EXPERT REVIEWS

Middle East respiratory syndrome: obstacles and prospects for vaccine development

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The recent emergence of Middle East respiratory syndrome (MERS) highlights the need to engineer new methods for expediting vaccine development against emerging diseases. However, several obstacles prevent pursuit of a licensable MERS vaccine. First, the lack of a suitable animal model for MERS complicates the *in vivo* testing of candidate vaccines. Second, due to the low number of MERS cases, pharmaceutical companies have little incentive to pursue MERS vaccine production as the costs of clinical trials are high. In addition, the timeline from bench research to approved vaccine use is 10 years or longer. Using novel methods and cost-saving strategies, genetically engineered vaccines can be produced quickly and cost-effectively. Along with progress in MERS animal model development, these obstacles can be circumvented or at least mitigated.

Keywords: betacoronavirus • *Coronaviridae* • coronavirus • MERS-CoV • Middle East respiratory syndrome • Nidovirales • nidovirus

High-consequence pathogens continue to emerge or re-emerge globally, leading to increased public health concerns about potential pandemics [1]. Current human viral disease outbreaks of concern include an ongoing Ebola virus disease epidemic in West Africa [2], avian influenza caused by a novel influenza A virus subtype (H7N9) in China [3], enterovirus D68 infections in the USA [4] and the topic of this review, Middle East respiratory syndrome (MERS) caused by Middle East respiratory syndrome coronavirus (MERS-CoV) (2012–present) [5]. Emerging infections often first present as limited disease outbreaks caused by rare or unknown pathogens, increasing the likelihood that their significance is overlooked. Consequently, financial resources are routed toward other infectious diseases that are deemed more 'pressing' at a given time. Examples of rare agents that caused very few human infections for many years, only to then erupt in outbreaks involving thousands are: Rift Valley fever virus (more than 100,000 cases from 1930 to 2015) [6] and Ebola virus (1581 cases and 1136 deaths since its discovery in 1976 until late 2013; 24,701 cases and 10,194 deaths from December 2013 to March 18, 2015) [2]. Other agents, such as severe acute respiratory syndrome coronavirus (SARS-CoV), emerge unexpectedly and eventually cause large epidemics (8096 cases and 774 deaths), only to seemingly disappear again [7]. Consequently, global public health professionals are challenged with the ever more important task of rapidly developing improved methods for infectious disease detection, surveillance, control, prevention and containment.

Through widespread efforts to provide a swift response to an emerging disease, MERS, an impressive spectrum of prevention and treatment strategies was established *in vitro* in a relatively short period of time. From antivirals to monoclonal and polyclonal antibodies and vaccines [8–18], *in vitro* and *in vivo* preclinical testing led us to the same predicament that the scientific community faced during the SARS epidemic. Laboratory research is all too often inefficiently translated into clinical testing of candidate therapeutics and prophylactics, delaying clinical licensure by the authorities and final administration during outbreaks. Here we summarize and evaluate

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the progress made in MERS vaccine development to provide an example of various challenges that are encountered on the path to medical countermeasure licensure.

Epidemiology of MERS

MERS was first recognized as a new disease in a 60-year-old Saudi male patient with respiratory distress admitted to a hospital in Jeddah, Saudi Arabia in June 2012 [19]. Soon after the index case was detected, more and more people were identified who suffered of the same ailment [20,21]. The majority of MERS infections were reported from Western Asia (in particular from Saudi Arabia and the United Arab Emirates, but also from Jordan, Lebanon, Kuwait, Oman, Qatar and Yemen). Later, MERS was also diagnosed in Europe (Austria, France, Germany, Greece, Italy, the Netherlands, UK), Northern Africa (Algeria, Egypt, Tunisia), Northern America (USA), southeastern Asia (Malaysia, Philippines) and southern Asia (Iran) among people with a travel history to Western Asia [5].

Human-to-human transmission of MERS-CoV is estimated to account for approximately 60% of the total MERS cases [22], and the origin of infection with MERS-CoV is unexplained in the rest of the cases. The risk of virus transmission is substantially greater from index cases than from secondary cases [22]. The increasing distribution of MERS cases within the Arabian Peninsula is worrisome. For instance, for the past 2 years, concerns about the initiation of a MERS pandemic prompted travel restrictions to Makkah, Saudi Arabia, for millions of Muslim pilgrims, preventing tens of thousands of potential travelers from making the religiously significant Hajj journey. Although a major outbreak of MERS has not occurred as a direct result of recent Hajjes, trepidations remained high [23,24].

Studies revealed that older men and individuals with comorbid conditions (e.g., diabetes; hypertension; chronic cardiac, lung or renal disease) are at greatest risk for developing fatal MERS, although the gender bias is epidemiologically unclear [20,25–29]. Whether physiological, genetic or cultural factors play a role in the increased risk toward men is unknown. At the time of writing, 1075 MERS cases were confirmed, including at least 404 deaths [5]. However, increasing evidence of subclinical infections [30] suggests that the actual number of human MERS cases is much higher than the currently confirmed number. The etiological agent of MERS, a novel betacoronavirus, was rapidly identified and named 'Middle East respiratory syndrome coronavirus (MERS-CoV)' [19,31,32].

