compared to IM prime-boost immunized ferrets, and no viral genomes were detectable after day 5 in both groups. In IN or IM prime immunized ferrets, viral genomes were detectable in oral swabs until day 5. In non-human primates (NHPs, rhesus macaques) [18], IN prime-boost (0–28 days) immunizations induced high levels of anti-S and anti-receptor binding domain (RBD) specific IgG and IgA antibodies in the serum, nasosorption samples and bronchioalveolar lavage fluids (BALFs). To apply the vaccine in the nasal cavity, a mucosal atomization device which produces aerosols from the liquid vaccine

Table 3
Mucosal COVID-19 vaccines in clinical trials with pending clinical data.

Route of administration	Name of vaccine	Vaccine platform	Developer/ manufacturer/ sponsor	Animal models (immunogenicity)	URT (protective efficacy in animals)	LRT (protective efficacy in animals)	Preclinical data	Phase	Status	Trial registries, public reports
IN	AdCOVID	Replication deficient Ad5 expressing SARS-CoV-2 RBD	Altimmune, Inc. (Maryland)	C57BL/6 mice	n/a	n/a	[49,65]	I	Completed	NCT04679909 (Phase I)
IM, IN, IH	SC-Ad6-1	Single-cycle adenovirus (replicating) expressing full-length SARS-CoV-2 S in its native form	Moat Biotechnology Corporation/ Tetherex Pharmaceuticals Corporation (Oklahoma, U.S.)	CD-1 mice Syrian hamsters	n/a ●Reduced amount of viral genomes detectable	n/a ●Reduced amount of viral genomes detectable	[52]	I	Recruiting	NCT04839042 (Phase I)
IN, IT	MV-014-212	Live attenuated RSV expressing full-length chimeric SARS-CoV-2 S (S+ cytoplasmic tail of RSV F)	Meissa Vaccines, Inc. (California, US)	BALB/c mice African green monkeys	n/a ●Reduced amount of viral genomes detectable	n/a ●Reduced amount of viral genomes detectable	[45]	I	Unknown	NCT04798001 (Phase I)
IN	B/HPIV3/S-6P	Recombinant, live attenuated HPIV3 expressing full-length SARS-CoV-2 S in a pre-fusion stabilized form	National Institute of Allergy and Infectious Diseases (NIAID, Maryland, US)	Syrian hamsters	n/a	●Reduced amount of viral genomes detectable	[46,66]	I	Active, not recruiting	NCT06026514 (Phase I)
IN	MPV/S-2P	MPV expressing SARS-CoV-2 S in a pre-fusion stabilized form	NIAID (Maryland, US)	Rhesus macaques	●No viral genomes detectable	●No infectious virus detectable ●No viral genomes detectable	[48]	I	Active, not recruiting	NCT06441968 (Phase I)
IN	Avacc® 10 (OMV-mC-Spike)	SARS-CoV-2 S in a pre-fusion stabilized form linked with mCramp to OMV of <i>Neisseria meningitidis</i>	Intravacc B.V. (Netherlands)	Human ACE2-transgenic mice BALB/c mice Syrian hamsters	n/a ●Reduced amount of viral genomes detectable	n/a ●Reduced amount of viral genomes detectable	[53]	I	Completed	NCT05604690 (Phase I)
IN	ACM-SARS-CoV-2- beta ACM CpG	Recombinant SARS-CoV-2 S (Beta)	ACM Biolabs (Singapore)	Syrian hamsters	●Reduced amount of viral genomes detectable	●No lung pathology observed	[54]	I	Completed	NCT05385991 (Phase I)
IN	3R _{AEW} -NC + KFDi.n.	Triple RBD (BA.2.86, EG.5, WIV 1) scaffold protein (3R-NC) adjuvanted with flagellin protein KFD	Shanghai Public Health Clinical Center (China)	New Zealand white rabbits BALB/c mice	n/a	n/a	[24]	I	Not yet recruiting	NCT06768697 (Phase I)
IH	MVA-SARS-2-ST	Non-replicating MVA vector expressing full-length SARS-CoV-2 S in a pre-fusion stabilized form	Hannover Medical School (Germany)	n/a	n/a	n/a	n/a	I	Terminated	NCT05226390 (Phase I)
IH	Recombinant COVID-19 vaccine (RCVi)	Recombinant COVID-19 vaccine based on a ChAd vector	Wuhan BravoVax Co., Ltd. (China)	n/a	n/a	n/a	n/a	I	Unknown	NCT05706324 (Phase I)
O	baCTRL-Spike	Live <i>Bifidobacterium longum</i> delivering DNA encoding full-length SARS-CoV-2 S	Symvivo Corporation (Canada)	n/a	n/a	n/a	n/a	I	Completed	NCT04334980 (Phase I)
O	CoV2-OGEN1	Recombinant SARS-CoV-2 S	USSF/Vaxform (Pennsylvania, U.S.)	n/a	n/a	n/a	n/a	I	Completed	NCT04893512 (Phase I)
IN	COV2/ FINCoVax 2.1	Ad5 vector expressing SARS-CoV-2 S	Rokote Laboratories Finland Oy (Finland)	n/a	n/a	n/a	n/a	I	Recruiting	NCT06851611 (Phase I)

(continued on next page)

Table 3 (continued)

