Systematic Review

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Comparison of the immunogenicity & protective efficacy of various SARS-CoV-2 vaccine candidates in non-human primates

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***Background & objectives*: The COVID-19 pandemic has emerged as a global public health crisis and research groups worldwide are engaged in developing vaccine candidates to curb its transmission, with a few vaccines having progressed to advanced stages of clinical trials. The aim of this systematic review was to compare immunogenicity and protective efficacy of various SARS-CoV-2 vaccine candidates tested in non-human primate (NHP) models.**

***Methods*: Literature on effect of SARS-CoV-2 vaccines in NHP models reported on PubMed and preprint platforms (medRxiv and bioRxiv) till October 22, 2020, was searched with the following terms: coronavirus vaccine, COVID-19 vaccine, SARS-CoV-2 vaccine, nonhuman primate, and rhesus macaque.**

***Results*: Our search yielded 19 studies, which reported immune response elicited by 18 vaccine candidates in NHP. All the vaccines induced detectable neutralizing antibody (NAb) titres in the serum of vaccinated animals, with some showing effective viral clearance from various organs. The vaccinated animals also showed nil to mild histopathological changes in their lungs compared to placebo groups in the trials that performed necropsy.**

***Interpretation & conclusions*: Our findings highlighted onset of quick immunogenicity and protective efficacy of mRNA-1273, followed by Ad26.CoV2.S, NVX-CoV2373, BNT162b2, RBD and BBV152 vaccine candidates in preclinical trials as compared to the others. NHP data also showed correlation with clinical trial data available for a few vaccines. Preclinical trials of COVID-19 vaccine candidates in NHPs yielded promising results, with some candidates faring better than others.**

Key words COVID-19 - immune response - inactivated vaccine - neutralizing antibody - non-human primate - protein subunit vaccine - T-cell response - vaccine - viral clearance

COVID-19 has emerged as a global pandemic and caused significant morbidity and mortality all over the world. Currently, no effective therapeutics or vaccines are available for this novel disease. Development of a safe and effective vaccine appears to be the most promising tool to help develop immunity against

the infection and reduce disease transmission. As per the World Health Organization (WHO) report, till October 29, 2020, 45 vaccine candidates were under clinical trials and 156 candidates were in pre-clinical evaluation stage. These include whole virion-inactivated vaccines, recombinant vaccines, viral vector vaccines, RNA- and DNA-based vaccines and sub-unit vaccines[1].

Vaccine development is a complex and time-consuming process. However, to control the pandemic, it is important to develop an effective vaccine candidate[2]. Various approaches are being tried to fast track the development of safe and efficacious COVID-19 vaccines without compromising scientific quality and ethics. One of such approaches is the use of well-established platforms, which have reduced the time taken to develop candidates and also helped in building trust since the beginning. DNA and RNA platforms are best suited for fast development, followed by subunit protein vaccines[2-5]. An important aspect of vaccine development is to assess the safety and protective efficacy of different candidates by conducting large animal challenge studies. Small animals such as rats, mice, rabbits and hamsters are useful for making preliminary evaluation of safety, immunogenicity and dosing of the vaccine candidates. Studies in large animals are more useful in understanding comparative immune responses in humans[6]. So far, non-human primates (NHPs) are the best models for assessing the protective efficacy of vaccine candidates as they mimic many diseases caused in humans. However, limited availability of approved primate facilities and cost are major deterrents[7]. Rhesus macaques have been established as an effective animal model for SARS-CoV-2, and their use in pre-clinical experiments is globally recognized as an acceptable way to fast track vaccine development[8,9].

Till now, pre-clinical studies in NHPs have been successfully completed for a few COVID-19 vaccine candidates. However, in view of the urgency to introduce vaccines for COVID-19, parallel Phase I/II clinical trials and NHP studies are ongoing/planned for several vaccine candidates. Pre-clinical studies in NHPs would be useful for all upcoming vaccines to enable decision-making process for quick licensure. In the interest of time and public health emergency, the Central Drugs Standard Control Organization (CDSCO) guidelines state that COVID-19 vaccine candidates with immunogenicity data demonstrating high neutralizing antibody (NAb) titres and T-helper (Th1)-type cell polarization may be allowed to proceed to first-in-human trials without first completing post-vaccination challenge studies in appropriate animal models. However, such studies are strongly advised in parallel to the initial trials[10]. Hence, there is an urgent need to undertake NHP studies for COVID-19 vaccine candidates. In this systematic review information has been compiled on various NHP studies conducted for COVID-19 vaccine candidates and performance of various candidates and platforms. The review also delineates the NHP species used, virus challenge doses, outcomes of the studies in terms of viral clearance, B- and T-cell responses, *etc*. In addition, wherever available, results of the clinical trials have also been included.

Material & Methods

A review of relevant literature was carried out as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[11]. The literature on SARS-CoV-2 vaccine studies in NHP models reported on PubMed and pre-print platforms (medRxiv and bioRxiv) in 2020 was searched using appropriate Medical Subject Heading (MeSH) terms. The searches were done using the following terms: coronavirus vaccine, COVID-19 vaccine, SARS-CoV-2 vaccine, nonhuman primate and rhesus macaque till October 22, 2020. Nineteen studies meeting the inclusion criteria were considered. These encompassed 18 vaccine candidates which were included in this review. The PRISMA flowchart depicting study selection is presented in the Figure.

All the articles were downloaded and read thoroughly by the authors. The data were compiled and reviewed to compare the degree of immunogenicity and protective efficacy of the various vaccine candidates based on the following parameters: (*i*) viral clearance from various organs and tissues of NHPs following challenge with live SARS-CoV-2; (*ii*) histopathology and immunohistochemistry (IHC) of the lung tissues of NHPs from vaccinated and placebo groups; and (*iii*) immune response induced by the vaccine, as evidenced by NAb and T-cell responses, measured by specific cytokines.

Results

Our search yielded a total of 318 studies and one record was added manually. Twelve articles

