REVIEW

Middle East Respiratory Syndrome coronavirus vaccine development: updating clinical studies using platform technologies

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Middle East Respiratory Syndrome coronavirus (MERS-CoV), a contagious zoonotic virus, causes severe respiratory infection with a case fatality rate of approximately 35% in humans. Intermittent sporadic cases in communities and healthcare facility outbreaks have continued to occur since its first identification in 2012. The World Health Organization has declared MERS-CoV a priority pathogen for worldwide research and vaccine development due to its epidemic potential and the insufficient countermeasures available. The Coalition for Epidemic Preparedness Innovations is supporting vaccine development against emerging diseases, including MERS-CoV, based on platform technologies using DNA, mRNA, viral vector, and protein subunit vaccines. In this paper, we review the usefulness and structure of a spike glycoprotein as a MERS-CoV vaccine candidate molecule, and provide an update on the status of MERS-CoV vaccine development. Vaccine candidates based on both DNA and viral vectors coding MERS-CoV spike gene have completed early phase clinical trials. A harmonized approach is required to assess the immunogenicity of various candidate vaccine platforms. Platform technologies accelerated COVID-19 vaccine development and can also be applied to developing vaccines against other emerging viral diseases.

Keywords: Middle East Respiratory Syndrome coronavirus (MERS-CoV), vaccine, platform technologies

Introduction

Middle East Respiratory Syndrome coronavirus (MERS-CoV) causes infectious respiratory diseases in humans (van Boheemen *et al.*, 2012). The clinical features of MERS-CoV infection can vary from asymptomatic or mild illness to death due to pneumonia and multiorgan failure, especially in people with underlying comorbidities (Zumla *et al.*, 2015). At the end of October 2021, a total of 2,578 laboratory-confirmed cases of MERS, including 888 associated deaths, were reported globally since its first identification in the Kingdom of Saudi Arabia in 2012, with a case-fatality ratio (CFR) of 34.4%. Most of these cases were reported in Saudi Arabia: 2178 cases, including 810 related deaths (CFR 37.2%). The largest outbreaks occurred in Riyadh and Jeddah in Saudi Arabia in 2014 (Memish *et al.*, 2020). The MERS outbreak outside the Middle East was reported in the Republic of Korea in 2015, with 186 laboratory-confirmed cases (185 cases in the Republic of Korea and 1 case in China) and 38 deaths (CFR 20.4%) that were spread from only one male returning from the Arabian Peninsula (Cho *et al.*, 2016). To date, intermittent sporadic cases among community clusters and healthcare facility outbreaks of MERS-CoV continue to occur (Memish *et al.*, 2020). Since 2015, MERS-CoV has been indicated as an infectious pathogen prioritized by the WHO R&D Blueprint for research and development in public health due to its epidemic potential and insufficient countermeasures (Mehand *et al.*, 2018). The Coalition for Epidemic Preparedness Innovations (CEPI) (Røttingen *et al.*, 2017), a global partnership formally founded in 2017 to accelerate the development of vaccines against emerging infectious disease and, therefore, establish investigational vaccine stockpiles before epidemics emerge, decided to fund the platform technology research for vaccine development focusing on MERS-CoV, Lassa fever, and Nipah virus in 2018 among diseases in the WHO R&D Blueprint list (Plotkin, 2017; Maslow, 2018; Wong and Qiu, 2018; Gouglas *et al.*, 2019) (Table 1). Later, the Chikungunya virus, Rift Valley Fever, and SARS-CoV-2 were also included for the CEPI investment (Bernasconi *et al.*, 2020).