Route of administration	Name of vaccine	Vaccine platform	Developer/manufacturer/sponsor	Animal models (immunogenicity)	URT (protective efficacy in animals)	LRT (protective efficacy in animals)	Preclinical data	Phase	Status	Trial registries, public reports
O, IN, SC	hAd5-S-Fusion+N-ETSD	Non-replicating hAd5 [E1/E2b/ E3 deletions] expressing full-length SARS-CoV-2 S + N	ImmunityBio, Inc. (California, US)	CD-1 mice	n/a	n/a	[50,51]	I/II	Terminated	NCT04732468 (Phase I)
				Rhesus macaques	●Reduced amount of viral genomes detectable	●Reduced amount of viral genomes detectable			Withdrawn	NCT04845191 (Phase I/II)
IN	Corfluevec	Recombinant H3N2 and recombinant H1N1pdm09 expressing full-length SARS-CoV-2 N	Research Institute of influenza (Russia)	n/a	n/a	n/a	n/a	I/II	Completed	NCT05696067 (Phase I/II)
IN	LVT001	Homo-trimeric protein (SARS-CoV-2 S + N and Fc domain) including S & N proteins and Fc domain	ANRS, Emerging Infectious Diseases (France)	n/a	n/a	n/a	n/a	I/II	Recruiting	NCT06821126 (Phase I/II)
IN	CoviLiv™	Live attenuated SARS-CoV-2 with deleted furin cleavage site in S protein	Codagenix/Serum Institute of India (India)	Syrian hamsters	●Reduced amount of viral genomes detectable	●Reduced amount of viral genomes detectable	[45]	III	Completed	NCT04619628 (Phase I)
									Completed	NCT05233826 (Phase I)
									Completed	ISRCTN15779782 (Phase III)
IH	mbO5	mRNA vaccine based on SARS-CoV-2 Beta and Omicron BA.4/5	Zhongnan Hospital (China)	n/a	n/a	n/a	n/a	n/a	Unknown	NCT05886790 (n/a)
IH	Ad5-NC05T-IH	Replication deficient hAd5 expressing SARS-CoV-2 S (Omicron BA.4/5 and ancestral)	Zhongnan Hospital (China)	n/a	n/a	n/a	n/a	n/a	Unknown	NCT05886790 (n/a)

ChAd: Chimpanzee adenovirus; (h)Ad5: (human) adenovirus serotype 5; EID₅₀: 50 % egg infectious dose; HPIV: bovine/human parainfluenza virus; IH: inhalation; IM: intramuscular; IN: intranasal; IU: infectious units; MPV: murine pneumonia virus; MVA: modified vaccinia virus Ankara; n/a: not applicable; LRT: lower respiratory tract; N: nucleocapsid; O: oral; OMV: outer membrane vesicles; PFU: plaque-forming units; PIV5: parainfluenza virus type 5; RBD: receptor-binding domain; RSV: respiratory syncytial virus; S: spike; SC: subcutaneous; TCID₅₀: 50 % tissue culture infectious dose; URT: upper respiratory tract; vps: viral particles.

formulation had been used. SARS-CoV-2-specific nAbs were observed in the serum of IN immunized rhesus macaques. NHPs were partially protected against SARS-CoV-2 infection, indicated by lower amounts of viral RNA and reduced infectious virus in nasal swabs. Furthermore, no infectious virus and only low levels of viral RNA were observed in BALFs. Although lower amounts of viral genome were found in lung tissue samples, no differences in the level of viral genomes were observed in nasal turbinates compared to control animals. In IN immunized rhesus macaques, no pulmonary pathology was observed in lung tissue and no viral antigen could be detected.

A single-center, open-label and partially-randomized phase I clinical trial [21] (NCT04816019) with 42 individuals, who either had no history of SARS-CoV-2 vaccination or who had received two IM doses of ChAdOx1 nCoV-19 or BNT162b2 mRNA vaccine, was conducted in mid-2021 in Oxford (United Kingdom, UK). The study aimed to explore safety, tolerability and the induction of mucosal immune responses of AZD1222 after IN application. For the first part of the study, SARS-CoV-2 vaccine naïve participants were IN immunized with different doses of AZD1222 (low dose, LD: 5×10^9 viral particles (vps); mid dose, MD: 2×10^{10} vps; high dose, HD: 5×10^{10} vps). A subset of participants received a booster immunization 28 days later (IN-IN), whereas another subset of individuals received an IM immunization with an mRNA COVID-19 vaccine between days 22 and 46 (IN-IM). For the second part of the study, pre-vaccinated (IM) individuals received an IN booster immunization with AZD1222 (IM-IM-IN). During the study, seven participants from different vaccine groups reported a SARS-CoV-2 infection, from which 6 of 7 individuals seroconverted. Overall, the IN vaccination was well tolerated. Frequently reported adverse reactions included headache, fatigue, nasal discharge and sore throat. Mucosal anti-S specific IgG or IgA antibodies were not detectable in nasal mucosal lining fluids (NMLFs) after a single IN immunization with ADZ1222, but were detectable in few individuals in the IN-IN group (4 of 13 participants). In the IN-IM vaccine group, anti-S specific IgA and IgG antibody responses were detectable in NMLFs of 5 of 11 and 11 of 11 participants, respectively. In the IM-IM-IN vaccine group, mucosal anti-S specific IgA and IgG antibodies in NMLFs were detectable in 2 of 11 participants. A higher systemic humoral immune response, measured by anti-S specific IgG and IgA antibodies in the serum, was observed in the IN-IM and IM-IM-IN vaccine groups compared to the IN and IN-IN vaccine groups. All vaccine groups elicited comparable levels of systemic cellular immune responses.

3.2. 3R-NC $\pm$ KFDi.n

3R-NC + KFDi.n. is based on a triple-receptor-binding domain (RBD) scaffold protein subunit platform, consisting of SARS-CoV-2 S-RBD from one Delta and two Gamma variant strains, which is IN administered together with the truncated flagellin variant KFD. KFD is derived from the non-pathogenic *E. coli* K12 strain and is a known Toll-like receptor 5 (TLR5) agonist, which is used as a mucosal adjuvant [22,23]. 3R-NC was generated by transfecting HEK293F cells and subsequent purification of the recombinant protein by affinity chromatography. BALB/c mice [24] were IN or IM prime-boost-boost (0–21–42 days) immunized with 4 μ g 3R-NC/1 μ g KFD or 4 μ g 3R-NC/200 μ g alum adjuvant, respectively. IN immunization induced high levels of anti-RBD specific serum IgG, salivary IgA and vaginal IgA antibody responses. Both IN and IM immunizations induced nAb responses against SARS-CoV-2 variant strains (ancestral, Alpha-Delta, BA.1, BA.2, BA.4/5) and SARS-CoV-1 in the serum. IN and IM immunizations induced comparable levels of anti-RBD specific serum IgG and IgG1 antibody responses, which remained stable up to 13 months after immunization. IN immunizations induced high levels of long-lasting anti-RBD specific serum IgG2a, salivary IgA, vaginal IgA, nasal IgG/IgA and BALF IgA antibody responses. IN immunization induced high numbers of long-lived plasma cells (LLPCs), as anti-RBD specific IgA secreting splenic and bone marrow derived cells were observed up to 13 months after immunization. Anti-RBD specific

IgA secreting cells in the bone marrow produced monomeric IgA, whereas in nasal tissue, dimeric or polymeric IgA were secreted. Following IN immunizations, an increased number of activated anti-RBD specific splenic CD4+ T cells could be detected. IN immunization provided long-lasting immunity (10 months between immunization and challenge) against SARS-CoV-2 Omicron infection in BALB/c mice, indicated by reduced viral loads and reduced lung pathology. Two IN booster immunizations of hACE2 mice following IM immunization with an inactivated SARS-CoV-2 vaccine, boosted both neutralizing and anti-RBD specific IgG antibody responses in the serum.