Epizootiology of MERS

How MERS-CoV was originally introduced into the human population and why MERS cases were not recorded before 2012 remain to be determined. One-humped camels (*Camelus dromedarius*) are currently suspected to be the animals from which MERS-CoV is transmitted to humans. This suspicion stems from the detection of MERS-CoV–neutralizing antibodies in one-humped camel herds of Egypt [33], Ethiopia [34], Kenya [35], Jordan [36], Nigeria [34], Oman [37], Qatar [36], Saudi Arabia [38,39], Somalia [40], Spain [37], Sudan [40], Tunisia [34] and United Arab Emirates [41,42]. MERS-CoV or MERS-CoV–like genome fragments and coding-complete genomes, highly similar to human MERS-CoV genomes, were detected in one-humped camels [38,39,43–49], and MERS-CoV was directly isolated from several one-humped camels and grown in tissue culture [39,49,50]. One-humped camels experimentally infected with MERS-CoV develop only minor clinical signs of respiratory disease, but MERS-CoV replicates in the upper airways [51]. Serologic evidence for MERS-CoV infection was not found in people with potential exposure to infected one-humped camels in three serosurveys [39,52,53]. Therefore, zoonotic transmission from one-humped camels to people might be a rare event. However, such a conclusion should be regarded with caution until further serosurveys are performed and published. It is, therefore, possible that MERS-CoV is widely distributed among one-humped camels, but that particular genotype of the virus had to evolve to allow a jump into the human population. A genomic study, indeed, revealed the presence of several genetic variants of MERS-CoV in individual one-humped camels, whereas MERS-CoV in humans exposed to these one-humped camels appears to be infected with clonal MERS-CoV populations [44]. As one-humped camels are frequently exported from Africa to the Arabian Peninsula of Western Asia, an animal native to Africa could be transmitting MERS-CoV to one-humped camels prior to exportation (Figure 1).

Based on the presence of genome fragments, genomes or replicating viruses, many betacoronaviruses seem to be maintained in Africa, Europe and Asia by phylogenetically highly diverse bats. These viruses include SARS-CoV and SARS-CoV–related viruses from horseshoe bats (*Rhinolophus* spp.) [54], but several viruses even more closely related to MERS-CoV have not been detected in humans [55–63]. Therefore, researchers speculate that MERS-CoV could be a bat-borne virus. The bat-origin hypothesis is based on betacoronavirus phylogeny and receptor usage [55,56,64–67] and isolation of MERS-CoV from one individual Egyptian tomb bat (*Taphozous perforatus*) [66]. However, these studies are suggestive and epidemiological evidence of bat-to-human or bat-to-one-humped camel transmission of MERS-CoV (Figure 1A) has yet to be gathered [68].

Clinical presentation of MERS

After an incubation period of 9–12 days, MERS generally presents in humans as a lower respiratory infection with fever (often with chills or rigors), dry or productive cough and dyspnea. More infrequently, patients develop chest pain, headaches, hemoptysis, myalgia and/or sore throat. In severe cases, the illness can quickly progress to severe atypical pneumonia, acute respiratory distress syndrome and severe hypoxemic respiratory failure [20,26,28,29,69]. MERS often includes extrapulmonary manifestations involving the circulatory, renal and/or gastrointestinal systems (abdominal pain/nausea, diarrhea, emesis) that can rapidly advance to septic shock, renal failure or refractory multiple organ failure [20,28,70]. Chest x-ray or computed tomography imaging often reveals subtle to extensive unilateral or bilateral abnormalities, such as consolidation, increased

bronchovascular markings, pleural or bronchial wall thickening, reticulonodular airspace opacities or cardiomegaly. Clinical chemistry is characterized by increased alanine transaminase and aspartate transaminase concentrations in some patients and increased L-lactate dehydrogenase concentration in about 50% of the cases. Low albumin and hemoglobin concentrations are frequent findings, as are lymphopenia and thrombocytopenia, whereas lymphocytosis occurs more rarely [20,26,28,29,69]. Viral loads are usually high in the respiratory tract (reaching $>10^6$ genome copies/ml) but may be low or absent in the blood [28,69,70].

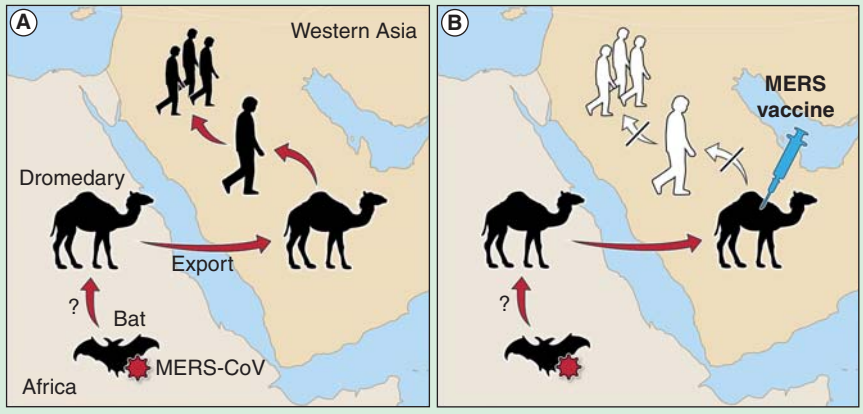

Figure 1. Hypothesized transmission of MERS-CoV from animal hosts to humans. **(A)** MERS-CoV is potentially transmitted by infected bats to African one-humped camels, which are often exported to the Arabian Peninsula. **(B)** Vaccination of one-humped camels could, therefore, prevent further transmission of the virus to humans and subsequent human-to-human transmission if one-humped camels are indeed the primary route of infection for humans.
MERS: Middle East respiratory syndrome; MERS-CoV: Middle East respiratory syndrome coronavirus.