Platform technology refers to a basic manufacturing system, such as DNA, mRNA, viral vectors, and proteins, that can be applied to various vaccine antigens. Once platform techniques are established, the same basic components can be rapidly adopted for various vaccines by inserting new genetic codes or proteins from other pathogens. As data on a new platform accumulate over time, regulatory authorities will likely become familiar with moving new vaccines to the same platform in clinical trials and can expedite the approval of novel vaccines against emerging epidemics or pandemics. In addition, platform technologies will facilitate quicker reactions to emerging variants of the same pathogen. CEPI has accelerated the development and manufacture of vaccines against unknown pathogens within 16 weeks of antigen identification (Vandeputte *et al.*, 2021). These platform technologies used in the development of MERS-CoV vaccines have led to a

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Table 1. Platform technologies for vaccines against emerging viral diseases funded by the Coalition for Epidemic Preparedness Innovations in 2018

Disease	Technology "Platform"	Partners
MERS-CoV	Adenovirus vector	Janssen & University of Oxford
	MVA	IDT Biologika
	Measles	Themis Bioscience
	DNA	Inovio Pharmaceuticals
	Protein Subunit	The University of Queensland
Lassa	Adenovirus vector	Janssen & University of Oxford
	VSV	Profectus Biosciences, Emergent Biosolutions and PATH
	VSV	International AIDS Vaccine Initiative (IAVI)
	Measles	Themis Bioscience
	DNA	Inovio Pharmaceuticals
	RNA	CureVac
Nipah	Adenovirus vector	Janssen & University of Oxford
	Measles	The University of Tokyo
	Protein Subunit	Profectus Biosciences, Emergent Biosolutions and PATH

This Table was presented at a Coalition for Epidemic Preparedness Innovations workshop in Oslo, Norway in 2019.

COVID-19 vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Kashte *et al.*, 2021). In this paper, a spike that is a MERS-CoV vaccine candidate molecule for neutralizing targets and the status of MERS-CoV vaccine development using several platforms will be reviewed.

MERS-CoV Spike as a Vaccine Target

Genome sequence analysis has shown that MERS-CoV belongs to the Betacoronavirus genus of the *Coronaviridae* family, which includes bat SARS-like coronavirus, human coronavirus (HCoV-OC43, HCoV-HKU1, SARS-CoV, and
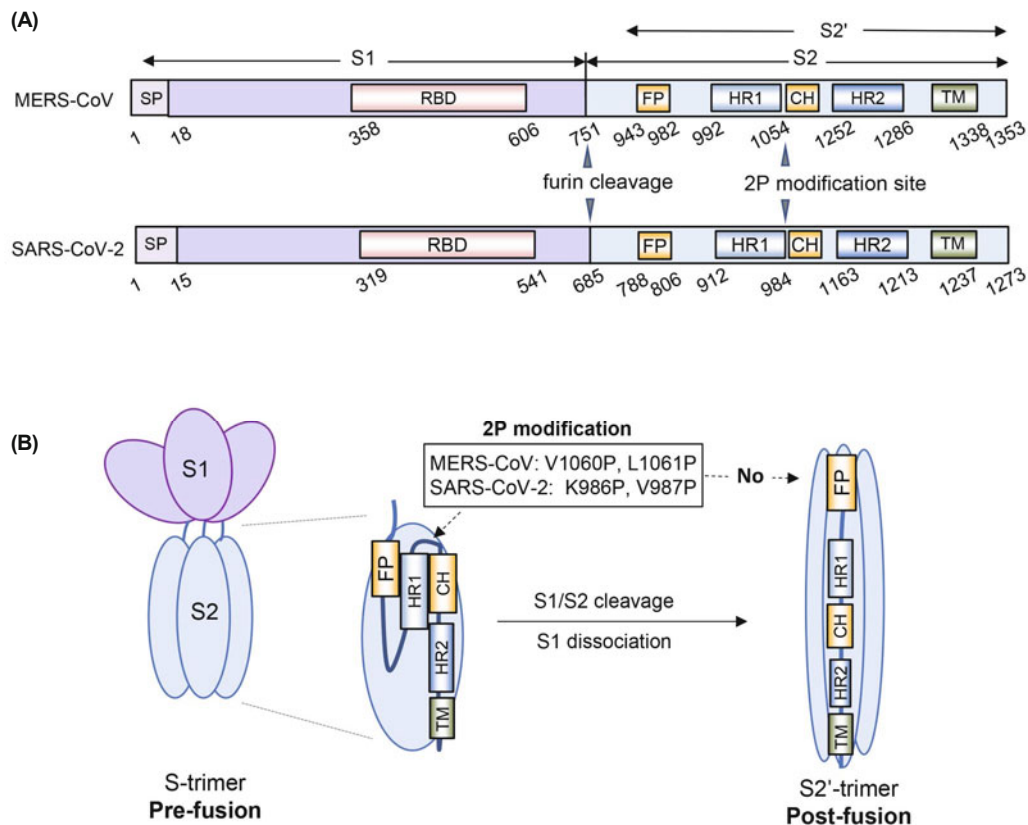