An investigator-initiated, non-controlled and open-labeled pilot study [24] (ChiCTR2400081625) with 6 individuals, who had received two or three immunizations with an inactivated SARS-CoV-2 vaccine and had no history of SARS-CoV-2 infection, was conducted by the end of 2022 in Shanghai (China). The study aimed to explore safety and immunogenicity of two IN booster immunizations with 3R-NC + KFDi.n. Individuals were IN prime-boost immunized with either 80 μ g 3R-NC/20 μ g KFD (2 individuals) or 160 μ g 3R-NC/40 μ g KFD (4 individuals) over a 21-day interval. The most frequently reported adverse reactions were a dry nose, running nose and nose ache. A single IN immunization increased SARS-CoV-2 variant strain and SARS-CoV-1 specific nAbs in the serum. In addition, an IN booster immunization increased anti-RBD specific IgG and IgA antibody responses in the serum. Furthermore, two IN immunizations increased anti-RBD specific IgA (12.5-fold increase compared to pre-boost), IgG and neutralizing antibodies in saliva samples. None of the participants reported a SARS-CoV-2 infection during the observation period.

3.3. Mambisa

Mambisa is a protein subunit vaccine based on ancestral SARS-CoV-2 RBD, which is produced in *Pichia pastoris* [25], and uses hepatitis B virus core (HBcAg) as scaffold. The vaccine formulation is administered intranasally without adjuvant.

A multi-center, randomized, prospective, open-label phase I/II clinical trial [26] (RPCEC00000382) with 1161 individuals was conducted in Havana (Cuba), Camagüey (Cuba), Pinar del Río (Cuba) and Santiago de Cuba (Cuba) in mid-2021. The study aimed to explore safety and immunogenicity of an IN booster immunization in COVID-19 convalescent individuals. Participants were boosted with a dose of 50 μ g RBD/40 μ g HBcAg into the nostrils using three different nasal delivery devices (atomizer (AZ), spray (S) or dropper (D)). An additional group received Abdala, which is based on ancestral SARS-CoV-2 RBD and adjuvanted with alum hydroxide gel [25], as IM booster. The most frequently reported adverse reactions were headache, rhinitis and nasal congestion. Both vaccines induced an increase in pre-existing anti-RBD specific serum IgG antibodies. In the Mambisa groups, the delivery via spray induced the highest antibody responses. Anti-RBD specific serum IgA antibodies were boosted in all groups, but individuals who had received the vaccine via dropper showed the lowest response. The sera from Abdala IM immunized individuals showed the highest RBD-angiotensin converting enzyme 2 (ACE2) interaction inhibition activity, followed by Mambisa AZ > S > D vaccine groups. NABs against SARS-CoV-2 variant strains (e.g., Beta, Delta, Omicron) increased in the serum from individuals of all vaccine groups, and overall, no differences were observed between the three intranasal delivery devices. However, a limitation of this study was the lack of data for mucosal immune responses.

3.4. VXA-CoV2-1

VXA-CoV2-1 is based on a replication deficient adenovirus type 5 (Ad5), which expresses full-length ancestral SARS-CoV-2 S and nucleocapsid (N) proteins. O or IN immunization [27] via a prime-boost regimen (0–28 days) provided partial protection against SARS-CoV-2 infection in Syrian hamsters, indicated by reduced weight loss,

reduced lung pathology and reduced viral loads in the lung. Furthermore, high levels of anti-S1 and anti-S specific serum IgG and SARS-CoV-2-specific nAbs were detected in both O and IN prime-boost vaccinated hamsters. In nasal washes, an increase in anti-S specific IgG antibody responses was observed after O or IN prime-boost immunizations. Overall, the anti-N specific serum antibody responses were low after O or IN immunizations in hamsters, most likely due to the human specific β -actin promoter which was used to express N.

O or IN prime-boost immunizations four weeks apart with VXA-CoV2-1-S [28], which expresses full-length ancestral SARS-CoV-2 S but not N, partially protected hamsters against SARS-CoV-2 infection, and induced high levels of anti-S specific IgG and IgA antibodies in the serum. In contrast to IM immunized hamsters, O and IN immunized hamsters displayed high levels of anti-S specific IgA antibodies in BALFs and showed lower levels of RNA in nasal swabs. However, infectious virus was detectable in all groups. Virus transmission through the air was reduced in both O and IN immunized hamsters.

An open-label phase I clinical trial [28] (NCT04563702) with 35 participants was conducted by the end of 2020 in Cypress (California, U. S.) to evaluate the safety and immunogenicity of VXA-CoV2-1 as oral vaccine. Individuals were immunized with VXA-CoV2-1 as an oral tablet vaccine via a prime regimen using a low dose (LD: 1×10^{10} infectious units (IU)) or high dose (HD: 5×10^{10} IU) or via a prime-boost regimen (0–28 days) with a LD of the vaccine. Increased levels of anti-S, anti-N and anti-RBD specific mucosal IgA in nasal or saliva samples could be observed. A ≥ 2 -fold increase of mucosal IgA antibodies in nasal or saliva samples was observed in 19/35 individuals. Furthermore, cross-reactive anti-S specific mucosal IgA antibodies against Middle East respiratory syndrome coronavirus (MERS-CoV), human coronavirus (hCoV)-HKU1, hCoV-OC43, hCoV-229E, hCoV-NL63 and SARS-CoV-1 could be detected.

