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 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...
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 airspaces opacities or cardiomegaly. Clinical chemistry is characterized by increased alanine transaminase and aspartate transaminase concentrations in some patients and increased t-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].

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.
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) [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
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

| Open reading frame | Expressed protein | Category | Function | Approach for candidate vaccine development |
|--------------------|------------------|----------|----------|-------------------------------------------|
| 1a, 1ab            | Polyproteins pp1a and pp1ab; proteolytically processed to Nsp 1–16 | Nonstructural | • RNA synthesis via RNA-dependent RNA polymerase (genome replication, transcription)  
• Proteolytic cleavage: interferon antagonist, deubiquitylation | 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] |
| S                  | 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 |

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

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

References
Papers of special note have been highlighted as:
* of interest
** of considerable interest
1. Brookes VJ, Hernandez-Jover M, Black PF, Ward MP. Preparedness for emerging infectious diseases: pathways from anticipation to action. Epidemiol Infect 2014;141:16. [Epub ahead of print]
2. World Health Organization. Global alert and response (GAR). Situation reports with epidemiological data: archives. 2015. Available from: www.who.int/csr/disease/ebola/situation-reports/archive/en/
3. World Health Organization. WHO risk assessment: human infection with avian influenza A(H7N9) virus as of 2 October 2014. 2014. Available from: www.who.int/influenza/human_animal_interface/influenza_h7n9/riskassessment_h7n9_2Oct14.pdf?ua=1
4. Foster CB, Friedman N, Carl J, Piedimonte G. Enterovirus D68: a clinically important respiratory enterovirus. Cleve Clin J Med 2015;82(1):26-31
5. World Health Organization. Global alert and response (GAR): Middle East respiratory syndrome coronavirus (MERS-CoV) – Oman. Disease outbreak news as of 23 January 2015, 2015. Available from: www.who.int/csr/don/23-january-2015-mers-oman/en/
6. Himeidan YE, Kweka EJ, Mahgoub MM, et al. Recent outbreaks of rift valley Fever in East Africa and the middle East. Front Public Health 2014;2:169
7. Cheng VC, Lau SK, Woo PC, Yuen KY. Severe acute respiratory syndrome coronavirus as an agent of emerging and reemerging infection. Clin Microbiol Rev 2007;20(4):660-94
8. FaiZarano D, de Wit E, Martellaro C, et al. Inhibition of novel beta coronavirus replication by a combination of interferon-alpha2b and ribavirin. Sci Rep 2013;3:1686
9. Ren Z, Yan L, Zhang N, et al. The newly emerged SARS-like coronavirus HCoV-EMC also has an 'Achilles' heel': current effective inhibitor targeting a 3C-like protease. Protein Cell 2013;4(4):248-50
10. Jiang L, Wang N, Zuo T, et al. Potent neutralization of MERS-CoV by human neutralizing monoclonal antibodies to the viral spike glycoprotein. Sci Transl Med 2014;6(234):234ra259
11. Spanakis N, Tsiodras S, Haagmans BL, et al. Virological and serological analysis of a recent Middle East respiratory syndrome coronavirus infection case on a triple combination antiviral regimen. Int J Antimicrob Agents 2014;44(6):528-32
12. Dyall J, Coleman CM, Hart BJ, et al. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 2014;58(8):4875-84
13. Chan JF, Chan KH, Kao RY, et al. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J Infect 2013;67(6):606-16
14. Tang XC, Agnihotram SS, Jiao Y, et al. Identification of human neutralizing antibodies against MERS-CoV and their role in virus adaptive evolution. Proc Natl Acad Sci USA 2014;111(19):E2018-26
15. Cheng KW, Cheng SC, Chen WY, et al. Thiopurine analogs and mycophenolic acid synergistically inhibit the papain-like protease of Middle East respiratory syndrome coronavirus. Antiviral Res 2015;115:9-16
16. Hart BJ, Dyall J, Postnikova E, et al. Interferon-beta and mycophenolic acid are potent inhibitors of Middle East respiratory syndrome coronavirus in cell-based assays. J Gen Virol 2014;95(Pt 3):571-7
17. Liu Q, Xia S, Sun Z, et al. Testing of Middle East respiratory syndrome coronavirus replication inhibitors for the ability to block viral entry. Antimicrob Agents Chemother 2015;59(1):742-4
Middle East respiratory syndrome

study. Lancet Infect Dis 2013;13(10):859-66

38. Alagaili AN, Briese T, Mishra N, et al. Middle East respiratory syndrome coronavirus infection in dromedary camels in Saudi Arabia. MBio 2014;5(2):e00884-14