Fig. 1. MERS-CoV Spike as a vaccine target. (A) Region between HR1 and CH unfolds after S binds to human dipeptidyl peptidase 4, the host cellular receptor, and human angiotensin-converting enzyme 2 (hACE2) via the RBD of MERS-CoV and SARS-CoV-2, respectively. (B) Cleavage at S1/S2 and S2′ sites causes irreversible structural changes from the pre-fusion to the post-fusion form. Replacement of two residues between HR1 and CH (V1060 and L1061 for MERS-CoV S; K986 and V987 for SARS-CoV-2) with proline stabilized S protein in the pre-fusion form. Prefusion S is beneficial for inducing neutralizing antibodies. RBD, receptor-binding domain; FP, fusion peptide; CH, central helix; HR, heptad repeat; TM, transmembrane domain.

SARS-CoV-2) (Hulswit *et al.*, 2019; Chen *et al.*, 2020; Bhat *et al.*, 2021). MERS-CoV is a zoonotic virus, and it is believed to have originated from bats and transmitted to camels (Hemida *et al.*, 2017). MERS-CoV has been identified in dromedaries in the Middle East, Africa, and South Asia (Mohd *et al.*, 2016). MERS-CoV can be transferred to humans from infected dromedary camels, and human-to-human transmission has been reported more frequently in healthcare facilities than among families living in the same household (Drosten *et al.*, 2014).

Like other viruses belonging to *Coronaviridae*, MERS-CoV is an enveloped virus with a positive-sense single-stranded RNA genome (de Groot *et al.*, 2013). It has four structural proteins: spike (S), envelope, membrane, and nucleocapsid, of which S glycoprotein is mainly used as a vaccine candidate for inducing neutralizing antibodies (Al-Amri *et al.*, 2017). The MERS-CoV S glycoprotein acts as a viral fusion protein that mediates attachment to the host receptor, which is human dipeptidyl peptidase 4 (hDPP4), and fusion of the viral and cellular membranes (Du *et al.*, 2013). S is synthesized as a single polypeptide chain of 1395 amino acids and is cleaved into the receptor-binding subunit S1 and the membrane-fusion subunit S2 by host furin protease during the infection process (Wang *et al.*, 2013, 2014; Yu *et al.*, 2015). The S1 and S2 subunits trimerize to form a prefusion spike (~600 kDa) with ~25 N-linked glycans per monomer (Pallesen *et al.*, 2017). S1 and S2 remain noncovalently bound in the prefusion conformation (Bosch *et al.*, 2003).

The S1 subunit comprises the apex of the S trimer, including the receptor-binding domains (RBDs), and stabilizes the prefusion state of the S2 fusion machinery, which is anchored in the viral membrane. The S2 subunit contains the fusion peptide, two heptad repeats, and a transmembrane domain, all of which are required to mediate fusion of the viral and host cell membranes. S is further cleaved by host proteases at the so-called "S2′" site located immediately upstream of the fusion peptide. This cleavage has been proposed to activate proteins for membrane fusion via large-scale, irreversible conformational rearrangements (Fig. 1) (Walls *et al.*, 2017).

The prefusion-stabilized MERS-CoV S protein (MERS S-2P) with V1060P and L1061P mutations still demonstrated high-affinity binding to hDPP4 and a panel of MERS-CoV neutralizing antibodies. Moreover, MERS S-2P induced high titers of neutralizing antibodies in mice (Pallesen *et al.*, 2017). Therefore, the successful production of pre-fusion-stabilized MERS-CoV S proteins will contribute to the development of efficient protective interventions against current and emerging variants (Fig. 1B).