3.5. NDV-HXP-S

NDV-HXP-S is a Newcastle disease virus (NDV)-based vaccine, encoding full-length ancestral SARS-CoV-2 S in a pre-fusion stabilized form. In addition, the transmembrane domain and the cytoplasmic tail were replaced by those from NDV fusion (F) protein. Syrian hamsters [29], IN prime-boost (0–22) immunized with 10^6 EID₅₀ (50 % egg infectious dose), showed high anti-S specific serum IgG antibodies already after the prime immunization, which did not increase after the booster immunization. Furthermore, nAbs against ancestral, Alpha and Beta SARS-CoV-2 variant strains were observed in the serum of immunized animals. IN prime-boost immunized hamsters were partially protected against SARS-CoV-2 infection, indicated by a lack of weight loss, reduced viral loads in nasal swabs (and lack of virus in other parts of the respiratory tract), and viral clearance 2 days after infection. In another hamster study [30], immunogenicity and protection against virus transmission conferred by IN prime-boost was compared with those by IM prime-boost, both at 10^6 EID₅₀ per animal. Then, naïve hamsters were infected with SARS-CoV-2 as donors, which were subsequently co-housed with vaccinated recipient hamsters. While IN and IM vaccination induced comparable levels of anti-S serum IgG in recipient hamsters, those that were IN vaccinated were less susceptible to viral transmission from infected donor hamsters, showing less weight loss and lower viral titers in the upper respiratory tissues than those that were IM vaccinated. Pigs [31] were immunized using a prime regimen (IN/IM simultaneously), an IN-IN (LD: 10^7 EID₅₀, MD: $10^{7.5}$ EID₅₀, HD: 10^8 EID₅₀) prime-boost regimen (0–21 days), an IN-IM prime-boost regimen or an IM-IM (10^7 EID₅₀, $10^{7.5}$ EID₅₀, 10^8 EID₅₀, $10^{8.5}$ EID₅₀) prime-boost regimen. MD and HD IN immunized, but not LD IN immunized pigs, had detectable levels of anti-RBD and anti-S ectodomain specific IgG antibody titers after one immunization, which increased only in the LD group after the booster immunization. The simultaneous IN/IM application induced only weak antibody responses. The IN prime and IM booster immunizations induced antibody levels comparable to the

highest IM vaccine dose. Sera from IN-IN immunized pigs showed inhibition in an ACE2-RBD interaction inhibition assay, however IM-IM and IN-IM immunizations showed better inhibitory activity. Similar patterns were observed in terms of induction of SARS-CoV-2 specific nAbs. Cross-reactive nAbs were observed against Alpha > Gamma > Beta SARS-CoV-2 variant strains. Again, HD IN-IN, IM-IM (all groups) and IN-IM vaccine groups showed the highest reactivity.

An open-label, non-placebo-controlled and non-randomized clinical phase I trial [32] (NCT04871737) with 91 individuals was performed in mid-2021 in Mexico to explore the immunogenicity and safety of IN-IN, IN-IM and IM-IM prime-boost immunizations (0–21 days) using three different doses (10^7 EID₅₀, $10^{7.5}$ EID₅₀, 10^8 EID₅₀). IM-IM immunizations induced higher anti-S specific serum IgG antibody responses compared to IN-IN immunizations. Sera from IM-IM immunized individuals showed better RBD-ACE2 interaction inhibitory activity compared to IN-IM > IN-IN. IFN- γ producing CD3⁺, CD4⁺ and CD8⁺ positive T cells were observed in all groups with a trend towards HD vaccine doses inducing higher amounts of IFN- γ producing cells. Breakthrough infections were observed in 10 individuals, however, there was no apparent trend towards a specific administration route or vaccine dose.

A mixed phase II study, with a single-blinded safety phase, and a placebo-controlled, double-blinded and randomized phase [33] (NCT05205746) with 229 individuals, who had received any COVID-19 vaccine (e.g., inactivated vaccines, mRNA vaccines) and had anti-S specific IgG antibody titers below 1200 U/ml, using a single IM or IN booster immunization, was conducted by the end of 2022 in Mexico City (Mexico) to explore immunogenicity and safety. In addition, individuals from the placebo group received an IM booster with AZ/ChAdOx-1-S later during the study. Both IM and IN booster immunizations induced an increase in anti-S IgG specific serum antibodies, however titers declined over time. By day 365 (IM) and day 180 (IN) the titers started to increase again (most likely due to SARS-CoV-2 exposure).

IM booster immunizations induced cross-reactive SARS-CoV-2 variant strains specific nAbs (Delta, Alpha, Beta, Omicron (Om) BA.2, Om BA.5), whereas IN booster immunization induced only cross-reactive neutralizing antibodies against the Alpha variant. Cellular immune responses were observed in all groups, which increased later during the study, likely due to SARS-CoV-2 exposure.

3.6. Ad5-S-Omicron BA.1 (NB2155)

Ad5-S-Omicron (Om) BA.1 is a replication deficient Ad5-based vaccine, which expresses full-length SARS-CoV-2 Om BA.1 S with a deleted furin cleavage site and 2 stabilizing point mutations at positions 983 and 984. BALB/c [34], IN or IM immunized with a single dose (5×10^9 vps), elicited high serum nAbs against SARS-CoV-2 Om BA.1 and, to a lower extent, against ancestral, Beta and Delta variant strains. Furthermore, IN immunization induced high levels of anti-S specific IgA and SARS-CoV-2 specific nAbs in BALF. A higher systemic cellular immune response against ancestral, Delta and Om BA.1 strains was observed in IM immunized mice, however, only IN immunized mice showed an increase in IFN- γ producing T cells in the respiratory tract. IN booster immunization with Ad5-S-Omicron BA.1 following IM prime immunization with an inactivated vaccine induced higher anti-S specific serum IgG antibodies against Om BA.1 and Om BA.2 compared to IM-IM immunized mice. In addition, higher levels of nAbs against SARS-CoV-2 ancestral, Beta, Delta, Om BA.1 and Om BA.2 were observed. Higher mucosal anti-S specific IgA antibodies and nAbs (ancestral, Beta, Delta, Om BA.1), as well as higher cell-mediated immune responses in spleen and BALF, were found after IM-IN immunization, compared to IM-IM immunization. IM-IM-IN-IN immunizations induced cross-reactive nAbs against several SARS-CoV-2 variant strains (ancestral, Beta, Om BA.1, Om BA.2, Om BA.5, Om BA.2.75, Om BF.7, Om BQ.1, Om BQ.1.1, Om XBB). hACE-2 transgenic mice, which were IM-IM-IN-IN immunized, showed reduced viral loads in the lung compared to IN-IN