Treatment of MERS

At the moment, specific antiviral agents for the treatment of MERS are not available. However, several *in vitro* studies identified drugs already in clinical use that potentially could be repurposed for treatment of MERS. These drugs include an inosine monophosphate dehydrogenase inhibitor (mycophenolic acid) used to treat other coronavirus infections [13,15,16]; a pan-coronavirus inhibitor that targets membrane-bound coronaviral RNA synthesis (K22) [71]; the guanosine analog ribavirin used in the treatment of hepatitis C, respiratory syncytial virus and arenavirus infections; interferon-β [13,16,72]; inhibitors of estrogen receptor 1 used for cancer treatment (toremifene citrate); inhibitors of dopamine receptors used as antipsychotics (chlorpromazine hydrochloride and triflupromazine hydrochloride); kinase signaling inhibitors (imatinib mesylate and dasatinib) [12]; endocytosis inhibitors (chlorpromazine and chloroquine) [18]; an antidiarrheal agent (loperamide); the HIV-1 protease inhibitor lopinavir [18]; and the transmembrane protease, serine 2 protease inhibitor camostat [73]. Unfortunately, few of these drugs have been evaluated in animal models of MERS (an exception is [72]), which, in part, is due to the absence of animal models that truly reflect the human disease (see below).

In the absence of approved specific antiviral agents against MERS-CoV, treatment, therefore, is based on supportive care. After initial laboratory blood tests and chest radiography, patients are treated with broad-spectrum antibiotics to control (often nosocomial) secondary bacterial infections. However, the majority of in-patient MERS cases escalate to respiratory failure, requiring intubation, mechanical ventilation or extracorporeal membrane oxygenation or renal replacement therapy and, therefore, admission to an Intensive Care Unit [20,26,28,29,69].

Potential costs associated with MERS

Treatment and disease management for MERS can be a tremendous financial burden to local economies. In the event of a pandemic, the financial burden may prove to be catastrophic, especially in countries with limited financial resources. Based on the small number of treatment–cost analyses available from the 2003 outbreak of SARS [74,75], at Tourcoing Hospital, France, additional hospital administration and material resource costs alone approximated US$79,150 per in-patient due to the need for increases in staff, containment apparatuses and personal protective equipment required when dealing with a high-consequence pathogen. Moreover, the average MERS in-patient incurs the added costs of a prolonged Intensive Care Unit stay (94.5% of cases) with mechanical ventilation (86% of cases) and renal replacement therapy (50% of cases) (Figure 2).

The World Bank estimates global economic losses of trillions of US dollars in the event of a severe influenza pandemic and considers MERS to be a pathogen of pandemic potential [14]. Global costs for the 2003 SARS epidemic alone were estimated at US$40 billion [76]. The cost of developing a successful vaccine is approaching US$500 million. An effective vaccine for a high-consequence pathogen has the potential to save 10 times its cost in a single year of use, as estimated for the smallpox vaccine. However, a true cost-to-benefit analysis must be performed to determine if vaccine development efforts will provide benefit. If we assume that MERS cases continue at the rate of roughly 450 per year with a 62% hospitalization rate [77] and that the additional cost per in-patient is approximately US$714,000 on average (cumulative average cost per day per intervention per in-patient multiplied by median stay per respective intervention), the cost of developing the vaccine would be justified within 2.5 years (Figure 2). Therefore, if sustained human-to-human MERS-CoV transmission were to occur, the benefit would outweigh the cost. In general, vaccine development is a worthwhile pursuit, and steps should be taken to expedite approval of safe and effective vaccines for emerging pathogens.