A technique involving the use of a molecular clamp consisting of a stable trimerization motif of 80 aa in length derived from HIV-1 gp41 was applied to viral fusion proteins such as respiratory syncytial virus fusion protein (McLellan *et al.*, 2013), influenza hemagglutinin (Isaacs *et al.*, 2021), and human immunodeficiency virus glycoprotein 140 (Pancera *et al.*, 2014) because the gp41 motif can be self-assembled into a stable six-helical bundle structure that is required to drive membrane fusion and cell entry of HIV-1 (Chan *et al.*, 1997). This technique was also planned for use in MERS-CoV vaccine development funded by the CEPI. With the urgent

Table 2. List of clinical trials of MERS-CoV vaccines

| Platform	Developer	Study Group	Vaccine description	Current stage of clinical evaluation/regulatory status: MERS-CoV	References	Current stage of clinical evaluation/regulatory status: COVID-19
DNA vaccine	Inovio Pharmaceuticals	GeneOne Life Science/ Inovio Pharmaceuticals/ International Vaccine Institute	GLS-5300 (INO-4700) with Electroporation device	Phase I, completed	Modjarrad *et al.* (2019) NCT02670187	
			GLS-5300 (INO-4700) with ID Electroporation device	Phase I/IIa, completed	NCT03721718	Phase I/II
Non-replicating viral vector	IDT Biologika GmbH	CTC North GmbH & Co. KG	MVA-MERS-S	Phase I, completed	Koch *et al.* (2020) NCT03615911	
			MVA-MERS-S_DF1	Phase Ib, recruiting	NCT04119440	Phase I
Non-replicating viral vector	University of Oxford	King Abdullah International Medical Research Center/ University of Oxford	ChAdOx1-MERS	Phase I, completed	Folegatti *et al.* (2020) NCT03399578 NCT04170829	Approved in Brazil. Emergency use in over 90 countries.
Non-replicating viral vector	Gamaleya Research Institute	Gamaleya Research Institute of Epidemiology and Microbiology/Acellena Contract Drug Research and Development	BVRS-GamVac-Combi	Phase I/II, recruiting	NCT04128059	Emergency use in over 70 countries.
			BVRS-GamVac	Phase I/II, recruiting	NCT04130594	
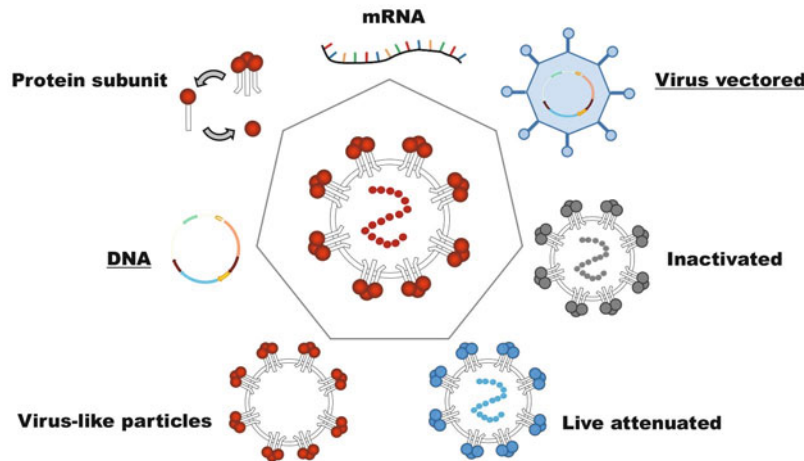
Fig. 2. Technologies used in vaccine development. DNA, mRNA, and virus-vectored vaccine coding antigen genes, protein subunit, virus-like particles, and inactivated and live attenuated viruses can be explored for novel vaccine development against emerging viral pathogens. Among these technologies, DNA and virus-vectored vaccines (with underline) with the full S gene are currently under clinical trials for MERS-CoV vaccine development.

need for the COVID-19 vaccine, however, this technique was quickly applied to the SARS-CoV-2 S to stabilize the authentic pre-fusion conformation that preserves neutralizing epitopes present on the virion surface. A clinical phase 1 study using this construct showed the limitation of the molecular clamp causing false positivity for HIV-1 in normal individuals. Gp41 motif-induced antibodies cross-reacted with some HIV diagnostics, although there were robust and highly correlated humoral and cellular immune responses (Chappell *et al.*, 2021). This false positivity led to abandon further clinical trials using gp41 as a trimerization motif. New clamp molecules need to be identified from other viruses or systems with similar levels of gp41 stability.