immunized mice. Five co-authors of this study, who had a history of vaccination with two doses of an inactivated COVID-19 vaccine, voluntarily immunized themselves with Ad5-S-Omicron via an intranasal spray. In nasal lavage fluids (NLFs), anti-S specific sIgA against SARS-CoV-2 variant strains (e.g., Beta, Delta, Om BA.1, Om BA.2, Om BA.3, Om BA.1.1) could be observed, with 11.2–29.5-fold increases compared to baseline levels. In addition, nAbs against SARS-CoV-2 variant strains (ancestral, Delta, Om BA.1, Om BA.2) were observed in NLFs. Nasal mucosal lining fluids (NMLFs), sampled from an IN immunized individual, partially protected hACE2 mice against SARS-CoV-2 infection upon IN instillation, indicated by reduced viral RNA in the lung.

An open-label, investigator-initiated, and single center clinical study [35] (ChiCTR2300070346) with 128 individuals, with no history of SARS-CoV-2 infection and who had received 2 or 3 immunizations with an inactivated COVID-19 vaccine prior to study start, was conducted by the end of 2022 in Guangzhou (China) to explore safety and immunogenicity of one or two (0–28 days) IN immunizations with NB2155. The most frequently reported adverse reactions were asthenia, fatigue, nasal congestion, and pharyngalgia. Forty-five and 43 participants reported a SARS-CoV-2 infection between days 1–14 and 15–28 after the first immunization, respectively. Thirty-one participants received a second immunization, after which 2 participants reported an infection 1–3 days after immunization. For further analysis, only individuals who did not report a SARS-CoV-2 infection were included. A single IN immunization induced anti-S (ancestral, Alpha, Beta, Delta, Om BA.1, Om BA.1.1, Om BA.2, Om BA.3, Om BA.5-like) specific mucosal sIgA antibody responses (> 3-fold increase), which were further boosted after the second immunization. SARS-CoV-2-specific (ancestral, Om BA.1, Om BA.5) nAbs were detectable after the first IN immunization and were boosted after the second immunization. NMLFs [36], collected 3–6 weeks after the second IN immunization from 8 participants, showed high SARS-CoV-2 (ancestral, Om BA.1, Om BA.5, Delta) specific nAbs and anti-S (ancestral, Om BA.1, Om BA.5, Delta) specific sIgA antibody responses. Isolated nasal sIgA antibodies showed higher neutralizing activity against Om BA.1, Om BA.5, and Om BF.7 than isolated serum IgG and IgA antibodies. However, sIgA, but not serum IgG and IgA antibodies showed neutralizing activity against other Omicron variants (XBB, XBB.1.5, BQ.1.1, EG.5, BA.2.86, JN.1, XBB.1.1). Passive IN immunization with isolated nasal IgA, but not serum IgG or IgA antibodies, partially protected K18-hACE2 against SARS-CoV-2 infection (Om XBB.1.1), indicated by reduced viral loads in the lung and reduced lung pathology.

3.7. ChAd-triCoV/Mac (AeroVax)

ChAd-triCoV/Mac [37] is based on a chimpanzee adenovirus type 68 (Tri:ChAd) backbone expressing SARS-CoV-2 S1, full-length N and truncated nsp12 (RNA-dependent RNA polymerase). The S1 protein was fused to the transmembrane/trimerization domain of vesicular stomatitis virus G protein (VSV-G). A single IN immunization of BALB/c mice induced higher serum anti-S and anti-RBD binding IgG antibody responses compared to IM immunization. In addition, IN, but not IM, immunization induced anti-S specific IgG and IgA antibody responses in BAL samples. Furthermore, IN immunization induced the generation of mucosal tissue-resident memory T cells. Prime-boost immunizations with ChAd-triCoV/Mac partially protected BALB/c mice against lethal infection with SARS-CoV-2, indicated by reduced body weight loss, reduced lung pathology and reduced viral loads in the lung. In K18-hACE2 transgenic mice, prime-boost immunizations with ChAd-triCoV/Mac protected against challenge with different SARS-CoV-2 variant strains (ancestral, B.1.1.7, B.1351), indicated by absence of body weight loss and the absence of viral loads in the lung or brain.

An open-label, multi-arm phase I clinical study (NCT05094609) [38] with 36 participants was conducted between 2022 and 2024 in Hamilton (Canada) to evaluate safety and immunogenicity of ChAd-triCoV. Individuals, who had been vaccinated with at least two doses of an

approved mRNA COVID-19 vaccine at least three months before recruitment and had not been infected (part I) or had been infected (part II) with SARS-CoV-2 were selected. For part I, individuals were immunized once with different doses (10^5 , 10^6 , 10^7 , 3×10^7 , 10^8 TCID₅₀) of ChAd-triCoV or a human Ad5 expressing SARS-CoV-2 S1, N and truncated nsp12 (huAd). For part II, individuals were immunized once with 3×10^7 (LD) or 6×10^7 TCID₅₀ (HD) of the ChAd-triCoV vaccine. The vaccines were applied using the Aeroneb® Solo Device to aerosolize the liquid vaccines, which are subsequently inhaled by the study participants. The vaccines were well tolerated and no severe adverse events were reported. The most frequently reported adverse reactions were headache, fatigue, vomiting and myalgia. Immunization with ChAd in uninfected, and vaccinated individuals induced T cell responses in the airways in a dose depended manner and was outperforming the huAd vaccine, which induced only weak T cell responses. In terms of humoral immunity, neither ChAd-triCoV nor huAd vaccine significantly boosted pre-existing anti-S or anti-RBD specific IgG and IgA antibody responses in BALF samples. Immunization of previously infected and vaccinated individuals using the HD of ChAd-triCoV induced a robust systemic and mucosal T cell response. Furthermore, HD vaccination boosted the anti-S and anti-RBD IgG antibody titers in the BALF samples, whereas anti-S or anti-RBD IgA antibodies were only slightly increased. Increased levels of neutralizing antibodies in BALF samples were detected against ancestral and XBB RBD. Systemic antibody responses (anti-S and anti-RBD IgG) were already high at baseline and could not be further boosted by the inhaled ChAd-triCoV vaccine.