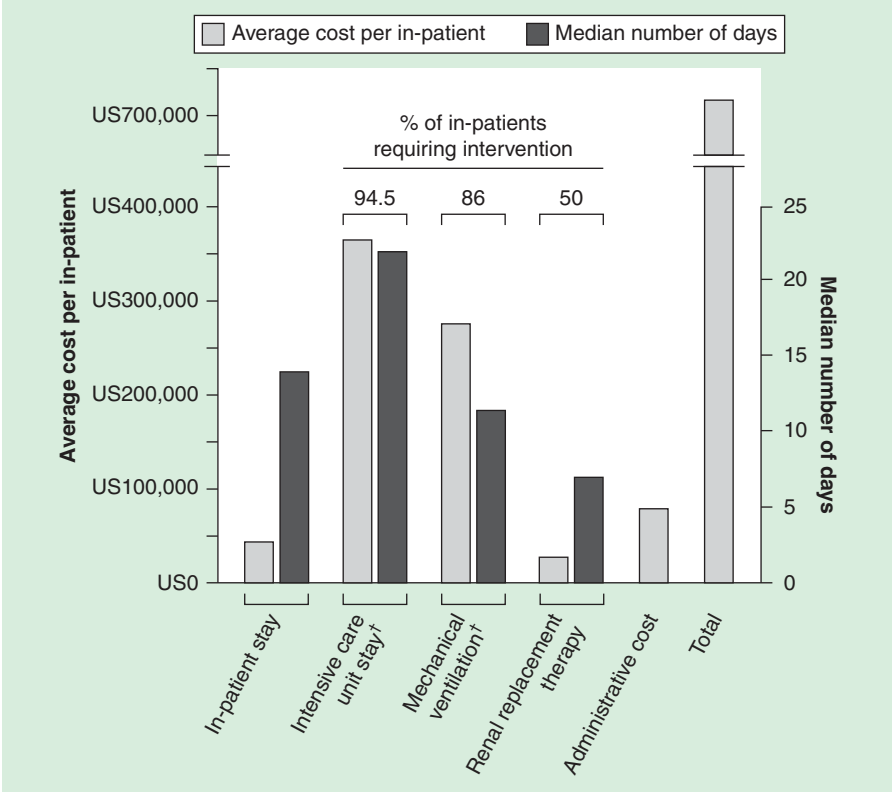

Figure 2. Estimation of major hospital costs affiliated with a MERS outbreak.
Average cost per day per in-patient [147,148] was multiplied by the median number of days for total cost per treatment. In-patient stay: US average of US$3145/day × 14 days median for a MERS in-patient = US$44,030.00; intensive care unit stay: †US$16,474 × 22 days = US$362,430; mechanical ventilation: †US$23,750 × 11.5 days = US$273,139; renal replacement therapy: US$3819 × 7 days = US$26,734; total: sum of in-patient costs after multiplying by the percentage required and adding the additional administrative costs of US$79,150 per in-patient = US$713,942. An in-patient requiring all interventions would incur expenses of more than US$785,000.
†Current cost estimate after adjustment for inflation from the time of report.

Overview of MERS-CoV genome & function of encoded proteins

The emergence of MERS-CoV infection has spurred a conglomeration of traditional and novel vaccine strategies, the success of which hinges on a thorough understanding of the genetic makeup and mechanism of action of this virus. MERS-CoV is a member of the genus *Betacoronavirus*, subfamily *Coronavirinae* (*Nidovirales*: *Coronaviridae*) [78]. Like all nidoviruses, MERS-CoV has a positive-sense, single-stranded RNA, linear, monopartite genome. The MERS-CoV genome encodes 16 nonstructural proteins, 4 major structural proteins and 7 accessory structural proteins (Table 1) [31]. The nonstructural proteins, produced through proteolytic cleavage of two polyproteins coded from two open reading frames, are the major components of the polymerase complex and manage replication and transcription.

Similar to many other coronaviruses, the major structural proteins, spike protein (S), envelope protein (E), membrane protein (M) and nucleoprotein (N), are the primary targets of the host antibody-mediated immune response and are the focus in vaccine development efforts. S is the surface glycoprotein of the MERS coronavirion. S mediates the attachment of the virion to target cells and its subsequent entry into the cell by fusing viral and host cell membranes [79]. These functions involve two distinct domains of S, referred to as S1 and S2, respectively. S1 contains the host cell receptor-binding domain [42,80,81], which engages the primary MERS-CoV cell-surface receptor CD26/DPP4 [11]. S2 contains epitopes that are cross-reactive with homologous epitopes of other group A and B betacoronaviruses [79,82], suggesting that the development of a general, multivalent, betacoronavirus vaccine might be possible. The E and the M work to secure the structural integrity of the virion. The nucleoprotein (N) encapsidates the viral genomic RNA [83].

Targeting the source of transmission through vaccination programs

The identification of the natural host reservoir of an emerging human pathogen is the first ideal step toward prevention of transmission. Then, the human population could be educated to avoid the host or to implement proper safety measures when coming in contact with the reservoir. If contact with the reservoir host cannot be avoided (e.g., abundance, economic importance), vaccination of such a host may be a straightforward approach to prevent host-to-human transmission. In addition, the development of an animal vaccine may ultimately be cheaper to produce and faster to achieve as regulatory hurdles to obtain licensure may not be as stringent as those in place for human vaccine development. For example, vaccination of wildlife reservoirs against rabies reduced human cases in the USA by 98% [84]. This strategy is also a relatively new pursuit for the eradication of tuberculosis in parts of Europe through vaccination of cattle and wildlife [85,86].

Unfortunately, as described above, the natural MERS-CoV reservoir and the MERS-CoV transmission cycle remain to be defined. Vaccination of one-humped camel herds could be feasible as these animals are often kept/raised/sold by humans, and wild animals could be identified relatively easily. However, some adult one-humped camels tested positive for MERS-CoV despite the presence of anti-MERS-CoV antibodies [38,39], and pre-existing neutralizing anti-MERS-CoV antibodies do not necessarily protect against re-infection with MERS-CoV [39]. This observation suggests that camel vaccination may have to

Table 1. Functions of nonstructural, major structural and accessory structural proteins of Middle East respiratory syndrome coronavirus.

Open reading frame	Expressed protein	Category	Function	Approach for candidate vaccine development
1a, 1ab	• Polyproteins pp1a and pp1ab; proteolytically processed to Nsp 1–16 [140,141]	Nonstructural	• RNA synthesis via RNA-dependent RNA polymerase (genome replication, transcription) • Proteolytic cleavage: interferon antagonist, deubiqutinylation	Conserved epitope among coronavirus strains [125]
S	Spike glycoprotein, proteolytically cleaved into S1 and S2 fragments	Major structural	• Mediates attachment and entry into host cells [142] • Elicits neutralizing antibodies	• VEEV replicons expressing S alone or with N [14,89,142] • Conserved S epitope found to interact with most MHC-1 alleles [17] • Adenovirus 5 vector expressing S or S1 [116] • RBD fused with IgG-Fc fragment [40,42,80,81,143]
3	3	Accessory structural	Unknown, but not essential for replication [101]	
4a	4a	Accessory structural	• Unknown, but not essential for replication [101] • Interferon antagonist [144,145]	
4b	4b	Accessory structural	• Unknown, but not essential for replication [101] • Interferon antagonist [144]	
5	5	Accessory structural	• Unknown, but not essential for replication [101,142] • Interferon antagonist [144]	
E	Envelope protein	Major structural	Structural integrity of the virion; required for propagation [101]	Recombinant MERS-CoV lacking E [14]
M	Membrane protein	Major structural	Structural integrity of the virions; interferon antagonist [144]	
N	Nucleoprotein	Major structural	Encapsidates viral RNA into ribonucleoprotein complexes	VEEV replicons expressing N alone or with S [14,142,146]
8b	8b	Accessory structural	Uncharacterized	