Clinical Trial Status of MERS-CoV Vaccines

All vaccine candidates described in the following clinical trial section used unmodified full-length S sequences (Table 2). In the absence of specific therapeutic agents, vaccination is expected to be an effective strategy for preventing MERS-CoV infection in both humans and animals. To date, several MERS-CoV vaccine candidates have been developed using various platform technologies and are undergoing clinical trials, including DNA and recombinant viral vector-based vaccines such as adenoviral vectors, modified vaccinia virus Ankara, and recombinant measles virus (Graham *et al.*, 2013; Modjarrad, 2016; Yong *et al.*, 2019) (Fig. 2).

MERS-CoV DNA vaccine

DNA vaccines generally refer to vaccines that have DNA expressing an immunogenic protein placed in a plasmid vector and delivered into host cells. Usually, DNA vaccines are delivered to host cells by electroporation (Aihara and Miyazaki, 1998). Compared with other vaccine technologies, DNA vaccines have the advantage of rapid development, and they may be useful against new emerging infectious diseases (Sardesai and Weiner, 2011; Rauch *et al.*, 2018). DNA vaccines also have the advantage of inducing both humoral and cell-mediated immune responses, but when used alone, immunogenicity is not strongly induced, and using an appropriate adjuvant is recommended (Liu, 2011). During the early stages of DNA

vaccine development, there are concerns that DNA vaccines are incorporated into the host chromosome and they cause mutations in host cells, leading to tumor generation (Nichols *et al.*, 1995; Rauch *et al.*, 2018). However, after repeated clinical trials using DNA vaccines, it has been demonstrated that the probability of inducing host cell mutations is lower than that of spontaneous gene mutations (Sheets *et al.*, 2006).

Vaccine development using DNA vaccine technology is also underway for MERS-CoV infection. In particular, the GLS-5300 (INO-4700) MERS-CoV DNA vaccine, which contains the full-length S gene, induces antigen-specific neutralizing antibodies in mice, monkeys, and camels, as well as strong cell-mediated immunity (Muthumani *et al.*, 2015). In addition, it was confirmed that macaque monkeys vaccinated with GLS-5300 (INO-4700) MERS-CoV DNA vaccine showed strong protective immunity against wild-type MERS-CoV challenge, thereby improving lung pathology and reducing viral load in the lungs (Muthumani *et al.*, 2015). Based on these pre-clinical experimental results, Phase 1 clinical trials of the GLS-5300 (INO-4700) MERS-CoV DNA vaccine have been completed (NCT02670187, NCT03721718). As a result, the GLS-5300 (INO-4700) DNA vaccine developed by Inovio Pharmaceuticals was confirmed to induce immunogenicity without severe side effects, and it was confirmed that serconversion by S1-ELISA was observed in 86% of clinical participants after the second immunization (Modjarrad *et al.*, 2019). Inovio Pharmaceuticals developed INO-4800 (Smith *et al.*, 2020), a candidate for a SARS-CoV-2 DNA vaccine with the same design as the MERS-CoV vaccine containing a full-length S gene, and it is currently in phase 1/2 clinical trials (NCT04447781, NCT04336410).

MERS–CoV viral vector vaccine

Viral vector-based vaccines involve the use of a recombinant unrelated virus that contains the targeted antigen gene or protein as a vaccine. Viral vector-based vaccines are generally known to induce strong antigen-specific antibody responses and cell-mediated immune responses, which have the advantage of inducing sufficient immune responses without adjuvants (Rollier *et al.*, 2011). In addition, viral vectors can easily insert large foreign genes, allowing flexibility in designing vaccine targets. However, the process of manufacturing the viral

vector vaccine is more complicated than the process of manufacturing other vaccines, and it requires considerable technical skills to select and optimize cells that are efficient for virus production, so it can act as a disadvantage in terms of productivity (Rauch *et al.*, 2018). In addition, most people already have antibodies against adenovirus or the measles virus; therefore, adenovirus or the measles virus can be incapacitated by the pre-existing antibodies when used as a viral vector. Therefore, the effectiveness of the vaccine may be reduced due to poor vaccine delivery efficiency. Therefore, it is necessary to choose a viral vector to avoid the interference by pre-existing antibodies for development of an effective vaccine (Knuchel *et al.*, 2013; Wong *et al.*, 2014).