3.8. CVXGA1

CVXGA1 is a recombinant viral vector vaccine based on a para-influenza virus 5 (PIV5) backbone expressing full-length ancestral SARS-CoV-2 S. IN prime immunization of BALB/c mice or C57BL/6 mice induced anti-S, and anti-RBD specific IgG as well as SARS-CoV-2 specific nAbs in the serum and systemic cellular immune responses [39]. IN prime-boost immunized and SARS-CoV-2 infected K18-hACE2 mice showed no infectious virus in the lung or brain, and lung pathology was reduced in vaccinated animals compared to the control groups. IN immunization of ferrets induced anti-S, anti-RBD specific IgG antibodies and nAb responses in the serum, however, only low levels of anti-S specific IgA antibodies in nasal washes. After SARS-CoV-2 infection, no viral RNA was detectable in neither nasal washes, lung nor trachea.

An open-label, dose-ranging phase I clinical trial (NCT04954287) [40] with 72 participants was conducted between 2021 and 2023 in Kentucky (U.S.), New York (U.S.), Ohio (U.S.), and Texas (U.S.) to evaluate the safety and immunogenicity of CVXGA1 in teens and adults. In addition, study participants were monitored for symptomatic SARS-CoV-2 infections during the follow-up period of the study. Individuals were divided into four groups and either vaccinated with 10^6 PFU (LD) or 10^7 PFU (HD) of CVXGA1. Group 1 included adult individuals without a history of prior SARS-CoV-2 infection and received one LD-immunization. Group 2 included adult individuals with or without a history of prior SARS-CoV-2 infection and received one HD-immunization. Group 3 included adult individuals with a history of SARS-CoV-2 infection or vaccination with mRNA-based COVID-19 vaccines and received one HD-immunization. Group 4 included adolescent individuals with a history of SARS-CoV-2 infection or vaccination with mRNA-based COVID-19 vaccines and received one HD-immunization. Overall, the two vaccine doses were well tolerated and no severe adverse events were reported. The most frequently reported adverse reactions included headache, fatigue, chills, and vomiting. In nasal swabs of fourteen participants, who had received the HD-immunization, vaccine-virus mRNA could be detected at day 1 post immunization. Among all groups, the pre-existing level of anti-S specific IgG and IgA, anti-RBD specific IgG and SARS-CoV-2 specific nAbs in the serum was only boosted in a small proportion of the study participants. In addition, also pre-existing nasal anti-S specific IgG and IgA antibodies were only

boosted in a small proportion of study participants among all groups. Overall, mucosal antibody responses were higher in the HD than the LD vaccine groups (52 % vs 21 %). Systemic anti-S specific T cell responses were boosted in both the LD and HD vaccine groups, with the HD-vaccine groups showing higher percentages of participants with anti-S specific CD4⁺ (94–100 % vs 54 %) T cells. The number of participants with anti-S specific CD8⁺ T cells were comparable among HD and LD vaccine groups (76–100 % vs 92 %). The SARS-CoV-2 infection rate was higher in group 1 > group 3 > group 4 > group 2 (73.3 % > 22.2 > 11.8 % > 11.1 %). The infections were graded as mild to moderate and none of the study participants had hypoxia or were hospitalized due to COVID-19.

3.9. S spores

S spores is a *Bacillus subtilis*-based COVID-19 vaccine, which expresses ancestral SARS-CoV-2 RBD on the outer surface of its spores. BALB/c mice [41] were orally (O) immunized with S spores (1×10^9 spores per dose) on days 1–4, 14–17 and 28–31 with or without adjuvants (*Astragalus membranaceus* Bge (AM) and *Coriolus versicolor* (CV)). AM [42] is a medicinal herb, which is used in the traditional Chinese medicine against diseases and body disorders. CV [43] is a mushroom with immunomodulatory properties, which is used in traditional Chinese medicine against diseases. After two immunizations, only low levels of anti-S specific serum IgG, IgA and IgM antibodies could be observed. However, high levels of anti-S specific IgA antibodies were found in feces and intestine lavage in both vaccine groups, with the adjuvanted vaccine outperforming the non-adjuvanted vaccine. Furthermore, upon three immunizations, no impairment in liver function or histopathological changes in different organs (liver, small intestine, lung) were observed in the vaccine groups. Next, BALB/c mice were O immunized with S spores over a 4-day course 14 days prior to a subsequent IM prime-boost immunization (0–14 days) with CoronaVac. The pre-immunization with S spores prior to the IM vaccination with CoronaVac induced a stronger anti-S specific IgG antibody response and higher SARS-CoV-2-specific nAbs in the serum, compared to the control groups (CoronaVac only or AM/CV+ CoronaVac).

A placebo-controlled, fixed dose, observer-blinded and investigator-initiated clinical trial [44] (NCT05158855) with 16 participants was conducted beginning in 2022 in Macau (China) to explore safety and immunogenicity of S spores. Individuals, who had been vaccinated twice with any approved COVID-19 vaccine prior to study begin and had no history of SARS-CoV-2 infection, were O immunized on three consecutive days with capsules containing 5×10^{10} inactivated spores. No adverse reactions were reported. In the control group, pre-existing SARS-CoV-2 nAbs decreased over time. In the vaccine group, the booster immunization increased the levels of SARS-CoV-2 specific nAbs in the serum.

4. Mucosal COVID-19 vaccines in clinical trials with records of preclinical data

Several mucosal COVID-19 vaccines are currently being tested in humans with pending clinical data. However, preclinical data of at least 10 vaccines are available, confirming their immunogenicity and efficacy in different animal models.

4.1. Live attenuated virus vaccines

The live attenuated vaccine COVI-VAC (CoviLiv) [45] has been developed by codon-pair deoptimization of full-length SARS-CoV-2 S protein, thereby introducing more than 280 silent point mutations. In addition, the furin cleavage site has been removed, which is the only difference in the amino acid sequence between the ancestral SARS-CoV-2 strain and the attenuated COVI-VAC vaccine. An IN prime immunization with COVI-VAC partially protected Syrian hamsters against SARS-

CoV-2 infection, indicated by reduced body weight loss and reduced viral genome in the lungs and olfactory bulb. Furthermore, IN prime immunization induced high titers of anti-S1 specific IgG and SARS-CoV-2 specific nAbs in the serum.