39. Hemida MG, Chu DK, Poon LL, et al. MERS coronavirus in dromedary camel herd, Saudi Arabia. Emerg Infect Dis 2014;20(7):1231-4

40. Muller MA, Corman VM, Jores J, et al. Antibodies against MERS coronavirus neutralizing antibodies in camels, Eastern Africa, 1983-1997. Emerg Infect Dis 2014;20(12):2093-5

41. Alexandersen S, Kobinger GP, Soule G, Wernery U. Middle East respiratory syndrome coronavirus antibody reactors among camels in Dubai, United Arab Emirates, in 2005. Transbound Emerg Dis 2014;61(2):105-8

42. Meyer B, Muller MA, Corman VM, et al. Antibodies against MERS coronavirus in dromedary camels, United Arab Emirates, 2003 and 2013. Emerg Infect Dis 2014;20(4):552-9

43. Azhar EI, El-Kafrawy SA, Farraj SA, et al. Evidence for camel-to-human transmission of MERS coronavirus. N Engl J Med 2014;370(26):2499-505

44. Briese T, Mishra N, Jain K, et al. Middle East respiratory syndrome coronavirus quasispecies that include homologues of human isolates revealed through whole-genome analysis and virus cultured from dromedary camels in Saudi Arabia. MBio 2014;5(3):e01146-14

45. Chu DK, Poon LL, Gomaa MM, et al. MERS coronaviruses in dromedary camels, United Arab Emirates, in 2005. Transbound Emerg Dis 2014;61(2):105-8

46. Haagmans BL, Al Dhahiry SH, Corman VM, et al. Replication and shedding of MERS-CoV in upper respiratory tract of inoculated dromedary camels. Emerg Infect Dis 2014;20(12):1999-2005

47. Alabiriaza AS, Mattes FM, Azhar EI, et al. Investigation of anti-middle East respiratory syndrome antibodies in blood donors and slaughterhouse workers in Jeddah and Makkah, Saudi Arabia, fall 2012. J Infect Dis 2014;209(2):243-6

48. Gierer S, Hofmann-Winkler H, Albuhi WH, et al. Lack of MERS coronavirus neutralizing antibodies in humans, eastern province, Saudi Arabia. Emerg Infect Dis 2013;19(12):2034-6

49. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature 2013;503(7477):535-8

50. Annan A, Baldwin HJ, Corman VM, et al. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. Emerg Infect Dis 2013;19(3):456-9

51. Corman VM, Ithete NL, Richards LR, et al. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. Emerg Infect Dis 2013;19(3):456-9

52. Yang Y, Du L, Liu C, et al. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. Proc Natl Acad Sci USA 2014;111(34):12516-21

53. Memish ZA, Mishra N, Olivai KJ, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis 2013;19(11):1819-23

54. Cai Y, Yu SQ, Postnikova EN, et al. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. Emerg Infect Dis 2013;19(3):456-9

55. Cotten M, Watson SJ, Kellam P, et al. Coronaviruses in bats. J Gen Virol 2013;94(Pt 5):1028-38

56. Anthony SJ, Ojeda-Flores R, Rico-Chavez O, et al. Coronaviruses in bats, Mexico. J Gen Virol 2013;94(Pt 5):1028-38

57. Yang L, Wu Z, Ren X, et al. MERS-related RNA synthesis reveals potent inhibition of diverse coronaviruses including the Middle East respiratory syndrome virus. PLoS Pathog 2014;10(5):e1004166

58. Cai Y, Yu SQ, Postnikova EN, et al. CD26/DPP4 cell-surface expression in bat cells correlates with bat cell susceptibility to Middle East respiratory syndrome coronavirus (MERS-CoV) infection and evolution of persistent infection. PLoS One 2014;9(11):e112060