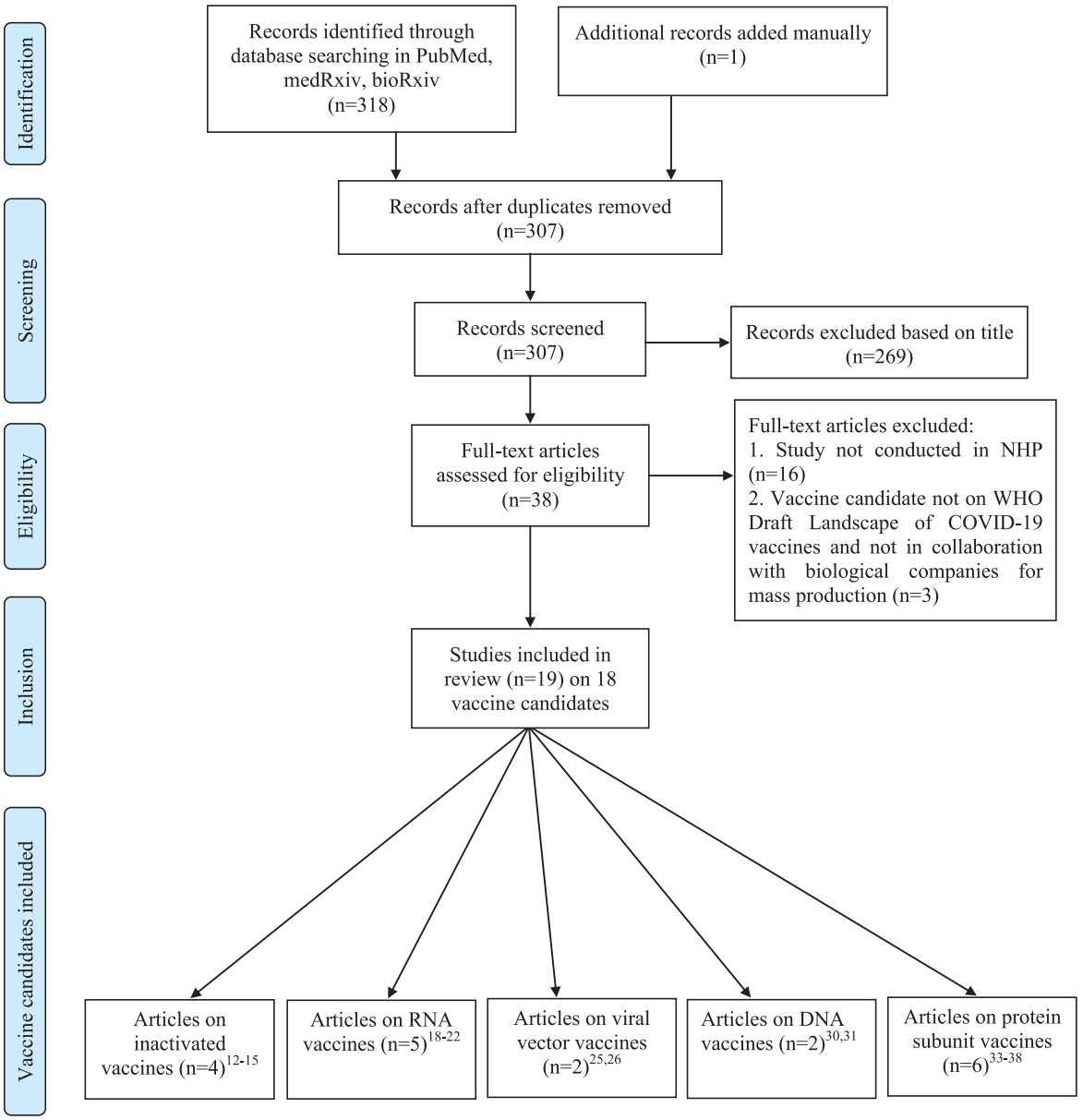

Figure. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart depicting study design.

were duplicated between the platforms and omitted. Vaccine studies related to other coronaviruses such as SARS-CoV and Middle East respiratory syndrome coronavirus, as well as *in vitro* studies, were excluded from the analysis. Based on the above criteria, 269 studies were eliminated. Sixteen studies were excluded further because those were not carried out in NHP. Two studies were not considered because those were neither on the WHO Draft Landscape of COVID-19 vaccine candidates nor were in collaboration with pharmaceutical or biological product companies to commence mass-scale production. The Figure depicts the methodology of study selection.

Nineteen studies including 18 vaccine candidates meeting the inclusion criteria were included in this review. NHP species used in the respective studies, site of experiment, details of vaccine administration and doses, virus challenge study and NHP sacrifice are enumerated in Table I.

Table I. Various parameters of COVID-19 candidate vaccines included in the review

Vaccine candidates	Species of NHP	Parameters						Proposed storage temperature (°C)
		Number of NHPs and groups (including control/ placebo/sham)	Site of animal experiment	Vaccine dosage and route	Time until challenge from the first dose (wk)	Live virus challenge dose	DPI of animal sacrifice	
Inactivated vaccines								
BBV152[12]	Rhesus macaques	20 (4 groups, 5 in each group)	ICMR-National Institute of Virology, Pune, India	3 µg+adjuvant B/6 µg+adjuvant A/6 µg+adjuvant B at 0, 14 days i.m. Two doses 14 days apart administered *via* i.m. route	4	$1\times10^{6.5}$ $TCID_{50}$/ ml, intranasally (0.5 ml: 0.25ml each nostril) and intratracheally (1 ml)	7	2-8
PiCoVacc[13]	Rhesus macaques	16 (4 groups, 4 in each group)	Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Beijing, China	Either 3 or 6 µg at 0, 7 and 14 days i.m. Three doses at intervals of seven days each administered *via* i.m. route	3	10^6 $TCID_{50}$/ ml, only intratracheally (volume not mentioned)	7	2-8
BBIBP-CorV[14]	Cynomolgus macaques and Rhesus macaques	Safety evaluation: 40 Cynomolgus monkeys (4 groups, 10 in each group) Challenge study: 10 Rhesus macaques (4 macaques each in 2 vaccinated group and 2 placebo)	Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Comparative Medicine Center, Beijing, China	2 or 4 or 8 µg of vaccine (0.5 ml) administered to Cynomolgus monkeys once a week for three weeks (4 doses) *via* i.m. route for safety profile 2 or 8 µg at 0 and 14 days administered to Rhesus macaques *via* i.m. route for viral challenge study Four or two doses given weekly and biweekly, respectively, to different NHPs *via* i.m. route	3	10^6 $TCID_{50}$/ml, intratracheally only (volume not mentioned)	Cynomolgus macaques: Day 25/36 Rhesus macaques: 7 DPI	Not known

Contd...

Vaccine candidates	Species of NHP	Parameters						Proposed storage temperature (°C)
		Number of NHPs and groups (including control/ placebo/sham)	Site of animal experiment	Vaccine dosage and route	Time until challenge from the first dose (wk)	Live virus challenge dose	DPI of animal sacrifice	
Inactivated Vaccine[15]	Rhesus macaques	20 (10 in vaccinated group and 10 in control group)	Institute of Medical Biology, Chinese Academy of Medical Sciences, Beijing, China	3 groups: 4 macaques given 200 EU i.m. at days 0 and 14 3 macaques given 100 EU i.m. at days 0 and 14 3 macaques given 20 EU i.m. at days 0 and 14 Two doses two weeks apart administered *via* i.m. route	2	2×10^5 CCID50 given to each monkey nasally	At different time points: 200 EU group: 1 animal each on 3, 5, 7, 9 DPI 100 EU group: 1 animal each on 3, 7, 15 DPI 20 EU group: 1 animal each on 5, 9 and 15 DPI Adjuvant group: 1 animal each on 3, 5, 7, 9 and 15 DPI*	Not known
RNA vaccines								
mRNA-1273[18]	Rhesus macaques	24 (3 groups, 8 in each group)	BIOQUAL, INC., Maryland, USA	Either 10 µg or 100 µg at 0 and 28 days i.m. Two doses 28 days apart administered via i.m. route	8	10^6 50% $TCID_{50}$ (1.9×10^5 pfu) intranasally (1 ml: 0.5 each nostril) and intratracheally (3 ml)	14/15 DPI	2-8 for 30 days, −20 for six months
BNT162b2[19]	Rhesus macaques	18 (3 groups, 6 in each group)	New Iberia Research Center, University of Louisiana, Louisiana, USA And Southwest National Primate Research Center, San Antonio, Texas, USA	30 or 100 µg on days 0 and 21 i.m. Two doses 21 days apart administered *via* i.m. route	11	1.05×10^6 pfu, intranasally (0.25 ml) and intratracheally (0.25 ml)	7/8 DPI	−70 Stable at RT for 24 h

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Vaccine candidates	Species of NHP	Parameters						Proposed storage temperature (°C)
		Number of NHPs and groups (including control/ placebo/sham)	Site of animal experiment	Vaccine dosage and route	Time until challenge from the first dose (wk)	Live virus challenge dose	DPI of animal sacrifice	
ARCoV[20]	Cynomolgus macaques	30 (10 each in two vaccinated groups and 10 in placebo group)	Academy of Military Medical Sciences, Beijing, China	100 or 1000 µg on days 0 and 14 Two doses 14 days apart administered *via* i.m. route	Not done	Not done	Not done	25 (RT) for one week
MRT5500[21]	Cynomolgus macaques	15 (4 monkeys in each of three vaccinated groups, 3 naïve macaques)	New Iberia Research Center, Louisiana, USA	15 or 45 or 135 µg on days 0 and 21 i.m. Two doses (500 µl each) 21 days apart administered *via* i.m. route	Not done	Not done	Not done	−20
LION/ repRNA-CoV2S[22]	Pigtail macaque	5 (3 in prime only and 2 in prime-boost group)	Washington National Primate Research Centre, Seattle, Washington, USA	2 groups: Prime only: 250 µg single dose i.m. Prime boost: Two doses of 50 µg each at weeks 0 and 4 i.m. Single dose or two doses at four weeks interval administered *via* i.m. route	Not done	Not done	Not done	Stable for one week at RT after mixing vaccine constituents
Viral vector vaccines								
Ad26.COV2.S	Rhesus macaques	32 (7 groups, 4-6 in each group) and 20 sham	BIOQUAL Inc., Maryland, USA	10^{11} viral particles of Ad 26 vector i.m. Single-dose administered *via* i.m. route	6	1.0×10^{5} $TCID_{50}$ intranasally (1 ml: 0.5 each nostril) and intratracheally (1 ml)	Not mentioned	Standard refrigeration
ChAdOx-1nCoV-19[26]	Rhesus macaques	18 (3 groups, 6 in each group)	Rocky Mountain Laboratories, Hamilton, Montana, USA	2.5×10^{10} viral particles ChAdO×1 nCoV-19, prime-only (28 days before challenge) and prime-boost regimens (56 and 28 days before challenge) administered *via* i.m. route Single dose or 2 doses at 28 days interval administered *via* i.m. route	4 (prime only) 8 (prime boost)	4×10^{5} $TCID_{50}$/ ml, 4 ml intratracheally and 1 ml intranasally and 1 ml orally and 0.5 ml ocularly	7 DPI	2-8