4.2. Replication competent recombinant viral vector vaccines

Several live attenuated recombinant vector vaccines, such as B/HPIV3/S-6P [46], MV-014-212 [47] or MPV/S-2P [48] are currently being tested in clinical trials. B/HPIV3/S-6P [46] is based on a bovine/human parainfluenza virus type 3 (B/HPIV-3) vector, which expresses full-length SARS-CoV-2 S in a pre-fusion stabilized form (S–6P). MV-014-212 [47] is based on a human respiratory syncytial virus (RSV) vector, encoding for a chimeric full-length SARS-CoV-2 S (S harboring the cytoplasmic tail of RSV F). MPV/S-2P [48] is based on a murine pneumonia virus (MPV) vector, expressing full-length SARS-CoV-2 in a pre-fusion stabilized form (S–2P). Prime or prime-boost IN/IT (intratracheal) immunizations with MV-014-212 [47] or B/HPIV3/S-6P [46], partially protected Syrian hamsters [46], or African green monkeys [47] against SARS-CoV-2 infection, which was associated with reduced body weight loss [46], reduced viral loads in the lung [46], BALF [47], or nasal turbinates [47]. IN/IT prime-boost immunizations of rhesus macaques with MPV/S-2P [48] provided complete protection against SARS-CoV-2 replication in the upper and lower respiratory tract. High levels of anti-S specific IgG [46–48] and IgA [46,48] antibodies were detected in the serum of immunized animals. Furthermore, anti-S specific IgG [48] and IgA [47,48], as well as anti-RBD specific IgG [48] and IgA [48] antibodies were detected in nasal swabs or nasal washes. In BALFs, anti-S specific IgG, IgA and dimeric IgA, as well as anti-RBD specific IgG and IgA antibodies could be detected in MPV/S-2P immunized rhesus macaques [48]. In human ACE2-transgenic mice [47], a single IN immunization with MV-014-212 induced anti-S specific serum IgG2 specific antibody responses and splenic IFN- γ producing T cells. SARS-CoV-2 specific nAbs were induced in the serum [46,48], which showed cross-reactive activities against SARS-CoV-2 variant strains [46,48]. Anti-S specific IFN- γ and/or tumor necrosis factor (TNF)- α producing CD4/CD8⁺ T cells were found in PBMCs [48] or BALF [48]. Furthermore, tissue-resident T cells could be found in BAL samples [48].

4.3. Replication incompetent viral vector vaccines

Replication incompetent adenoviral vector vaccines, including AdCOVID [49], and hAd5-S-Fusion+N-ETSD [50], are currently being tested in clinical trials. AdCOVID [49] is an adenovirus type 5-vectored vaccine expressing ancestral SARS-CoV-2 RBD. hAd5-S-Fusion+N-ETSD [50] is based on a human adenovirus serotype 5 expressing full-length SARS-CoV-2 S and N. The latter was fused to an enhanced T cell stimulation domain (ETSD) for improved activation of MHC class II immune responses. The adenoviral vector vaccines were tested in K18-hACE2 mice [49], CD-1 mice [49,50], or rhesus macaques [51] over a prime-boost immunization schedule using heterologous [50,51] or homologues [50] immunization strategies. High levels of anti-S [49–51] or anti-N [50] specific IgG antibodies and SARS-CoV-2 specific nAbs [49] could be detected in the serum of immunized animals. In addition, anti-S [49,50] or anti-N [50] specific IgA or IgG antibodies, as well as anti-RBD specific IgA-secreting cells [49] could be found in BALFs. In terms of cellular immunity, maturation of anti-RBD specific IFN- γ , anti-S specific IFN- γ or IL-4, or anti-N specific T cells was observed in the spleen [49,50], the lungs [49] or PBMCs [51] of immunized animals. Furthermore, tissue-resident memory T cells could be found in the lung [49]. In terms of protective efficacy, a single IN immunization with AdCOVID19 protected K18-hACE2 mice against weight loss and mortality after SARS-CoV-2 challenge [49]. Prime-boost immunizations of rhesus macaques with hAd5-S-Fusion+N-ETSD partially protected against SARS-CoV-2 infection, indicated by reduced viral loads in nasal swabs and BALFs.

IN or IM immunizations of Syrian hamsters or BALB/c mice [52] with the single-cycle replicating adenovirus vector AC-Ad6-1, expressing full-length ancestral SARS-CoV-2 S in its native form, induced anti-S specific serum IgG antibodies and anti-S specific T cells in the spleen. Furthermore, high anti-S specific IgG and SARS-CoV-2 specific nAbs were found in BALFs. However, only IN immunization induced anti-S specific IgA antibodies and CD8/CD4⁺ T cells in BALFs. IN or IM immunization partially protected Syrian hamsters against SARS-CoV-2 challenge infection, indicated by reduced body weight loss and reduced viral loads in the lung and nasal turbinates.

4.4. Protein subunit vaccines

Several protein subunit vaccines are currently being evaluated in clinical trials. Avacc® 10 (OMV-mC-Spike) [53] is based on full-length SARS-CoV-2 S (ancestral strain) in a pre-fusion stabilized form (6P), which was linked via the peptide sequence mCramp to outer membrane vesicles (OMV) of *Neisseria meningitidis*. The recombinant protein has been produced in Chinese hamster ovary (CHO) cells and subsequently linked to the OMVs. ACM-SARS-CoV-2-beta ACM CpG [54] is based on recombinant full-length SARS-CoV-2 (Beta variant B.1.351) in a pre-fusion stabilized form (2P) with a deleted furin cleavage site. The recombinant protein is co-administered with a CpG adjuvant, and encapsulated within synthetic nanovesicles, named artificial cell membrane (ACM) polymersomes. ACM facilitates delivery and internalization of target antigens which improves immune responses. Recombinant SARS-CoV-2 S was produced in Sf9 insect cells, which were infected with recombinant baculovirus containing the genetic information of SARS-CoV-2 S B.1.351. 3R_{AEW}-NC + KFD1.i.n. is a modified version of the aforementioned triple-RBD scaffold vaccine 3R-NC + KFD1.i.n, based on RBDs from BA.2.86, EG.5 and SARS-like coronavirus WIV 1. IM or IN immunization of with the protein subunit vaccines partially protected Syrian hamsters [53,54] against SARS-CoV-2 infection, indicated by reduced body weight loss [53], reduced viral loads in throat/oral swabs [53,54], the lung [53] and nasal turbinates [53], and the absence of lung pathology [54]. Furthermore, the vaccines were highly immunogenic in BALB/c mice [24,53] and were proven safe in New Zealand white rabbits [54]. High levels of anti-S or anti-RBD specific IgG [24,53,57] antibodies were detected in the serum of immunized animals. Furthermore, high titers of anti-S or anti-RBD specific IgG or IgA antibodies were found in nasal washes [53], saliva [24] and BALF [53]. SARS-CoV-2 specific nAbs were detected in the serum [24,56,54] or nasal washes [54], which showed cross-reactive activity against SARS-CoV-2 variant strains (e.g., Delta, Omicron) [24,54].