59. Chastel C, [Middle East respiratory syndrome (MERS): bats or dromedary, which of them is responsible?]. Bull Soc Pathol Exot 2014;107(2):69-73

60. Kapoor M, Pringle K, Kumar A, et al. Clinical and laboratory findings of the first imported case of Middle East respiratory syndrome coronavirus to the United States. Clin Infect Dis 2014;59(11):1511-18

61. Corman VM, Rasche A, Dallal TD, et al. Highly diversified coronaviruses in neotropical bats. J Gen Virol 2013;94(Pt 9):1984-94

62. Lau SK, Poon RW, Wong BH, et al. Coexistence of different genotypes in the same bat and serological characterization of Rousettus bat coronavirus HKU9 belonging to a novel Betacoronavirus subgroup. J Virol 2010;84(21):11385-94

63. Corman VM, Watson SJ, Kellam P, et al. Middle East respiratory syndrome coronavirus in bats. J Gen Virol 2013;94(Pt 9):1984-94

64. Corman VM, Watson SJ, Kellam P, et al. Transmission and evolution of the Middle East respiratory syndrome coronavirus in Saudi Arabia: a descriptive genomic study. Lancet 2013;382(9909):1993-2002

65. Yang Y, Du L, Liu C, et al. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. Proc Natl Acad Sci USA 2014;111(34):12516-21

66. Memish ZA, Mishra N, Olivai KJ, et al. Middle East respiratory syndrome coronavirus in bats, Saudi Arabia. Emerg Infect Dis 2013;19(11):1819-23

67. Cai Y, Yu SQ, Postnikova EN, et al. CD26/DPP4 cell-surface expression in bat cells correlates with bat cell susceptibility to Middle East respiratory syndrome coronavirus (MERS-CoV) infection and evolution of persistent infection. PLoS One 2014;9(11):e112060

68. Chastel C, [Middle East respiratory syndrome (MERS): bats or dromedary, which of them is responsible?]. Bull Soc Pathol Exot 2014;107(2):69-73

69. Kapoor M, Pringle K, Kumar A, et al. Clinical and laboratory findings of the first imported case of Middle East respiratory syndrome coronavirus to the United States. Clin Infect Dis 2014;59(11):1511-18

70. Drosten C, Seilmaier M, Corman VM, et al. Clinical features and virological analysis of a case of Middle East respiratory syndrome coronavirus infection. Lancet Infect Dis 2013;13(9):745-51

71. Lundin A, Dijkman R, Bergstrom T, et al. Targeting membrane-bound viral RNA synthesis reveals potent inhibition of diverse coronaviruses including the Middle East respiratory syndrome virus. PLoS Pathog 2014;10(5):e1004166

72. Falzarano D, de Wit E, Rasmussen AL, et al. Treatment with interferon-alpha2b and ribavirin improves outcome in...
MERS-CoV-infected rhesus macaques. Nat Med 2013;19(10):1313-17

73. Shirato K, Kawase M, Matsuyama S. Middle East respiratory syndrome coronavirus infection mediated by the transmembrane serine protease TMRPSS2. J Virol 2013;87(23):12552-61

74. Achoua C, Laporte A, Gardam MA. The financial impact of controlling a respiratory virus outbreak in a teaching hospital: lessons learned from SARS. Can J Public Health 2005;96(1):52-4

75. Yadanapanyah Y, Daval A, Alfandari S, et al. Analysis of costs attributable to an outbreak of severe acute respiratory syndrome at a French hospital. Infect Control Hosp Epidemiol 2006;27(11):1282-5

76. Lee J, McKibbin W. Estimating the global costs of SARS. In: Knobler S, Mahmoud A, Lemon S, et al. editors. Learning from SARS: preparing for the next disease outbreak: workshop summary. National Academies Press; Washington, DC: 2004. Available from: www.ncbi.nlm.nih.gov/books/NBK92473/ [Last accessed 31 October 2014]

77. Biylek SR, Allen D, Alvarado-Ramy F, et al. First confirmed cases of Middle East respiratory syndrome coronavirus (MERS-CoV) infection in the United States, updated information on the epidemiology of MERS-CoV infection, and guidance for the public, clinicians, and public health authorities - May 2014. MMWR Morb Mortal Wkly Rep 2014;63(19):431-6