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Vaccine candidates	Species of NHP	Parameters						Proposed storage temperature (°C)
		Number of NHPs and groups (including control/ placebo/sham)	Site of animal experiment	Vaccine dosage and route	Time until challenge from the first dose (wk)	Live virus challenge dose	DPI of animal sacrifice	
DNA vaccines								
INO-4800[30]	Rhesus macaques	10 (2 groups, 5 in each group)	BIOQUAL Inc., Maryland, USA	1 mg at days 0 and 28 ID Two doses 28 days apart administered *via* i.d. route	17	1.1×10^4 pfu intranasally (1 ml: 0.5 each nostril) and intratracheally (1 ml)	Not mentioned	Stable at RT for >one year
GX-19[31]	Cynomolgus macaques	3 vaccinated macaques and 3 macaques previously not exposed to the virus used as controls in viral challenge study	Korea National Primate Research Centre, Korea Research Institute of Bioscience and Biotechnology, Cheongju, Chungcheongbuk, Republic of Korea	3 mg GX-19 given i.m. at weeks 0, 3 and 5.5 Three doses three weeks apart administered *via* i.m. route	15.5	2.6×10^7 $TCID_{50}$/ ml given intranasally and intratracheally and orally and intravenously and ocularly (volumes not mentioned)	4 DPI	Stable at RT (4-25)
Protein subunit vaccines								
NVX-CoV2373[33,34]	Olive Baboon and Cynomolgus macaques	10 (4 groups, 2-3 in each group) baboons Virus clearance study in 16 Cynomolgus macaques (4 groups of 4 macaques each)	BIOQUAL Inc., Maryland, USA	Three groups of Baboons: 1 µg/5 µg/25 µg NVX-CoV2373 (with 50 µg Matrix-M adjuvant) i.m. on days 0 and 21 One group of baboons: 2.5 µg NVX-CoV2373 without adjuvant, i.m. on days 0 and 21 Two groups of macaques: 5 µg/25 µg NVX-CoV2373 (with 50 µg Matrix-M adjuvant) i.m. on days 0 and 21 One group of macaques: 2.5 µg NVX-CoV2373 (with 25 µg Matrix-M adjuvant) i.m. on days 0 and 21 Two doses 21 days apart administered *via* i.m. route	6	1.04×10^4 pfu intranasally and intratracheally (0.25 ml each)	7 DPI	2-8 for six months 24 h at RT

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Vaccine candidates	Species of NHP	Parameters						Proposed storage temperature (°C)
		Number of NHPs and groups (including control/ placebo/sham)	Site of animal experiment	Vaccine dosage and route	Time until challenge from the first dose (wk)	Live virus challenge dose	DPI of animal sacrifice	
S-Trimer[35]	Rhesus macaques	18 (6 in each group)	Kunming Institute of Zoology, Chinese Academy of Medical Sciences, Kunming, Yunnan, China	2 groups: 30 µg S-Trimer (with 0.25 ml AS03 adjuvant) on days 0 and 21 given i.m. 30 µg S-Trimer (with 1.5 mg CpG 1018 + 0.75 mg alum adjuvant) on days 0 and 21 given i.m. Two doses 21 days apart administered *via* i.m. route	5	2.6×10^6 $TCID_{50}$ intranasally (40%) and intratracheally (60%)	5 DPI (2 from each group) and 7 DPI (4 from each group)	2-8
RBD[36]	Rhesus macaques	12 (7 vaccinated macaques in two dose groups, 2 macaques in placebo group and 3 in untreated group)	Kunming National High-level Biosafety primate Research Centre, Kunming, Yunnan, China	20 µg or 40 µg on days 0 and 7 i.m. Two doses seven days apart administered *via* i.m. route	4	10^6 pfu/ml only intranasally (0.5 ml)	Not mentioned	
Sad23L-nCoV-S/ Ad49L-nCoV-S[37]	Rhesus macaques	8 (5 vaccinated and 3 control macaques)	Huangzheng Laboratory Animal Breeding Centre, Guangzhou, China	5×10^9 pfu Sad23L-nCoV-S at week 0 i.m., followed by 5×10^9 pfu Ad49L-nCoV-S at week 4 i.m. Two doses four weeks apart administered *via* i.m. route	Not done	Not done	Not done	
S1-Fc[38]	Cynomolgus macaques	2	Xieerxin Biotech, China	500 µg on days 1, 5 and 23 i.m. Three doses four days and 22 days from first dose. Route of administration not mentioned	Not done	Not done	Not done	-

*2 animals were also injected with RBD peptide. The data is not included in the table in view of inadequate number of macaques; TCID, tissue culture infective dose; EU, entropy units; CCID, cell culture infective dose; pfu, plaque-forming unit; LION, lipid inorganic nanoparticles; DPI, days post-inoculation; i.m., intramuscular; NHPs, non-human primates; RT, room temperature

Table II. Cellular immune response elicited by COVID-19 candidate vaccines included in the review

Vaccine	Time points post-first dose of vaccination	Groups of NHP	Th1 response				Th2 response				Th response	Pre-dominant T-cell response type
			Anti-inflammatory	Pro-inflammatory			Anti-inflammatory			Pro-inflammatory		
				TNFα	IFN γ	IL 2	IL 4	IL 13	IL 6	IL 5		
Inactivated vaccines												
BBV152	4-5 weeks	Vaccinated	-	N	N	N	N	N	↓	↑[#]	Y	-
		Unvaccinated	-	N	N	N	N	N	↑[*]	↓	-	
PiCoVacc	Weeks 1, 3, 5	Vaccinated	-	NS	NS	NS	NS	-	NS	NS	-	-
		Unvaccinated	-	NS	NS	NS	NS	-	NS	NS	-	
BBIBP-CorV	Day 1 till day 36	Vaccinated	-	NS	NS	NS	NS	-	NS	NS	-	-
		Unvaccinated	-	NS	NS	NS	NS	NS	NS	NS	-	
Inactivated Vaccine	Days 3, 5, 7, 9, 15	Vaccinated with 200 EU	-	↓	-	↓	↓	-	NS	NS	-	-
		Vaccinated with 100 EU	-	↓	-	↓	↓	-	NS	NS	-	
		Vaccinated with 20 EU	-	↓	-	↓	↓	-	NS	NS	-	
		Control	-	↑	-	↑	↑	-	NS	NS	-	
RNA vaccine												
mRNA-1273	Eight weeks	Vaccinated	-	Y	Y	Y	N	N	-	-	Y	Th1-biased response
		Unvaccinated	-	N	N	N	N	N	-	-	-	
BNT162b2	Peak response at four weeks	Vaccinated	-	↑	↑	↑	↓	-	-	-	-	Th1-biased response
		Unvaccinated	-	N	N	N	N	-	-	-	-	
ARCoV	Three weeks	Vaccinated	-	-	↑↑	-	NS	-	-	-	-	Th1 biased
		Unvaccinated	-	-	-	-	NS	-	-	-	-	
MRT 5500	Three weeks	Vaccinated	-	-	↑↑	-	-	x	-	-	-	Th1 biased
		Unvaccinated	-	-	-	-	-	x	-	-	-	
LION/repRNA-CoV2S	2, 4, 6 weeks	Vaccinated	-	-	NS	-	-	-	-	-	-	-
		Unvaccinated	-	-	-	-	-	-	-	-	-	
Viral vector vaccine												
Ad26.COV2.S	Four weeks	Vaccinated	-	-	Y	-	N	-	-	-	-	Th1-biased response
		Unvaccinated	-	-	N	-	N	-	-	-	-	
ChAdOx-1nCoV-19	1, 3, 5, 7 DPI	Vaccinated	-	NS	-	NS	NS	NS	NS	NS	-	Low-Th1/Th2 response
		Unvaccinated	-	NS	-	NS	NS	NS	NS	NS	-	