5. Future perspective and conclusion

The COVID-19 pandemic triggered tremendous innovation and progress towards the development of mucosal vaccines over the last years. Preclinical and clinical evaluation of mucosal vaccines has demonstrated their potential as effective countermeasures against SARS-CoV-2, by inducing a robust and durable systemic and mucosal immune response. The hope is that the mucosal immune response induced by these vaccines in the upper respiratory tract could prevent infection, inhibit transmission and, in the long run, reduce virus circulation in the population. SARS-CoV-2 has become an endemic virus over the last years, and a complete eradication of the virus is unlikely. However, a reduction of viral circulation by vaccination might be beneficial to reduce morbidity and mortality in vulnerable population groups. A high proportion of the population has been naturally infected with and vaccinated against SARS-CoV-2, which led to the induction of hybrid immunity in these individuals. The combination of natural infection and vaccination with parenteral vaccines has been shown to induce a high and durable level of protection and broad reactive humoral and cellular immune responses [for review: [55,56]]. This phenomenon has been widely studied with approved parenteral COVID-19 vaccines, but data

on the induction of hybrid immunity with mucosal COVID-19 vaccines are sparse. As summarized in this review, current mucosal vaccines are not providing sterilizing immunity, as vaccinated study participants still got infected with SARS-CoV-2. These individuals infected with SARS-CoV-2 and vaccinated with mucosal COVID-19 vaccines might also develop a type of hybrid immunity. This could be beneficial as the pre-existing mucosal immunity (e.g., secretory IgA antibodies) acquired due to a natural SARS-CoV-2 infection [57] might get boosted by the mucosal vaccines, providing a more robust and balanced systemic and mucosal immune response compared to parenteral vaccines.

There are still several obstacles which hamper the success of mucosal COVID-19 vaccines (Fig. 1) [58,59]: 1) the human mucosa provides a strong barrier and nasal clearance hinders the delivery of vaccine antigens and induction of mucosal immune responses, 2) safety concerns, as facial nerves innervate the nasal cavity, 3) difficulty of sampling from the lower respiratory tract (BAL(F), tracheal aspirates) to assess immunogenicity in humans, 4) difficulty to administer accurate doses, 5) most candidates are based on viral vectors and pre-existing vector immunity might impair immune responses and 6) mucosal vaccines may induce weak systemic immune responses, which may lead to discontinuation of clinical development due to inferiority of serum antibody responses compared to injected vaccines. Another obstacle is the fact that immunogenicity and efficacy studies in animals, especially in small animals such as mice or hamsters, only have low predictive value for vaccine effectiveness and immunogenicity in humans. Although many of the described vaccines were capable to induce high levels of systemic and mucosal immune responses and protective efficacy in different animal species (Table 1, Table 3), the evaluation of these vaccines in clinical trials did not reflect the preclinical results. Nevertheless, the evaluation of vaccines in different animal species is crucial before being tested in humans to e.g., identify the optimal vaccination route, to evaluate the safety and toxicity of the vaccines, or to determine the quality of induced immune responses. For the development of future mucosal vaccines, it is important to focus not only on the vaccine design, but also establish a suitable animal model to allow for adequate bridging studies [60,61].

Another obstacle is the fact that phase III trials, if done well, need to evaluate the efficacy in terms of protection from infection, not just symptomatic disease. This involves frequent testing of study subject to also capture asymptomatic infections and this is associated with very high costs. Furthermore, with the emergence of new SARS-CoV-2 variant strains, the effectiveness of mucosal vaccines primarily based on ancestral SARS-CoV-2 strains is waning. Due to that, modification and adaption of vaccine formulations have to be made. Several candidate vaccines currently being tested in clinical trials are already based on SARS-CoV-2 variant strains, such as mbO5, ACM-SARS-CoV-2 beta+ ACM CpG or 3R-NC_{AEW} + KFD1. Additional mucosal vaccines, including OCU500, which is based on a novel chimpanzee adenovirus (ChAd36), and CDO-7 N-1 [62], a live attenuated intranasal vaccine [63,64], will be tested in clinical trials in the near future.

Importantly, the development and improvement of these next generation COVID-19 vaccines – if successful – could also be applied to other respiratory viruses to curb virus spread in the population.

CRedit authorship contribution statement

Alina Tscherné: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Weina Sun:** Writing – review & editing, Writing – original draft. **Sean T.H. Liu:** Writing – review & editing, Writing – original draft. **Florian Krammer:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization.

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Declaration of competing interest

Florian Krammer declares the following conflicts of interest. The Icahn School of Medicine at Mount Sinai has filed patent applications regarding influenza virus vaccines on which FK is listed as inventor. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays, NDV-based SARS-CoV-2 vaccines influenza virus vaccines and influenza virus therapeutics which list FK as co-inventor and FK has received royalty payments from some of these patents. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2 and another company, Castlevax, to develop SARS-CoV-2 vaccines. FK and WS are co-founders and scientific advisory board member of Castlevax. FK has consulted for Merck, GSK, Gritstone, Sanofi, Curevac, Seqirus and Pfizer and is currently consulting for 3rd Rock Ventures and Avimex. The Krammer laboratory is also collaborating with Dynavax on influenza vaccine development.

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Data availability

No data was used for the research described in the article.

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