78. International Committee on Taxonomy of Viruses. The international code of virus classification and nomenclature. In: King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy - classification and nomenclature. In: Lemon S, et al. editors. Learning from SARS: preparing for the next disease outbreak? PLoS One 2014;9(7), (2015)

79. Qian Z, Dominguez SR, Holmes KV. Role of the spike glycoprotein of human Middle East respiratory syndrome coronavirus (MERS-CoV) in virus entry and syncytia formation. PLoS One 2013;8(10):e76469

80. Du L, Kou Z, Ma C, et al. A truncated receptor-binding domain of MERS-CoV spike protein potently inhibits MERS-CoV infection and induces strong neutralizing antibody responses: implication for developing therapeutics and vaccines. PLoS One 2013;8(12):e81587

81. Du L, Zhao G, Kou Z, et al. Identification of a receptor-binding domain in the S protein of the novel human coronavirus Middle East respiratory syndrome coronavirus as an essential target for vaccine development. J Virol 2013;87(17):9939-42

82. Chan KH, Chan JF, Tse H, et al. Cross-reactive antibodies in convalescent SARS patients' sera against the emerging novel human coronavirus EMC (2012) by both immunofluorescent and neutralizing antibody tests. J Infect 2013;67(2):130-40

83. Zhang N, Jiang S, Du L. Current advancements and potential strategies in the development of MERS-CoV vaccines. Expert Rev Vaccines 2014;13(6):761-74

84. Review of MERS vaccine development with a focus on the receptor-binding domain as a vaccine target. Centers for Disease Control and Prevention. Human rabies. 2014. Available from: www.cdc.gov/rabies/location/usa/surveillance/human_rabies.html [Last accessed on 10 December 2104]

85. Burtle MM, Wedlock DN, Denis M. Progress in the development of tuberculoses vaccines for cattle and wildlife. Vet Microbiol 2006;112(2-4):191-200

86. Beltran-Beck B, Ballesteros C, Vicente J, et al. Progress in oral vaccination against tuberculosis in its main wildlife reservoir in Iberia, the Eurasian wild boar. Vet Med Int 2012;2012:978501

87. Al-Gethamy M, Corman VM, Hussain R, et al. Cross-reactive antibodies in convalescent MERS S and its use as a vaccine target. Al-Gethamy M, Corman VM, Hussain R, et al. A case of long-term excretion and subclinical infection with Middle East Respiratory Syndrome Coronavirus in a healthcare worker. Clin Infect Dis 2015; 60(6):973-4

88. Faure E, Poissy J, Goffard A, et al. Immunity to viruses: learning from SARS: preparing for the next disease outbreak? PLoS One 2014;9(2):e88716

89. Zhao J, Li K, Wohlford-Lenane C, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc Natl Acad Sci USA 2014;111(13):4970-5

90. Pulendran B, Oh JZ, Nakaya HI, et al. Cross-reactive antibodies in convalescent MERS S and its use as a vaccine target. Pulendran B, Oh JZ, Nakaya HI, et al. Immunity to viruses: learning from SARS: preparing for the next disease outbreak? PLoS One 2014;9(2):e88716

91. Zhao J, Li K, Wohlford-Lenane C, et al. Cross-reactive antibodies in convalescent MERS S and its use as a vaccine target. Zhao J, Li K, Wohlford-Lenane C, et al. A case of long-term excretion and subclinical infection with Middle East Respiratory Syndrome Coronavirus in a healthcare worker. Clin Infect Dis 2015; 60(6):973-4

92. Faure E, Poissy J, Goffard A, et al. Immunity to viruses: learning from SARS: preparing for the next disease outbreak? PLoS One 2014;9(2):e88716
104. Bolles M, Deming D, Long K, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol 2011;85(23):12201-15

105. Yaqub O, Castle-Clarke S, Sevdalis N, Chataway J. Attitudes to vaccination: a critical review. Soc Sci Med 2014;112: 1-11

106. Dube E, Gagnon D, Nichols E, et al. Mapping vaccine hesitancy – country-specific characteristics of a global phenomenon. Vaccine 2014;32(49):6649-54