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Vaccine	Time points post-first dose of vaccination	Groups of NHP	Th1 response				Th2 response				Th response Y	Pre-dominant T-cell response type
			Anti-inflammatory	Pro-inflammatory			Anti-inflammatory			Pro-inflammatory		
				TNFα	IFN γ	IL 2	IL 4	IL 13	IL 6	IL 5		
DNA vaccine												
INO-4800	Monitored till week 15	Vaccinated	-	-	Y	-	-	-	-	-	-	-
		Unvaccinated	-	-	N	-	-	-	-	-	-	
		Unvaccinated	-	NS	NS	NS	NS	NS	NS	NS	-	
GX-19	Weeks 5.5 and 8	Vaccinated	-	↑	↑	↑	-	-	-	-	-	Th1 biased
Protein subunit vaccine												
NVX-CoV2373	Four weeks	Vaccinated (with adjuvant)	-	↑	↑	↑	↓	-	-	-	-	Th1 biased
		No adjuvant	-	↓	↓	↓	↓↓	-	-	-	-	
S-Trimer	Five weeks, continued till sacrifice	Vaccinated (with adjuvant)	X	X	X	X	X	X	X	X	X	Better lymphocyte response in CpG-1018 + alum group
		No adjuvant	X	X	X	X	X	X	X	X	X	
Sad23L-nCoV-S/ Ad49L-nCoV-S	2, 4, 5, 6, 8 weeks	Vaccinated	-	↑	↑↑	↑	↓	-	-	-	-	Th1 biased

*Compared to vaccinated groups, 3µg+adjuvant B and 6 µg+adjuvant A; #Compared to the group 6 µg+adjuvant A. T cell response induced by RBD and S1-Fc were not available. Th, T helper response; Y, detected; N, Not detected; ↑, increased as compared to placebo; ↓, decreased compared to vaccinated; NS, not significant; DPI: days post-infection; X, not studied; IFN, interferon; LION, lipid inorganic nanoparticles; TNF-α, tumour necrosis factor-alpha, IL, interleukin

Source: Refs 11-14, 17-21, 24, 25,29, 30, 32-37

Comparison of viral clearance, histopathology findings and immune response of these candidate vaccines was also done:

Inactivated vaccines

Four inactivated vaccine candidates were considered in this review: BBV152[12], PiCoVacc[13], BBIBP-CorV[14] and Inactivated Vaccine[15].

Viral clearance from various organs: Robust viral clearance from upper respiratory tract (nasopharynx and oropharynx), lungs, bronchoalveolar lavage (BAL) fluid, pharynx, trachea, tonsil, mediastinal and cervical lymph nodes and extrapulmonary organs such as gastrointestinal tract (GIT), urinary bladder and skin was observed with 3 µg+adjuvant B and 6 µg+adjuvant B formulations of BBV152, with none of the vaccinated animals showing sub-genomic RNA (sgRNA) by seven-day post-infection (DPI). 6 µg+adjuvant A formulation of BBV152 showed less efficacy than the aforementioned formulations in viral clearance[12]. 6 µg dose group of PiCoVacc effectively cleared the virus from nasopharynx, GIT and lung, while the animals vaccinated with 8 µg of BBIBP-CorV were unable to completely clear the virus from the GIT, though it was successful in virus clearance from the oropharynx and lungs by seven DPI[13,14]. The lower dose groups of both these vaccines were less effective in viral clearance, but animals in the lower dose groups still fared better than the placebo groups. Inactivated Vaccine was excluded from this analysis due to unavailability of the number of vaccinated macaques at different time points in post-virus challenge study[15].

Histopathology of lungs on necropsy: Nil-to-mild focal histopathological changes were observed in lung tissues of vaccinated animals compared to the placebo groups in all four trials[12-15]. The unvaccinated animals showed evidence of pneumonia, which ranged from moderate to severe.

Immune response in vaccinated animals

Neutralizing antibody (NAb) response: NAbs were measured using viral neutralization assays such as plaque reduction neutralization test (PRNT), microneutralization test and live SARS-CoV-2 assays. NAbs first appeared in the serum of animals injected with BBIBP-CorV and Inactivated Vaccine injected animals at one week, PiCoVacc-vaccinated macaques at two weeks and BBV152-vaccinated animals at three weeks following administration of the first dose of the vaccine[12-15]. The levels gradually rose, with PiCoVacc-vaccinated animals showing peak NAb levels at three weeks. The antibody titres were observed and detected till five weeks after administration of the first dose of BBV152, PiCoVacc and BBIBP-CorV[12-14]. Data from the Inactivated Vaccine trial were not clear on the duration of protection offered by the antibodies. Control animals showed nil/minimal NAb titres compared to vaccinated animals in all the studies[12-15].

T-cell response: The inactivated vaccines did not elicit an effective cellular immune response in vaccinated NHPs; however, BBV152 produced T-helper cell response[12]. Table II depicts T-cell response and cytokine profile of various vaccine candidates.

Progress to clinical trial: Except Inactivated Vaccine, which is still in Phase I/IIa, the other three candidates have entered Phase III clinical trials. Data from Phase II clinical trials of PiCoVacc and BBIBP-CorV are available in the public domain[16,17].

PiCoVacc Phase II trial: A total of 600 participants enrolled in the study were given either placebo or the vaccine candidate (renamed CoronaVac in clinical trials) in doses of 3 or 6 µg two or four weeks apart[16].

(*i*) NAb response: Although higher NAb response was observed with the 6 µg group, the different vaccination schedules did not produce any significant difference in NAb response in the respective dosage groups. With the day 0,14 dosage schedule, NAb was detected first at 14 days. Response was higher with the 0,28 day dosage schedule[16].

(*ii*) T-cell response: Cellular immune response data were not available.

BBIBP-CorV Phase I/II trial: Phase I trial enrolled 192 participants and they were inoculated with 2, 4 or 8 µg BBIBP-CorV four weeks apart. Phase II was carried out in 448 participants who received 8 µg single dose of BBIBP-CorV or two doses of 4 µg each on days 0,14/0,21 and 0,28[17].

(*i*) NAb response: Phase I trial yielded better response in 18-59-yr-old participants, with 79-96 per cent seroconversion by two weeks and 100 per cent seroconversion by four weeks. Response was poorer in participants aged 60 yr and above. Phase II participants had detectable NAb starting from 1 to 2 wk after first dose, with better response seen in the 4 µg dosage schedules on days 0,21 and 0,28.

(*ii*) T-cell response: Cellular immune response data were not available.

RNA vaccines

The five RNA vaccine candidates included were mRNA-1273[18], BNT162b2[19], ARCoV[20], MRT5500[21] and lipid inorganic nanoparticles (LION)/repRNA-CoV2S[22].

Viral clearance from various organs: mRNA-1273 successfully cleared virus from tissues, as evidenced by the absence of sgRNA in throat swab and BAL of vaccinated macaques by 5-7 DPI and lung tissue of vaccinated animals at necropsy on 14/15 DPI. The 100 µg dose group showed better response than the 10 µg group, and both fared better than the control group[18]. BNT162b2 showed the absence of sgRNA in nasal swabs at three DPI and throat swabs and BAL fluid at six DPI (throat swab of one vaccinated animal tested positive at 10 DPI)[19]. Virus challenge study was not conducted in the ARCoV, MRT5500 and LION/repRNA-CoV2S vaccine trials[20-22].