MERS-CoV: Middle East respiratory syndrome coronavirus coronavirus; Nsp: Nonstructural proteins; RBD: Receptor-binding domain; VEEV: Venezuelan equine encephalitis virus.

be repeated regularly or may not be effective at all. Probably even more unrealistic is the vaccination of bats due to sheer number of these relatively small and (dependent on species) often quite abundant animals.

The absence of a clearly defined zoonotic virus ecology prompts researchers to contemplate development of human vaccines. As MERS tends to be acute and is not yet widespread, targeting specific human populations at high risk of infection for vaccination is a logical strategy for prevention or limitation of infections. Development and distribution of vaccines should, therefore, be expedited for one-humped camel handlers and herders, healthcare workers and veterinarians, and travelers to areas where MERS-CoV infection is prevalent. If widespread infection throughout the general population is expected, other high-risk populations (e.g., aged people, persons with cormorbidities) should be targeted for vaccination.

MERS-CoV immunology

Given the lack of a suitable MERS animal model and relatively few clinical data on MERS-CoV patients, the nature of a successful immune response to MERS-CoV infection is difficult to establish. Serology and PCR-based assays indicate that many

people may test positive for MERS-CoV despite being asymptomatic [25,30,87]. It is unknown if all of the asymptomatic, positive individuals were transiently exposed, established a carrier state, or developed a subclinical, easily controlled infection. Few human patient data are available; however, Faure *et al.* performed an *ex vivo* comparison of two MERS patients to determine the immune response to infection [88]. The experiment was based on bronchoalveolar lavages obtained from two patients and identified that the one who succumbed to MERS did not develop a Th1 response. This patient had lower concentrations of interferon-α, interleukin-12 and interferon-γ, compared to the patient who did not succumb. Although the experiment involved a limited sample size, the data support the necessity of a Th1 response.

Zhao *et al.* investigated knockout mice to evaluate the immunological requirements of clearance of MERS-CoV [89]. The authors demonstrated that interferon-α– and MyD88-deficient mice could not clear MERS-CoV as rapidly as wild-type mice. Similarly, T-cell and B-cell knockout mice could not clear the virus as efficiently as control mice. Furthermore, vaccinated mice had reduced viral titers, and serum transfer experiments provided protection against homologous MERS-CoV infection. These data suggest that both an efficient T- and B-cell response are required for protection. Indirect or direct B- and T-cell functional response should be included as criteria for candidate vaccine evaluation. Until more clinical data become available, correlates of protection will be difficult to establish.

Vaccine design strategies applicable to MERS

Historically, the first vaccines against viral pathogens were homotypic live-attenuated viruses. The virus isolate was passaged in an animal host or cell line until a nonvirulent (live-attenuated) strain evolved, and this nonvirulent virus was then used for vaccination [90]. A more recent method for developing vaccines is to genetically engineer the virus to be avirulent or replication incompetent [91,92]. Replication-deficient vaccine virus constructs were generated that expressed human parainfluenza virus-3 or human respiratory syncytial virus [93,94]. Similarly, replication-deficient adenoviruses were developed as vaccines against Ebola virus and HIV-1 infection [95,96]. A Phase IIa clinical trial was initiated to evaluate the efficacy of a replicating modified vaccinia Ankara (MVA) expressing influenza A virus proteins [97]. Replicating MVA has also been used to boost the effect of replication-deficient adenovirus-based vaccine responses [98].

The main concern with replicating (live) viral vaccines is the possibility of disseminated infection in immunosuppressed populations (e.g., disseminated vaccinia virus infection) with heterotypic vaccine platforms or the possibility of reversion to virulence in the case of homotypic candidates [99]. New methods of replicating vaccine development typically incorporate fail-safe mechanisms. Such mechanisms include deletion of a gene encoding a protein required for viral propagation or introduction of a sufficient number of genomic mutations to make reversion extremely unlikely [91,100]. For instance, Almazan *et al.* engineered a recombinant MERS-CoV lacking the E open reading frame as a MERS candidate vaccine; however, its protective efficacy has not yet been demonstrated [101].

Whole inactivated virion preparations often provide the immunogenicity of a replicating viral vaccine without the possibility of reversion to a virulent phenotype. Virions are killed or inactivated by chemical or radiological methods prior to use in a vaccine. While the preparation is not able to replicate in the host, the recipient's immune system still mounts a response against the presented antigens. Although examples of inactivated MERS-CoV virions are not yet published, inactivated SARS-CoV particle vaccines have been tested with minimal success in laboratory mice and domestic ferrets [102–104].