107. Appaiahgari MB, Vrati S. Adenoviruses as gene/vaccine delivery vectors: promises and pitfalls. Expert Opin Biol Ther 2015;15(3): 337-51

108. Lichy BD, Power AT, Stojdl DF, Bell JC. Vesicular stomatitis virus: re-inventing the bullet. Trends Mol Med 2004;10(5):210-16

109. Altenburg AF, Kreijtz JH, de Vries RD, Lichty BD, Power AT, Stojdl DF, Bell JC. Antibody quality and protection from lethal Middle East respiratory syndrome coronavirus vaccine in BALB/c mice. Vaccine 2014;32(26):3169-74

110. Poon LL, Leung YH, Nicholls JM, et al. Vaccinia virus-based multivalent H5N1 avian influenza vaccines adjuvanted with IL-15 confer sterile cross-clade protection in mice. J Immunol 2009;182(5):3063-71

111. Song F, Fux R, Provacia LB, et al. Middle East respiratory syndrome coronavirus spike protein delivered by modified vaccinia virus Ankara efficiently induces virus-neutralizing antibodies. J Virol 2013;87(21):11950-4

112. Trivedi S, Jackson RJ, Ranasinghe C. Different HIV pox viral vector-based vaccines and adjuvants can induce unique antigen presenting cells that modulate CD8+ T cell avidity. Virology 2014;468-470: 479-89

113. Blaney JE, Mazi A, Willet M, et al. Antibody quality and protection from lethal Ebola virus challenge in nonhuman primates immunized with rabies virus based bivalent vaccine. PLoS Pathog 2013;9(5):e1003389

114. Papaneri AB, Wirilich C, Cooper K, et al. Further characterization of the immune response in mice to inactivated and live rabies vaccines expressing Ebola virus glycoprotein. Vaccine 2012;30(43):6136-41

115. Escioni N, Callender B, Lorin V, et al. Protection from SARS coronavirus conferred by live measles vaccine expressing the spike glycoprotein. Virology 2014;452-453:32-41

116. Kim E, Okada K, Kenniston T, et al. Immunogenicity of an adenoviral-based Middle East Respiratory Syndrome coronavirus vaccine in BALB/c mice. Vaccine 2014;32(45):5975-82

117. Coleman CM, Liu YV, Mu H, et al. Purified coronavirus spike protein nanoparticles induce coronavirus neutralizing antibodies in mice. Vaccine 2014;32(26):3169-74

118. Hutnick NA, Myles DJ, Bian CB, et al. Selected approaches for increasing HIV DNA vaccine immunogenicity in vivo. Curr Opin Virol 2011;1(4):233-40

119. Health Promotion Board, Singapore. FAQs on human papilloma virus (HPV) and HPV vaccination. 2014. Available from: www.hpb.gov.sg/HOPPortal/health-article/8768 [Last accessed on 2 December 2014]

120. European Centre for Disease Prevention and Control. Vaccine schedule: recommended immunisations for human papillomavirus infection. Available from: http://vaccine-schedule.cdc.europa.eu/Pages/Scheduler.aspx [Last accessed on 2 December 2014]

121. Cancer Council Australia. The HPV vaccine. Available from: www.hpvvaccine.org.au/about-the-vaccine/vaccine-background.aspx [Last accessed 2 December 2014]

122. Ladner J, Besson MH, Rodrigues M, et al. Performance of 21 HPV vaccination programs implemented in low and middle-income countries, 2009-2013. BMC Public Health 2014;14:670

123. Ladner J, Besson MH, Hampshire R, et al. Assessment of eight HPV vaccination programs implemented in lowest income countries. BMC Public Health 2012;12:370

124. Available from: http://ir.inovio.com/news/news-releases/news-releases-details/2013/Inovio-Pharmaceuticals-DNA-Vaccine-for-the-Deadly-MERS-Virus-Induces-Robust-Immune-Response-in-Preclinical-Trial/default.aspx

125. Sharron R, Islam AB. A highly conserved WDYPKCDRA epitope in the RNA directed RNA polymerase of human coronaviruses can be used as epitope-based universal vaccine design. BMC Bioinformatics 2014;15:161