Histopathology of lungs on necropsy: Mild inflammation was noted in the lung tissues of mRNA-1273-vaccinated animals on necropsy, while the control animals showed moderate-to-severe pneumonia[18]. Histopathological findings were not available for BNT162b2 vaccine, while the other trials did not conduct necropsy[19].

Immune response in vaccinated animals

Neutralizing antibody response: Different virus neutralization methods such as Plaque Reduction and Neutralization Assay (PRNT), microneutralization assay, live virus neutralization assay, as well as pseudovirus neutralization assay were used to assess NAb induced by RNA vaccines. NAbs were first detected in the serum of MRT5500-injected macaques at one week, mRNA-1273, ARCoV and LION/repRNA-CoV2S–vaccinated animals at two weeks and BNT162b2-vaccinated animals at three weeks after first vaccination. NAb titres peaked at six weeks with mRNA-1273 and LION/repRNA-CoV2S vaccines and were detected in the serum of vaccinated animals till 8 and 10 wk, respectively, from the first vaccine dose[18-22] while with the remaining three vaccines, peaks were observed at 4-5 wk (four weeks for ARCoV, five weeks for MRT5500 and 4-5 wk for BNT162b2)[19-21]. NAb response persisted up to eight weeks with BNT162b2 and six weeks with MRT5500. Control animals in the mRNA-1273 trial had poor NAb titres compared to vaccinated animals. Vaccinated macaques had NAb titres 84 times higher than that of convalescent human serum panel used in the study[18]. No data on NAb titres in the control animals were available for BNT162b2, ARCoV, LION/repRNA-CoV2S and MRT5500, although LION/repRNA-CoV2S and low-dose group of ARCOV had NAb titres comparable with convalescent human serum[19-22]. MRT5500 apparently produced a dose-dependent response, but there was no significant difference in titres between vaccinated groups[21].

T-cell response: mRNA-1273, BNT162b2, ARCoV and MRT5500 elicited Th1 skewed cellular immune responses, while T-cell response was not significant for LION/repRNA-CoV2S vaccine. Details of T-cell response elicited by vaccine candidates are enumerated in Table II.

Progress to clinical trial: mRNA-1273 and BNT162b1 (which showed similar results to BNT162b2 in animal trials) have progressed to Phase III clinical trials. Others are in Phase I/II or in pre-clinical stage. Data from published Phase I/II clinical trials of mRNA-1273 and BNT162b1 clinical trials are as follows:

mRNA-1273 Phase I trial: Forty five participants were inoculated with two doses of 25/100 µg or 250 µg vaccine, four weeks apart[23].

(*i*) NAb response: First detected at two weeks in 50 per cent participants and gradually increased. Higher response was seen with higher dose groups.

(*ii*) T-cell response: Cellular immune response data for the 25 and 100 µg groups showed a Th1-biased immune response.

BNT162b1 Phase I/II trial: Forty five participants enrolled in the trial were given two doses of 10 µg/30 µg vaccine three weeks apart or a single shot of 100 µg vaccine[24].

(*i*) NAb response: First detected at three weeks and the titres gradually rose.

(*ii*) T-cell response: Cellular immune response data were not available.

Viral vector vaccines

Ad26.COV2.S[25] and ChAdOx1 nCoV-19[26] were the two viral vector vaccines included in this review.

Viral clearance from various organs: Ad26.COV2.S showed remarkable clearance of the virus from the nose and BAL fluid starting from two DPI[25]. ChAdOx1 nCoV-19 showed less efficacy, with 50 and 16 per cent vaccinated animals showing the presence of genomic RNA (gRNA) in the nasal swabs and BAL fluid, respectively, on five DPI[26]. Although ChAdOx1 nCoV-

19 effectively cleared the virus from the trachea and tonsils on necropsy at seven DPI, it was less effective in doing so from the pharynx, mediastinal lymph node, tonsil, GIT and bladder[26].

Histopathology of lungs on necropsy: Lung pathology was not studied for Ad26.COV2.S, while no vaccinated animal in the ChAdOx1 nCoV-19 vaccine trial showed evidence of pneumonia on histopathology, which is in contrast with the control group where two of the three animals developed viral interstitial pneumonia[25,26].

Immune response in vaccinated animals

Neutralizing antibody response: Live virus neutralization assay and pseudovirus neutralization assay were used to measure NAb response elicited by both the vaccines. NAb response was induced within two weeks of administration of the first dose, with NAb peak achieved at four weeks with Ad26.COV2.S and six weeks with ChAdOx1 nCoV-19. While in the Ad26.COV2.S trial NAb titres were not measured beyond four weeks, NAbs induced by ChAdOx1 nCoV-19 were detectable till eight weeks[25,26]. The response was higher with the prime-boost regimen of ChAdOx1 nCoV-19, and animals vaccinated with these candidates had higher titres than control animals and convalescent human serum. Ad26.COV2.S-vaccinated animals had higher NAb titres than control animals[25,26].

T-cell response: Ad26.COV2.S induced Th1-biased response in vaccinated macaques, while ChAdOx1 nCoV-19 produced low Th1/Th2 immune response. T-cell responses elicited by these vaccines are described in Table II.

Progress to clinical trial: Both candidates are currently under evaluation in Phase III clinical trials. Phase I data from both vaccine trials and Phase II/III data from ChAdOx1 nCoV-19 trial are discussed below.

Ad26.COV2.S (renamed JNJ-78436735) Phase I trial: Of the 796 participants enrolled, immunogenicity results were available for 390 vaccine recipients. 5×10^{10} or 1×10^{11} virus particles were given as single dose or two doses four weeks apart[27].

(*i*) NAb response: First testing carried out at four weeks showed 98 per cent participants had detectable NAb.
(*ii*) T-cell response: It was predominantly Th1 skewed.

ChAdOx1 nCoV-19 Phase I/II trial: A total of 1077 participants were given either a single dose of 5×10^{10} virus particles or two doses four weeks apart[28].

(*i*) NAb response: All participants tested at four weeks had detectable NAb.
(*ii*) T-cell response: Interferon (IFN)-γ was detected at seven days and peaked at 14 days.

ChAdOx1 nCoV-19 Phase II/III trial: Data were available from 560 participants in low-dose (2.2×10^{10} virus particles) and standard-dose ($3.5\text{-}6.5\times10^{10}$ virus particles) cohorts in single and double dose regimens (28 days apart)[29].

(*i*) NAb response: First detected at four weeks and peaked at six weeks after first dose.
(*ii*) T-cell response: IFN-γ peaked at 14 days after first dose.

DNA vaccines

This category included INO-4800[30] and GX-19[31] vaccines.

Viral clearance from various organs: INO-4800–vaccinated animals did not demonstrate effective clearance of the virus from tissues, with sgRNA still detectable in the nasal swab and BAL fluid of vaccinated animals at seven DPI. Findings were similar to GX-19, which showed viral copies in the nasal and throat swabs of all vaccinated animals till euthanasia on four DPI[30,31].

Histopathology of lungs on necropsy: Lungs of GX-19–vaccinated animals showed mild changes compared to the placebo group, which developed moderate-to-severe inflammation[31]. Necropsy studies were not carried out for INO-4800[30].

Immune response in vaccinated animals

Neutralizing antibody response: The INO-4800 trial employed pseudovirus neutralization assay, while the GX-19 trial employed PRNT assay for measuring NAb. Both vaccines induced NAb response in vaccinated macaques, with the first detection at four weeks with INO-4800 and 5.5 wk with GX-19, starting from administration of the first dose of the vaccines. INO-4800 showed peak NAb response at six weeks after the first dose, and the antibodies were detectable for INO-4800 and GX-19 till weeks 12 and 8, respectively, from the first vaccination[30,31]. INO-4800–immunized animals had higher NAb titres than placebo animals.