Use of inactivated virion preparations has prompted several concerns. One concern is that toxic reagents used in virion inactivation must be completely removed from the product before administration. Another concern is that irradiation, used as an alternative to toxic agents, may destroy crucial epitopes and, therefore, render the preparation nonimmunogenic. The third concern is that inactivation could be, for whatever reason, incomplete, resulting in preparations containing fully virulent viruses. These concerns are exacerbated by the increasing stigma that exists among the general public regarding vaccines, in general, and chemical additives in vaccine preparations, in particular [105,106].

Recombinant viral vectors are an upgrade to the replicating viral vaccine strategy. The ability to optimize for safety and immunogenicity via bioengineering is an obvious benefit. Using vaccine platforms such as adenoviruses [107], vesiculoviruses [108] or MVA [109–112], the foreign gene of immunological interest can be inserted into a heterotypic viral genome with proven success as a vaccine. The recombinant virus will express the foreign protein, which will stimulate a protective immune response in the inoculated host. This approach has also been used successfully to develop multivalent vaccines. Such vaccines can confer protection against not only multiple variants or strains of the same pathogen but also multiple pathogens simultaneously [98,113–115]. Recently, Escriou *et al.* showed evidence of bivalent protection of laboratory mice from measles virus and SARS-CoV infection [115]. For such constructs, codon optimization for host recognition may increase attenuation and protein expression, yielding greater safety and immunogenicity of the vaccine. For instance, two vaccines, an MVA vaccine and an adenovirus-based vaccine, expressing codon-optimized MERS-CoV S elicited serum antibodies in laboratory mice that were used to neutralize MERS-CoV *in vitro* [111,116].

As an alternative method to immunogen presentation by a viral vector, nanoparticles of the protein of interest can be formulated with a suitable adjuvant for use as a candidate vaccine. For instance, micellular nanoparticles with MERS-CoV S trimers expressed on the surface (Novavax, Inc., Gaithersburg, MD, USA) were concentrated from preparations of a recombinant baculovirus (autographa californica multicapsid nucleopolyhedrovirus [AcMNPV]) expressing MERS-CoV S. The AcMNPV genes were codon-optimized for expression in the insect cells in which the virus was propagated [117]. Again, MERS-CoV–neutralizing antibodies were induced in immunized laboratory mice.

DNA vaccines typically consist of a viral genomic segment encoding a neutralizing epitope contained in a plasmid and combined with an adjuvant for administration [118]. While early fabrications failed to yield protective immune responses, more recent studies testing DNA vaccines corrected this shortcoming by optimizing constituents and delivery methods. Also, due to the relatively simple and low-cost processes for production and manufacturing, DNA vaccines are a competitive pursuit. One such DNA vaccine, a quadrivalent vaccine against HPV infection caused by types 6, 11, 16 and 18, is widely used in vaccination programs in numerous countries [119–123]. Unpublished data from Inovio Pharmaceuticals suggest strong neutralizing antibody elicitation, possible multiple strain coverage and broad $CD8^+$ T-cell responses in mice after immunization with a MERS-CoV DNA vaccine. Inovio's proprietary technology involves using the SynCon DNA vaccine platform. Inovio's proprietary technology involves using the SynCon DNA vaccine platform. This platform incorporates DNA from multiple strains and/or antigens. The DNA is transfected by electroporation, resulting in a more efficient delivery into muscle or skin cells [124].

Other novel vaccine development strategies of interest use immunoinformatics to predict the most immunogenic parts of a virus that should be included in a vaccine to achieve the most potent and relevant neutralization. The resulting candidate vaccines are also known as subunit vaccines. Current MERS candidate vaccines focus on the receptor-binding domain of S1 as a precise source from which the adaptive immune response would generate effective neutralizing antibodies [40,42,81]. Using immunoinformatics, Sharmin and Islam chose to focus on an epitope found in RNA-dependent RNA polymerase (RdRp) that is conserved across all human coronaviruses [125]. While blocking viral replication by affecting the RdRp may be effective *in vitro*, this method has yet to be proven effective in protecting a host from infection by an RNA virus. One drawback to this approach is that the RdRp epitopes may not be readily detected by the host immune system as the RdRp is usually not a major structural component of virions. Also, antigenic processing should lead to a wide range of T-cell and B-cell immunoreactive epitopes. Therefore, focusing on RdRp epitopes would not provide broad, effective cellular and humoral responses.

In combination with the aforementioned immunoinformatics technology, a more realistic approach is based on the use of B-cell and T-cell epitope predictions based on viral genome sequence. Neutralizing antibodies to MERS-CoV structural proteins, primarily S, are believed to be required to inhibit infection [81,111,116,117]. In addition to humoral immunity, T-cell responses are now considered to be a major determinant of protection from infectious diseases [126,127]. Such an approach using B-cell and T-cell epitopes was used by researchers such as Terry *et al.* and Oany *et al.* to develop a MERS candidate vaccine [128,129]. While this approach is innovative, no actual *in vivo* MERS-CoV neutralization has been demonstrated to date.

In vivo evaluation of MERS-CoV candidate vaccines

The evaluation of any candidate vaccine against a high-consequence viral disease is critically dependent on the availability of animal models. The US FDA 'Animal Rule' permits use of efficacy data from animal models that closely mimic human disease for approval of medical countermeasures, when evaluation in humans is not ethically feasible due to the high lethality of the virus under evaluation [130]. To the best of our knowledge, the FDA has not determined that MERS medical countermeasure development falls under the Animal Rule. However, the identification and development of a suitable animal model for any human disease has many challenges and caveats. Upon exposure to MERS-CoV, animals should display respiratory distress, fever, tussis, dyspnea, gastrointestinal signs such as vomiting and diarrhea, and renal failure [20,26,29].