126. Weiskopf D, Angelo MA, Bangs DJ, et al. The human CD8+ T cell responses induced by a live attenuated tetravalent dengue vaccine are directed against highly conserved epitopes. J Virol 2015;89(1):120-8

127. Slfka MK, Amanna I. How advances in immunology provide insight into improving vaccine efficacy. Vaccine 2014;32(25): 2948-57

128. Oany AR, Emran AA, Jyoti TP. Design of an epitope-based peptide vaccine against spike protein of human coronavirus: an in silico approach. Drug Des Devel Ther 2014;8:1139-49

129. Terry FE, Moise L, Martin RF, et al. Time for T? Immunoinformatics addresses vaccine design for neglected tropical and emerging infectious diseases. Expert Rev Vaccines 2015;14(1):21-35

• Evaluation of the role of immunoinformatics in improving vaccine development for emerging infectious diseases.

130. Food and Drug Administration. Guidance for industry: product development under the animal rule. Draft guidance. 2014. Available from: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM399217.pdf [Last accessed 15 December 2014]

131. Raj VS, Smits SL, Provacia LB, et al. Adenovirus dmanniase as a natural antagonist for dipeptidyl peptidase 4-mediated entry of the Middle East respiratory syndrome coronavirus. J Virol 2014;88(3):1834-8

132. de Wit E, Rasmussen AL, Falzarano D, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. Proc Natl Acad Sci USA 2013;110(41):16598-603

133. Yao Y, Bao L, Deng W, et al. An animal model of MERS produced by infection of rhesus macaques with MERS coronavirus. J Infect Dis 2014;209(2):236-42

134. Munster VJ, de Wit E, Feldmann H. Pneumonia from human coronavirus in a macaque model. N Engl J Med 2013;368(16):1560-2

135. Falzarano D, de Wit E, Feldmann F, et al. Infection with MERS-CoV causes lethal pneumonia in the common marmoset. PloS Pathog 2014;10(8):e1004250

136. Graham RL, Donaldson EF, Baric RS. Making vaccines “on demand”: a potential solution for emerging pathogens and biodefense? Hum Vaccin Immunother 2014;39(9):1877-84

137. De Groot AS, Einck L, Moise L, et al. Immunoinformatics in improving vaccine development for emerging infectious diseases.

138. Fries LF, Smith GE, Glenn GM. A recombinant viruslike particle influenza
A (H7N9) vaccine. N Engl J Med 2013; 369(26):2564-6

139. Serdobova I, Kieny MP. Assembling a global vaccine development pipeline for infectious diseases in the developing world. Am J Public Health 2006;96(9):1554-9

140. Kilianski A, Mielech AM, Deng X, Baker SC. Assessing activity and inhibition of Middle East respiratory syndrome coronavirus papain-like and 3C-like proteases using luciferase-based biosensors. J Virol 2013;87(21):11955-62

141. Yang X, Chen X, Bian G, et al. Proteolytic processing, deubiquitinase and interferon antagonist activities of Middle East respiratory syndrome coronavirus papain-like protease. J Gen Virol 2014; 95(Pt 3):614-26

142. Scobey T, Yount BL, Sims AC, et al. Reverse genetics with a full-length infectious cDNA of the Middle East respiratory syndrome coronavirus. Proc Natl Acad Sci USA 2013;110(40):16157-62

143. Zhao G, Du L, Ma C, et al. A safe and convenient pseudovirus-based inhibition assay to detect neutralizing antibodies and screen for viral entry inhibitors against the novel human coronavirus MERS-CoV. Virol J 2013;10:266

144. Yang Y, Zhang L, Geng H, et al. The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. Protein Cell 2013;4(12):951-61

145. Niemeyer D, Zillinger T, Muth D, et al. Middle East respiratory syndrome coronavirus accessory protein 4a is a type I interferon antagonist. J Virol 2013;87(22):12489-95

146. Agnihothram S, Yount BL Jr, Donaldson EF, et al. A mouse model for betacoronavirus subgroup 2c using a bat coronavirus strain HKU5 variant. MBio 2014;5(2):e00047-14

147. Available from: http://kff.org/other/state-indicator/expenses-per-inpatient-day/#

148. Available from: www.economist.com/blogs/graphicdetail/2013/06/daily-chart-18