Viral vector-based vaccines are also being studied for targeting S proteins, similar to DNA vaccines. Various viral vector vaccines are being used to develop MERS-CoV vaccines (Enjuanes *et al.*, 2008; Schindewolf and Menachery, 2019), and among them, we take a closer look at the viral vectors currently undergoing clinical trials as MERS-CoV vaccines.

The first vaccine uses the modified vaccinia virus Ankara (MVA) as a viral vector. The MVA-based MERS-CoV vaccine (MVA-MERS-S) contains a full-length S protein as an antigen, similar to the DNA vaccine (Sutter and Moss, 1992; Stittelaar *et al.*, 2001). The MVA-MERS-S vaccine has been found to induce antigen-specific neutralizing antibodies, as well as CD8$^+$ T cell responses, in mice (Song *et al.*, 2013). Furthermore, its protective immunity effect was confirmed using the wild-type MERS-CoV challenge test in hDPP4 transgenic mice (Volz *et al.*, 2015); it was confirmed that inflammation and lymph node hyperplasia caused by viral infection were reduced (Langenmayer *et al.*, 2018). Similarly, in dromedary camels, it was confirmed that antigen-specific neutralizing antibodies were induced after the MVA-MERS-S vaccination. In addition, it was confirmed that the release of infectious virions was effectively blocked when the nasal cavity was infected by wild-type MERS-CoV after the MVA-MERS-S vaccination. Therefore, the MVA-MERS-S vaccine is considered to effectively control the transmission of MERS-CoV from camels to humans (Haagmans *et al.*, 2016). Based on the pre-clinical experimental results, Phase I clinical trials were conducted (NCT03615911), and the safety and immunogenicity of the MVA-MERS-S vaccine were confirmed in a phase 1 clinical trial (Koch *et al.*, 2020). A phase 1b clinical trial is underway to confirm the immune response by homologous prime-boost immunization in more volunteers (NCT-04119440). Currently, Phase 1 clinical trials are in progress for the SARS-CoV-2 (MVA-SARS-2-ST) vaccine, which was developed using the same MVA as the vaccine vector used to develop the MERS-CoV vaccine (NCT04895449).

Adenovirus is a widely used platform for MERS-CoV vaccine studies. Adenovirus vectors have been used for a long time to develop vaccines against various diseases and have been used in HIV-1 vaccine clinical trials (Hammer *et al.*, 2013). The effectiveness of the immunogenicity of the antigen translated to a protein from the adenovirus vector itself is not bad, but it is known that the immune response of the adenovirus vector vaccine is reduced by the pre-existing antibodies against the adenovirus in most people (Chirmule *et al.*, 1999; Mercier *et al.*, 2004; Mast *et al.*, 2010). The adenovirus type 5-based vaccine, which is related to the development of the MERS-

CoV vaccine, induced antigen-specific neutralizing antibodies in mice, but protective immune responses were not confirmed in hDPP4 transgenic mice. In addition, humoral immune responses, as well as cell-mediated immune responses, have been reported in camels administered the same vaccine (Guo *et al.*, 2015; Jung *et al.*, 2018).

To avoid pre-existing antibodies, vaccine development using low prevalence vectors in humans, such as adenovirus type 26 or other animal origin adenoviruses such as chimpanzee adenovirus, and the heterologous prime-boost method using various types of adenoviruses is being explored. The BVRS-GamVac-Combi vaccine, a heterologous prime-boost immune vaccine using recombinant adenovirus type 26 and recombinant adenovirus type 5, induced high titers of antigen-specific neutralizing antibodies in mice (Dolzhikova *et al.*, 2020). It was subsequently confirmed that the antibody titer was maintained for up to 18 months. In an experiment to confirm the protective efficacy of hDPP4 transgenic mice, the protective immune response against the lethal MERS-CoV infection was confirmed, and the protective immunity was maintained even 7 months after the last immunization (Dolzhikova *et al.*, 2020). Currently, phase 1/2 clinical trials using the BVRS-GamVac-Combi vaccine are in progress, and the results of clinical trials are expected to be announced in December 2021 (NCT04128059, NCT04130594). The vaccine against SARS-CoV-2, which uses the same platform as the BVRS-GamVac-Combi vaccine, has also been developed and is being used as an emergency vaccine under the name of Sputnik V (Gam-Covid-Vac) in more than 70 countries, including Russia.