T-cell response: GX-19 produced a Th1 cell response in vaccinated macaques, while INO-4800 induced only IFN-γ which was detected in the serum of vaccinated animals but not control animals. Table II describes the cellular immune response elicited by these vaccines.

Progress to clinical trial: Both candidates are currently in Phase I/IIa clinical trials with limited data available for INO-4800.

INO-4800 Phase I trial: Phase I trial on 40 healthy volunteers demonstrated immune response in 94 per cent of participants six weeks after two doses of INO-4800[32].

Protein subunit vaccines

NVX-CoV2373[33,34], S-Trimer[35], RBD[36], Sad23L-nCoV-S/Ad49L-nCoV-S[37] and S1-Fc[38] were the protein vaccines included in the review.

Viral clearance from various organs: NVX-CoV2373 demonstrated robust viral clearance in Cynomolgus macaques, with all vaccinated animals showing the absence of sgRNA by four DPI. S-Trimer vaccinated macaques (16-64 per cent of animals from AS03 and CpG-1018/alum adjuvant groups) showed the presence of gRNA in the nasal swabs, throat swabs, anal swabs and trachea till seven DPI. There was complete clearance of the virus from lungs with S-Trimer, and RBD (20 and 40 µg doses) showed absence of the virus in the throat and anal swabs, with no detectable sgRNA (though gRNA was detected) by six DPI[35,36]. Neither gRNA nor sgRNA could be detected in the lung tissues of RBD vaccinated animals in both groups following virus challenge. Virus challenge study was not performed for Sad23L-nCoV-S/Ad49L-nCoV-S vaccine[37]. No data were available for viral clearance from tissues and organs for S1-Fc[38].

Histopathology of lungs on necropsy: Lungs of control animals showed evidence of pneumonia, while nil-to-mild changes were observed in lung tissue of animals vaccinated with NVX-CoV2373, S-Trimer and RBD vaccines[34-36]. No data were available for Sad23L-nCoV-S/Ad49L-nCoV-S candidate vaccine. Further, data were not available for histopathological findings of lung tissue in the S1-Fc trial[37,38].

Immune response in vaccinated animals

Neutralizing antibody response: In NVX-CoV2373 vaccinated animals NAbs were measured using live virus neutralization assay. S-Trimer and RBD trials employed live virus as well as pseudovirus neutralization assays. Sad23L-nCoV-S/Ad49L-nCoV-S trial used surrogate viral neutralization assay and pseudovirus neutralization assays to measure NAb. Both S-Trimer and Sad23L-nCoV-S/Ad49L-nCoV-S induced NAb response within two weeks of first vaccination, while it took only one week with RBD, three weeks with NVX-CoV2373 and one week with S1-Fc. The titres gradually rose, with peaks at five weeks for S-Trimer and S1-Fc and 4-6 wk for Sad23L-nCoV-S/Ad49L-nCoV-S, and were detectable till five weeks with NVX-CoV2373 and RBD, six weeks with S-Trimer and 10 wk with Sad23L-nCoV-S/Ad49L-nCoV-S[33,35-38]. NAb titres were higher in animals vaccinated with NVX-CoV2373, S-Trimer and RBD compared to placebo, while control data were not available for Sad23L-nCoV-S/Ad49L-nCoV-S. NVX-CoV2373–vaccinated animals had higher NAb titres than control animals, with increasing titres on booster dose administration[33,35-38]. Sad23L-nCoV-S/Ad49L-nCoV-S response was not enhanced after booster dose.

T-cell response: NVX-CoV2373 and Sad23L-nCoV-S/Ad49L-nCoV-S produced Th1-biased responses, and better lymphocyte response was seen with the CpG-1018+alum–vaccinated macaques in S-Trimer study[33,35,37], while no data were available for RBD and S1-Fc[36,38]. The cellular immune responses produced by these candidate vaccines are described in Table II.

Progress to clinical trial: NVX-CoV2373 is being evaluated in Phase III clinical trials, with results of Phase I/II trial already published[39]. Others are in Phase I or pre-clinical stage, with Sad23L-nCoV-S/Ad49L-nCoV-S not picked up for further trials.

NVX-CoV2373 Phase I trial: A total of 131 participants were injected with placebo or 5 or 25 µg vaccine (renamed rSARS-CoV-2) with Matrix-M1 adjuvant three weeks apart[39].

(*i*) NAb response: Detected at week three and peaked at week five, with higher response in 5 µg group.

(*ii*) T-cell response: Predominantly Th1-biased response.

The vaccines are ranked according to their efficacy in NHP models in Table III.

Discussion

Pre-clinical trials of vaccines and other biologicals are important before embarking on clinical trials, as these may allow correct prediction of outcome in clinical trials**.** Rhesus macaques and Cynomolgus macaques are the two monkey species most frequently used for pre-clinical studies. There is genetic similarity between humans and Rhesus macaques, including sharing of pathogen genomes. It is also easy to handle rhesus monkeys, and therefore, they have been widely used in biomedical

Table III. Ranking of COVID-19 vaccine candidates according to their efficacy in non-human primates

Rank	Vaccine candidate	Number of doses needed (should not be >2, as per the WHO)	Viral clearance	Lung histopathology	T cell response	NAb response	
						First detected at (should be detected within two weeks, as per the WHO)	Detected till
1	mRNA-1273 (100 µg)	2	Complete clearance from pulmonary and extra-pulmonary organs by 7 DPI	Mild inflammation compared to placebo group	Th1-biased response. Additional T-helper cell response	Week 2	Week 8
2	Ad26.COV2.S	1	Complete clearance from pulmonary organs, 2 DPI onwards	Not studied	Th1-biased response	Week 2	Week 4
3	NVX-CoV2373 (25 µg + 50 µg M-1) and (5 µg + 50 µg M-1)	2	Complete clearance from pulmonary organs, not detectable 2 DPI onwards	Nil-to-mild changes in vaccinated animals compared to placebo group	Th1-biased response	Week 3	Week 5
4	BNT162b2	2	Complete viral clearance starting from 3 DPI and lasting upto 10 DPI in pulmonary organs, with 1 vaccinated animal showing presence of virus in throat swab on 10 DPI	Data not available	Th1-biased response	Week 3	Week 8
5	RBD	2	Undetectable sgRNA levels in pulmonary organs and GIT starting from 2 DPI and lasting up to 6 DPI, thus depicting viral clearance. gRNA was, however, detectable in vaccinated animals	Nil-to-mild changes in vaccinated animals compared to placebo group	Data not available	Week 1	Peak at week 5
6	BBV152 (3µg + adjuvant B and 6 µg+adjuvant B)	2	Complete viral clearance from pulmonary and extra-pulmonary organs by 7 DPI	Nil-to-mild histopathological changes in vaccinated group compared to placebo group	T-helper cell response	Week 3	7 DPI (week 5)
7	BBIBP-CorV (8 µg)	2 or 4	Complete clearance from throat and lungs and incomplete clearance from GIT in *R. macaques* by 7 DPI	Nil-to-mild histopathological changes in vaccinated group compared to placebo group	Not significant in *C. macaques*	Week 1	Week 5

Contd...