Since MERS-CoV was first identified, several groups have attempted to develop such MERS animal models. Laboratory mice, Syrian hamsters (*Mesocricetus auratus*) and domestic ferrets (*Mustela putorius furo*) were evaluated as potential models for medical countermeasure screening and for understanding MERS-CoV–induced pathogenic mechanisms [117,131]. Initial experiments included screening of wild-type BALB/c and STAT-1 knockout laboratory mice for susceptibility to MERS-CoV infection and development of disease. However, the mice did not develop clinical signs of disease, and infectious virus could not be recovered [117]. Zhao *et al.* overcame this hurdle by transducing BALB/c mice with human CD26/DPP4 using an adenovirus construct [89]. Transduced mice were permissive to MERS-CoV infection and demonstrated minimal weight loss. However, candidate medical countermeasures in this mouse model can only be evaluated by comparing the changes in viral titer in the lungs at 4 days post-inoculation between treated and untreated animals. Transduction-based models may also prove quite variable due to infection efficacy of the transducing vector.

Nonhuman primates, such as rhesus monkeys (*Macaca mulatta*), crab-eating macaques (*Macaca fascicularis*), grivets (*Chlorocebus aethiops*) or common marmosets (*Callithrix jacchus*), are frequently used in the development of animal models for human viral disease because of their immunological similarity to humans. MERS-CoV inoculation into rhesus monkeys gave varied results, leading to disease with limited similarity to human disease [72,132,133] For instance, a study by Yao *et al.* revealed that intratracheal inoculation of rhesus monkeys with MERS-CoV resulted in a nonlethal disease, and some limited pathology could be observed at 28 days post-inoculation [133]. de Wit *et al.* described a study of nonhuman primates following inoculation with a combination of intratracheal, intranasal, oral and conjunctival routes [132]. Rhesus monkeys were euthanized on days 3 and 6 post-inoculation with MERS-CoV and evaluated for virological, immunological and histopathological changes [132,134]. At the euthanasia time points, the animals had signs of pneumonia, and replicating virus could be demonstrated in tissues and mucous membranes by quantitative PCR. However, inherent in the serial-sampling design, the disease progression induced by viral infection was truncated, limiting the data gleaned from the study.

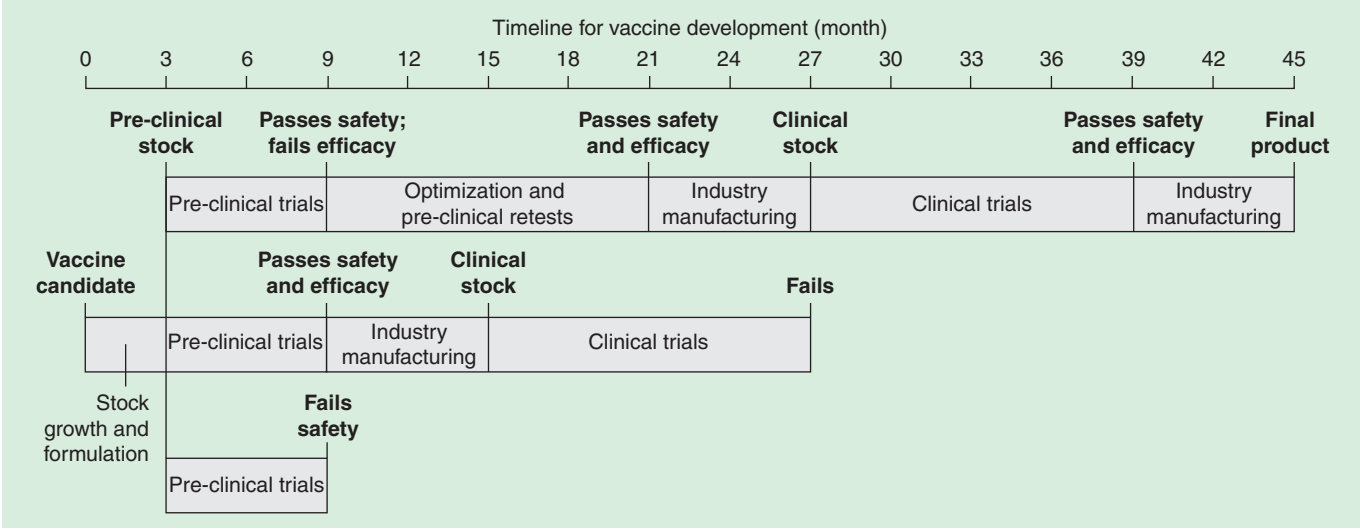

Figure 3. Idealized vaccine development timeline from post-discovery to pre-regulatory submission. A simplified timeline illustrates the potential pitfalls encountered throughout the development process. Optimistic estimates for vaccine development from candidate selection to industrial production fall between 3.5 and 4 years, depending on the type of vaccine. After adding 2–3 years for research prior to candidate selection and 2–3 years for regulatory submission and licensure once a final formulation is in hand, total time is approximately 10 years. As discovery methods and bureaucratic processes and approvals are accelerating, the overall timeline could realistically shrink to 6–7 years.