Chimpanzee adenoviruses are being explored to overcome pre-existing antibodies against adenovirus in humans. In the development of the MERS-CoV vaccine, the ChAdOx1-MERS vaccine based on the chimpanzee adenovirus vector (ChAdOx1) was found to induce high titers of antigen-specific neutralizing antibodies, as well as a high cell-mediated immune response, in mice. In a hDPP4 transgenic mouse study, the ChAdOx1-MERS vaccine showed high protective efficacy against wild-type MERS-CoV lethal infection (Alharbi *et al.*, 2017; Munster *et al.*, 2017). It was confirmed that the ChAdOx1-MERS vaccine significantly reduced the viral load caused by the wild-type MERS-CoV virus infection in dromedary camels and also reduced the viral load in monkeys and showed a protective immune response against wild-type MERS-CoV virus infections (Alharbi *et al.*, 2019; van Doremalen *et al.*, 2020). Based on pre-clinical experimental results, Phase 1 clinical trials were conducted using the ChAdOx1-MERS vaccine (NCT03399578, NCT04170829), and the results confirmed that ChAdOx1-MERS was safe and an immune response was well-induced for all test doses (Folegatti *et al.*, 2020). The ChAdOx1 virus vector-based SARS-CoV-2 vaccine has been used as an emergency vaccine in more than 90 countries (Folegatti *et al.*, 2020). It was developed by the University of Oxford research team, which is also the developer of ChAdOx1-MERS, and officially approved in Brazil under the name AZD1222 (or ChAdOx1-nCoV-19).

Several MERS-CoV vaccines based on viral vectors, such as adenovirus (Jung *et al.*, 2018), Venezuelan equine encephalitis virus (Walls *et al.*, 2017; Agnihothram *et al.*, 2018), vesicular stomatitis virus (VSV) (Liu *et al.*, 2018), measles

virus (Malczyk *et al.*, 2015), Newcastle disease virus (Liu *et al.*, 2017), and rabies virus (Wirblich *et al.*, 2017), have been developed. Additionally, various viral vectors, in addition to the viral vectors described above, are being explored in ongoing MERS-CoV vaccine development.

Four vaccines are currently undergoing clinical trials for MERS-CoV, which include a DNA vaccine (INO-4700) and the three virus vector-based vaccines as described above. The vaccine platforms used in clinical trials for the MERS-CoV vaccine have also been used to develop SARS-CoV-2 vaccines. Among them, two vaccines (a heterologous prime-boost immune vaccine using recombinant adenovirus type 26 and recombinant adenovirus type 5 and chimpanzee adenovirus vector [ChAdOx1] vaccine) have been developed and are being used as emergency vaccines in several countries (Li *et al.*, 2020).

MERS-CoV inactivated, live attenuated and protein-based vaccine development

Traditional inactivated vaccines (Agrawal *et al.*, 2016; Deng *et al.*, 2018), live attenuated vaccines (Almazán *et al.*, 2013), and recombinant protein-based vaccines (Coleman *et al.*, 2017; Jiaming *et al.*, 2017) have also been explored for MERS-CoV, but most are still in the pre-clinical experimental stage.