Rank	Vaccine candidate	Number of doses needed (should not be >2, as per the WHO)	Viral clearance	Lung histopathology	T cell response	NAb response	
						First detected at (should be detected within two weeks, as per the WHO)	Detected till
8	ChAdOx-1nCoV-19	1 or 2	Incomplete clearance from pulmonary and extra-pulmonary organs by 7 DPI	No pneumonia in vaccinated animals. Evidence of viral interstitial pneumonia in placebo group	Low Th1/Th2 response	Week 2	Week 8
9	PiCoVacc (6 µg)	3	Complete viral clearance from pulmonary and extra-pulmonary organs by 7 DPI	Nil-to-mild histopathological changes in vaccinated group compared to placebo group	Not significant	Week 2	7 DPI (week 5)
10	MRT 5500	2	Not done	Not done	Th1-biased response	Week 1	Week 5
11	Sad23L-nCoV-S/ Ad49L-nCoV-S	2	Not done	Not done	Th1-biased response	Week 2	Week 10
12	ARCoV	2	Not done	Not done	Th1-biased response	Week 2	Peak at Week 4
13	S-Trimer	2	Ineffective viral clearance, with 1 or more vaccinated animal showing presence of virus in pulmonary organs by 7 DPI in both vaccinated groups. However, no vaccinated animal had detectable virus in lung tissue on 7 DPI	Nil-to-mild changes in vaccinated animals compared to placebo group	Better lymphocyte response in CpG-1018 + alum-vaccinated group	Week 2	Week 6
14	INO-4800	2	Ineffective viral clearance from pulmonary organs by 7 DPI	Not done	IFN-γ raised in vaccinated animals	Week 4	Week 12
15	GX-19	3	Ineffective viral clearance from pulmonary organs by 4 DPI (necropsy)	Mild changes as compared to placebo group	Th1-biased response	5.5 wk	Rising at week 8
16	LION/ repRNA-CoV2S	1 or 2	Not done	Not done	Not significant	Week 2	Week 10
17	S1-Fc	3	Not done	Not done	Data not available	2-3 wk	Peak at week 5
18	Inactivated vaccine	2	Not possible to comment	Nil-to-mild histopathological changes in vaccinated group compared to placebo group	Not significant	Not possible to comment	Not possible to comment

DPI: days post-infection; R. macaques, Rhesus macaques; C. macaques, Cynomolgus macaques; IFN, interferon; LION, lipid inorganic nanoparticles; GIT, gastrointestinal tract; sgRNA, sub-genomic RNA; Nab, neutralizing antibody

Source: Refs 11-14, 17-21, 24, 25, 29, 30, 32-37

research. Cynomolgus monkeys bred exclusively for laboratory use are the most commonly used NHP models in pre-clinical trials these days, despite the unavailability of extensive genomic data for this species, unlike Rhesus monkeys[40].

While Muñoz-Fontela *et al*[41] have commented on the lack of available data for choosing the best NHP model to study SARS-CoV-2, a recent pre-print study has shown that Rhesus and Cynomolgus monkeys are equally effective models for studying COVID-19[42]. The third primate species discussed here is the Baboon. They are excellent models for vaccine trials because of their phylogenetic similarity to humans[43]. Most of the vaccine candidates considered here were evaluated in Rhesus macaques (*Macaca mulatta*) and Cynomolgus macaques (*Macaca fascicularis*), with part of the NVX-CoV2373 trial conducted in olive baboons and LION/repRNA-CoV2S tested in pigtail macaques.

Target product profile (TPP) of the WHO for COVID-19 vaccines advocates the administration of vaccine by any route as long as it is safe. The maximum parenteral dose should not exceed 1 ml, and the regimen should consist of no more than two doses, with booster doses permitted for long-term effect[44]. Schedule of administration of various vaccines along with the doses are provided in Table I. According to the WHO TPP, vaccine-induced protection should last for a minimum of six months and the vaccine should clearly demonstrate its efficacy, ideally with about 50 per cent point estimate, with 70 per cent efficacy considered preferable. Endpoint may be assessed by presence of severe disease and/or virus shedding/transmission[44]. Based on the review of available data of NHP studies, the performance of different vaccine candidates has been compared based on their compliance with the WHO TPP.

Viral clearance from various organs and tissues of NHPs following challenge with live SARS-CoV-2

Virus challenge study was conducted initally with Inactivated Vaccine at two weeks after the first vaccination and last with GX-19 at 15.5 wk post-vaccination[15,31]. Challenge studies were not carried out for ARCoV, MRT 5500, LION/repRNA-CoV2S, Sad23L-nCoV-S/Ad49L-nCoV-S and S1-Fc vaccine candidates[20-22,37,38]. Route and dose of virus challenge are provided in Table I. The challenge doses used in different studies may be much higher than natural infective doses. Not only can this approach lead to erroneous estimation of NAb needed to stifle natural infection, but it may also necessitate the detection of sgRNA to differentiate actively replicating virus from leftover inoculum[45].

Thirteen vaccine candidates were tested for their efficacy in viral clearance from various tissues. To demonstrate this, the studies relied on detection of gRNA and/or sgRNA from pulmonary and extrapulmonary tissues of vaccinated and placebo animals. As mentioned earlier, sgRNA detection is considered as evidence of active viral replication[45,46]. The vaccinated animals fared better than the placebo groups, with 50-100 per cent animals showing complete viral clearance within seven DPI in all studies. Inactivated Vaccine was excluded from this analysis due to unavailability of data[15].

Of the inactivated vaccines, 3 µg+adjuvant B and 6 µg+adjuvant B regimens of BBV152 and 6 µg dose of PiCoVacc showed the best clearance of gRNA and sgRNA, and gRNA clearance, respectively[12,13]. Both these vaccines showed effective viral clearance from the upper respiratory tract (nasopharynx and oropharynx) and lungs (BAL fluid). Complete clearance of the virus from extrapulmonary tissues of vaccinated groups was seen with 3 µg+adjuvant B formulation of BBV152 whereas the 6 µg+adjuvant B formulation showed impaired clearance. gRNA clearance from throat of NHP with 8 µg dose of BBIBP-CorV was seen within seven DPI, with impaired rectal clearance[12-14]. mRNA-1273 and BNT162b2 were the only RNA vaccine candidates to evaluate viral clearance (using sgRNA) and both showed comparable efficacy within seven DPI[18,19]. In the viral vector vaccine group, Ad26.COV2.S was more efficacious in clearing viral sgRNA from tissues within seven DPI than sgRNA and gRNA clearance by ChAdOx1 nCoV-19[25,26], which also showed impaired clearance of the virus from extrapulmonary organs. None of the DNA vaccines (INO-4800 and GX-19) could successfully eliminate sgRNA or gRNA from tissues of vaccinated macaques within seven DPI[30,31]. Among the protein subunit vaccines, RBD and NVX-CoV2373 both demonstrated better results in terms of clearing sgRNA and gRNA at six DPI and sgRNA at seven DPI, respectively, than S-Trimer[34-36]. However, viral gRNA was detectable in the throat and anal swabs and lung tissue of RBD vaccinated macaques till six DPI. Further, sgRNA was not detected from these samples, suggesting an absence of viral replication. The viral load in vaccinated animals was lower than the placebo groups in all the studies.

From the available data, it was inferred that BBV152, PiCoVacc, mRNA-1273, BNT162b2, Ad26.COV2.S, NVX-CoV2373 and RBD showed the most promising results in viral clearance from tissues and organs of vaccinated macaques.

Histopathology and immunohistochemistry of lung tissues of NHPs

Ten vaccine candidates - BBV152, PiCoVacc, BBIBP-CorV, Inactivated Vaccine, mRNA-1273, ChAdOx1 nCoV-19, GX-19, NVX-CoV2373, S-Trimer and RBD - were compared for lung pathology in vaccinated and placebo groups of animals. The vaccinated animals showed nil to mild histopathological changes compared to the placebo groups in all the studies[12-15,18,26,31,34-36]. IHC performed on lung tissues demonstrated no SARS-CoV-2 antigen detection in the animals vaccinated with BBV152[12].

Neutralizing antibody response

All the vaccine candidates elicited NAb response after the administration of first dose in NHP, which was detected between 1 and 4 wk of vaccination and lasted up to 5-12 wk[12-15,18-22,25,26,30,31,33,35-38]. All the studies reported significantly higher NAb titres in vaccinated groups compared to panels of human convalescent serum used in the respective trials, except LION/repRNA-CoV2S, which showed no significant difference in titres with convalescent human serum. WHO TPP[44] suggests that the vaccine candidate should induce immune response, preferably within two weeks of administration, and should provide protection for a minimum of six months.