A follow-up study by Falzarano *et al.* demonstrated that administration of anti-inflammatory immunomodulators, interferon-α2b and ribavirin, reduced the viral burden and lessened disease severity following intratracheal, intranasal, oral and ocular challenge with MERS-CoV [72]. X-ray radiographs indicated lung infiltrates at days 3 and 5 post-inoculation, suggesting virus-induced lung disease. However, these two studies did not include mock-inoculated controls to demonstrate that the observed clinical signs were not due to generalized inflammation from inoculation and handling procedures. More recently, Falzarano *et al.* described intratracheal inoculation of common marmosets with MERS-CoV, which resulted in partially lethal disease [135]. However, the animals received a large volume bolus of MERS-CoV (0.5 ml) intratracheally, which was disproportionate based on the small lung volume (15–25 ml) of a common marmoset [Johnson RF, Unpublished Observation measured by computed tomography (N = 10)]. In addition, the experiment did not include animals that only received control inocula. Therefore, the extent of virus-induced pathology compared to pathology due to animal manipulation remains unclear. Overall, a suitable nonhuman primate model of human MERS is still lacking.

If one-humped camels are the reservoir of MERS-CoV (see above), then their vaccination against MERS-CoV infection may provide an intervention opportunity (Figure 1B). Indeed, three one-humped camels, inoculated by intranasal, intratracheal and conjunctival routes with MERS-CoV developed benign clinical signs, but shed large quantities of the virus from the upper respiratory tract [51]. Comparisons drawn from a uniformly lethal animal model against this one-humped camel model would be interesting.

A MERS animal model based on one-humped camels has many challenges. The large size of these animals, their relative scarcity in the Western world and the classification of MERS-CoV as a WHO Risk Group 3 pathogen (requiring biosafety level 3 [BSL-3] containment) limit the number of facilities that could perform such studies. Colorado State University, Kansas State University, United States Department of Agriculture (Ames, IA, USA) and Commonwealth Scientific and Industrial Research Organisation (commonly known as CSIRO, Clayton South, Australia) all have BSL-3 labs that could handle such large animals. An alternative is the use of other camelids, such as alpacas (*Vicugna pacos*), guanacos (*Lama guanicoe*), llamas (*Llama glama*) or vicuñas (*Vicugna vicugna*), as these camelids are smaller and may be easier to obtain than bactrian camels (*Camelus bactrianus*). Such camels are as big as one-humped camels and rarer). However, the consequence of MERS-CoV inoculation in these animals requires evaluation.

Expert commentary

The recent emergence of MERS-CoV and the re-emergence of several other high-consequence pathogens in recent years have spurred a retooling of current vaccine strategies and development procedures. Many current MERS vaccine development strategies are based on SARS research [136]. However, in the 11 years since the first description of SARS, no vaccine to prevent SARS-CoV infection has been approved. This fact does not bode well for researchers and clinicians. While researchers are not deficient in MERS candidate vaccines, more emphasis should be placed on improving translational research, licensure procedures and animal model development for emerging pathogens [89,133].

For transient outbreaks of infectious diseases, such as MERS, that appear to subside relatively quickly on their own, justification of funding and research efforts for vaccine development is

not always straightforward. Unfortunately, vaccine development often seems reactionary rather than prospective. For instance, vaccine development against Ebola virus disease was overall a niche activity until the current 2013–2015 outbreak affected thousands of people. For any rare or emerging pathogen, cost–benefit analyses for vaccine development must be calculated based on a limited knowledge of its pandemic and/or re-emergence potential. As the example of MERS-CoV shows, even with sparse economical data available, cost estimates as shown in Figure 2 warrant MERS vaccine development, as economic losses from even small infectious disease outbreaks far outweigh the costs associated with vaccine development. However, faster methods to move a candidate vaccine from the laboratory bench into the clinic are essential. Such on-demand acceleration strategies are in current evaluation, and support for these efforts should be advanced [137–139].

Five-year view

In light of the 2014 Ebola virus disease outbreaks, added efforts to accelerate clinical trials, regulatory filings and licensure approvals will affect vaccine development for high-consequence pathogens in future. Companies like Novavax and Inovio Pharmaceuticals have an advantage of established pipelines for developing an approved MERS vaccine. A MERS wildlife vaccine targeting one-humped camels should also be evaluated. Considering the encouraging rate at which new technologies are being developed for emerging pathogen treatment strategies, a licensed MERS vaccine is feasible within 5 years after an appropriate MERS animal model becomes available. A typical timeline for vaccine development by the manufacturer prior to regulatory submission is approximately 3.75 years (Figure 3).

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Key issues

- The control of emerging pathogen diseases is a perpetual challenge requiring constant re-evaluation and ingenuity in containment, treatment and prevention methods.
- The lack of a proper animal model for Middle East respiratory syndrome (MERS) is inhibiting the progress of vaccine testing. Animal models thus far have demonstrated limited pathology.
- The prediction of clinical trial success based on *in vitro* research results remains a major obstacle to timely vaccine development, particularly for vaccines against emerging diseases with high lethality as seen with MERS.
- Emerging pathogen control via accelerated on-demand vaccine development is an idealistic approach.
- The usual lag in disease identification from accurate clinical reporting of presentation and pathogenesis should be shortened, and the difficulties related to regulatory submission and licensure for vaccines should be addressed before on-demand methods can be practically implemented.
- Thus far, the most promising MERS vaccine candidates are those that are proven to elicit MERS coronavirus (MERS-CoV)-neutralizing antibodies and that use an approved platform for administration. These include vaccines by Novavax (MERS-CoV S nanoparticles) and Inovio Pharmaceuticals (DNA MERS vaccine targeting multiple antigens). MERS vaccines that focus on targeting the MERS-CoV receptor-binding domain also look promising.

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