Inactivated vaccines are made from virions, which are usually killed by chemical or radiational inactivation (Li *et al.*, 2020). In general, inactivated vaccine is safer than the attenuated vaccine, but has the disadvantage that it may be accompanied by structural change during the inactivation process (Bolles *et al.*, 2011; Tseng *et al.*, 2012). Various studies have been conducted to develop an inactivated MERS-CoV vaccine (Agrawal *et al.*, 2016; Deng *et al.*, 2018). Among such previous studies, gamma-irradiated MERS-CoV vaccine administered with alum or MF59 adjuvant induced neutralizing antibodies in mice. However, it has been confirmed that wild-type MERS-CoV infection after vaccination causes eosinophil-related lung pathology in mice (Agrawal *et al.*, 2016). In contrast, a recent study found that the alum adjuvanted formalin-inactivated MERS-CoV vaccine prevented eosinophil-related lung pathology (Deng *et al.*, 2018). Currently, there are no ongoing clinical trials for an inactivated MERS-CoV.

The live attenuated vaccine, which utilizes the actual virus from which the pathogenicity has been removed, is the most immunogenic vaccine, and has been applied to prevent various infectious diseases (Minor, 2015). However, it was pointed out as a disadvantage that there are always opportunities for the pathogenicity to be recovered, which can cause infection in immunocompromised patients (Minor, 2015). In the development of the MERS-CoV vaccine, the live attenuated vaccine efficacy was confirmed in mice (Almazán *et al.*, 2013), but it did not progress to clinical trials.

Recombinant protein-based vaccine is composed of a recombinant protein containing one or more antigenic proteins, and has the advantage of easy production and high safety (Hansson *et al.*, 2000). Regarding the development of the MERS-CoV vaccine, a vaccine using the RBD or full S protein was developed, and it was confirmed that the neutralizing antibody titer was highly induced in mice, and the protective immunity effect was also confirmed in hDPP4 mice

(Coleman *et al.*, 2014, 2017; Tai *et al.*, 2016; Jiaming *et al.*, 2017). However, no recombinant protein-based vaccine has entered into clinical trials yet.

Conclusion

Vaccine development should be prioritized for MERS-CoV considering its CFR and the absence of a therapeutic agent. Several MERS-CoV vaccines using different platform technologies, such as DNA and viral vectors, are enrolled in clinical studies. However, most MERS-CoV vaccine clinical trials remain in the early phase 1/2 studies although they were initiated earlier than trials for SARS-CoV-2. Furthermore, the platform technology targeted at MERS-CoV vaccine development is being used successfully for SARS-CoV-2 vaccines.

Given the nature of RNA viruses, a variant S can emerge during the transmission of MERS-CoV from animals to humans or humans to humans. In contrast to monoclonal antibodies that cannot neutralize pseudoviruses even with one amino acid mutation in S (Goo *et al.*, 2020), a vaccine targeting one viral strain S can induce cross-protective immune responses against multiple viral strains with mutation in S (Choi *et al.*, 2020). However, if a new S variant escapes the neutralizing antibodies developed in the original MERS-CoV vaccines, it should be possible to quickly produce a vaccine against a mutant strain using the identified viral sequence once the platform technology is established. To induce more efficacious neutralizing antibodies, the pre-fusion form of S-like COVID-19 vaccines developed by Moderna, Pfizer/BioNTech, and Jassen need to be adopted for MERS-CoV vaccines (Dai and Gao, 2021).

Given the cases of other vaccine licenses such as the Hepatitis B vaccine (Eyigun *et al.*, 1998), the evaluation of vaccine efficacy can be made easier if the immune correlate of protection is identified. Using the WHO International Standard for MERS-CoV antibodies released in 2020, the pooling of sera from MERS-recovered patients can be a useful tool for harmonizing the assessment of immunogenicity, especially binding and neutralizing antibody responses to different vaccine platforms. This approach was established by the International Vaccine Institute, NIBSC, and funded by CEPI (WHO/BS/2020.2398 Establishment of 1st WHO International Standard for anti-MERS-CoV antibody). In addition, further studies are needed to determine the critical cellular immune responses induced by vaccines against MERS-CoV infection. Considering the characteristic immune responses induced by each platform technique, heterologous priming/boosting may also be considered to maximize protective immunity (Choi *et al.*, 2020).

In conclusion, the platform technologies tested during MERS-CoV vaccine development can be used for other unknown emerging pathogens in the future.

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Conflicts of Interest

The authors declare they have no potential conflicts of interest to disclose.

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