BBIBP-CorV, RBD and MRT 5500 induced NAb response at week one post-vaccination[14,21,36], followed by PiCoVacc, mRNA-1273, Ad26.COV2.S, ChAdOx1 nCoV-19, S-Trimer, ARCoV, S1-Fc, LION/repRNA-CoV2S and Sad23L-nCoV-S/Ad49L-nCoV-S at two weeks[13,18,20,22,25,26,35,37,38] and BBV152, BNT162b2, Inactivated Vaccine and NVX-CoV2373 at three weeks[12,15,19,33]. NHPs injected with INO-4800 elicited NAb response at four weeks of administration of the first dose, while with GX-19, it was first detected at 5.5 wk after the first vaccination. All vaccine candidates elicited persistent NAb response in vaccinated animals until they were euthanized. In the trials where live virus challenge was not performed, NAbs mostly reached peak values at 5-7 wk and were detectable up to wk 6-10. INO-4800 induced NAb that were detected up to 12 wk post-vaccination. Antibody response was higher with AS03 adjuvant containing S-Trimer–vaccinated group as compared to CpG-1018/alum adjuvant.

T-cell response

The T-cell responses induced by the vaccine candidates were measured using an array of Th1 and Th2 cytokines. These included Th1 cytokines such as TNF-α, IFN-γ and IL-2 and Th2 cytokines IL-4, IL-5, IL-6 and IL-13, which were measured in varying degrees in different trials.

BBV152 was the only inactivated vaccine candidate to produce significant T-cell response in vaccinated non-human primates[12]. It induced T helper cell response. Except LION/repRNA-CoV2S, the remaining RNA vaccines (mRNA-1273, BNT162b2, ARCoV and MRT5500) produced a desirable Th1 skewed response in vaccinated animals[18-21]. Unlike ChAdOx1 nCoV-19, Ad26.COV2.S showed Th1-biased response in the viral vector vaccine category[25,26]. GX-19 also demonstrated a predominant Th1 response among the two DNA vaccines[31]. Of the protein subunit vaccines, NVX-CoV2373 and Sad23L-nCoV-S/Ad49L-nCoV-S both induced Th1-dominant response[33,37].

Eight candidates - mRNA-1273, Ad26.COV2.S, BNT162b2, NVX-CoV2373, GX-19, ARCoV, MRT 5500 and Sad23L-nCoV-S/Ad49L-nCoV-S - induced a predominantly Th1-biased cellular immune response in vaccinated animals; BBV152 and mRNA-1273 produced additional T helper cell response. INO-4800 induced detectable levels of IFN-γ (a Th1 cytokine) in vaccinated animals, but the authors did not specify if the cellular immune response was predominantly of the Th1 type[30]. Cytokine profile of NHPs was not available for S-Trimer, though vaccinated animals showed increased lymphocyte frequency, which was higher with the CpG-alum–adjuvant group. T-cell response induced by RBD and S1-Fc was not available.

A good COVID-19 vaccine candidate should result in effective clearance of the virus from various tissues and organs, induce potent NAb response, and should not elicit a Th2 skewed cellular immune response. The last condition is of some urgency as vaccine-associated enhanced respiratory disease has been observed with some respiratory virus vaccines, which predominantly stimulate a Th2 response path[47].

Ranking of the vaccines according to their performance in NHP models: It was observed that RNA vaccine, mRNA-1273, followed by viral vector

vaccine, Ad26.COV2.S, performed well, keeping in line with the TPP of WHO[44]. Both demonstrated robust viral clearance from various tissues and induced effective NAb response, which was detectable within two weeks of administration of the first dose of the vaccine[18,25]. These two vaccine candidates also induced Th1 polarized cellular immune response in vaccinated macaques. mRNA-1273–vaccinated macaques also showed mild inflammation on lung histopathology compared to the placebo group, while no such information was available for Ad26.COV2. S–immunized animals.

Protein subunit vaccine, NVX-CoV2373 and RNA vaccine, BNT162b2 both induced Th1 immune response in vaccinated NHPs, but both elicited NAb response in vaccinated animals after three weeks of administration of the first dose[19,33] which does not comply with the TPP of WHO. However, these showed effective viral clearance from the respiratory tract, thereby making them valuable vaccine candidates. NVX-CoV2373 vaccination led to mild histopathological changes in the lungs following viral challenge[34], while histopathological data were not available for the BNT162b2 NHP trial.

Protein subunit vaccine, RBD, led to NAb production within one week of the first dose, and the vaccinated animals also showed effective clearance of the virus from various organs following viral challenge, but there is no cellular immune response data available for this candidate, and it is important to know the T-cell response to avoid vaccine-associated adverse effects.

Among the inactivated vaccines, BBV152 performed better than the other candidates. BBV152 produced NAbs in vaccinated animals, which were first detectable three weeks after administration of the first dose of the vaccine (not in line with the WHO guidelines)[44] but it induced significant T-helper cell response. There was robust clearance of virus in vaccinated groups five DPI onwards, demonstrating the strong protective efficacy of this vaccine candidate[12].

DNA vaccines did not perform as well as these candidates; however, response was better with INO-4800 than GX-19[30,31].

After taking into account the immune responses generated by these candidate vaccines in NHPs, namely, NAb response, T-cell response, and viral clearance, as well as the number of doses required to achieve the said response, it may be concluded that mRNA-1273 and Ad26.COV2.S are the most promising candidates. NVX-CoV2373 and BNT162b2 are close behind them. RBD and BBV152 are two other candidates to look out for. The data demonstrate that these six vaccines are the most promising among the candidate vaccines analyzed.

Human trial data available for some of these candidates also showed results that corroborated NHP studies, with mRNA-1273, Ad26.COV2.S and NVX-CoV2373 showing early NAb response (measured first at three weeks) and Th1 immune response[23,27,39]. BNT162b1 induced NAb response first detected at three weeks, with unavailability of cellular immunity data[24]. Besides these six vaccine candidates, clinical trial data are also available for other upcoming vaccines. ChAdOx1 nCoV-19 showed better T-cell response in human trials compared to NHP studies[28,29], and PiCoVacc (CoronaVac) and BBIBP-CorV, which required three doses in NHP, generated NAb with two doses in human trials[16,17], thus complying with WHO guidelines. However, their T-cell response data were not available for comparison.

Apart from the efficacy demonstrated by these vaccines, a major factor that needs to be considered before large-scale rollout of these candidates for mass vaccination is storage temperature. While some candidates such as ARCoV and LION/repRNA-CoV2S are stable at room temperature for a week and INO-4800 at room temperature for a year, other candidates such as BBV152, PiCoVacc, Ad26. COV2.S and ChAdOx1 nCoV-19 need refrigeration at 2-8°C[20,48-52]. MRT5500 and BNT162b1 require storage at −20°C and −70°C, respectively. Storage temperature of mRNA-1273 has recently been updated by the manufacturers to 2-8°C[52]. Vaccine manufacturers are still upgrading their formulations to enable storage at higher temperatures, which has to be given special consideration in African and Asian countries.

Recently, Krammer[53] published an article comparing the immune response and protective efficacy of six COVID-19 vaccine candidates (PiCoVacc, mRNA-1273, Ad26.COV2.S, ChAdOx1n CoV-19, BBIBP-CorV and NVX-CoV2373) in NHPs, which provided an insight into the effectiveness of various vaccine candidates. A systematic review of candidate vaccines for SARS-CoV-2 by Dong *et al*[54] discussed the results of clinical trials, with a brief mention of results obtained in NHP models. Our

review compares and analyzes the immunogenicity and protective efficacy of 18 COVID-19 vaccine candidates in NHPs and provides an elaborate comparison of pre-clinical data obtained from the individual studies.

To conclude, COVID-19 vaccine studies in NHPs yielded promising results, with some candidates faring better than the others. This review highlights the immunogenicity and protective efficacy of various candidates who are frontrunners in the vaccine race. Successful preclinical NHP studies with these vaccine candidates demonstrating their immunogenicity and efficacy have boosted the confidence of researchers across the world to fast track clinical trials and to produce a safe and effective vaccine for SARS-CoV-2. However, it should be remembered that there are logistical challenges that need to be addressed before commencing mass vaccination, and a suitable vaccine candidate has to be chosen as per the infrastructure available in the country.

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