Research and Development Activities for
Middle East Respiratory Syndrome: The Current Landscape

Kayvon Modjarrad¹

¹U.S. Military HIV Research Program, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA.
kmmodjarrad@hivresearch.org

Keywords: Middle East Respiratory Syndrome, Coronavirus, Vaccines, Therapeutics, Diagnostics, Research, Development

Competing Interests and Disclosures:
The author is a co-inventor on a patent application for MERS-CoV vaccines and monoclonal antibodies developed by the US National Institutes of Health Vaccine Research Center. The author is also the Principal Investigator of an upcoming first-in-human Phase I clinical trial being conducted by the Walter Reed Army Institute of Research in collaboration with GeneOne LifeScience Inc. The opinions expressed herein are those of the authors and should not be construed as official or representing the views of the US Department of Defense or the Department of the Army.

Abstract
Middle East respiratory syndrome coronavirus (MERS-CoV), an emerging infectious disease of growing global importance, has caused severe acute respiratory disease in more than 1500 people, resulting in more 500 deaths. The high case fatality rate, growing geographic distribution and vaguely defined epidemiology have created an urgent need for effective public health countermeasures. Despite the relatively few number of cases to date, research and development of diagnostic, prophylactic and therapeutic products for MERS-CoV are advancing quickly. This review surveys the current landscape of these efforts and examines the gaps in knowledge and technical capacity that will have to be overcome in order to control the resilient MERS-CoV epidemic.
Middle East Respiratory Syndrome (MERS-CoV) was first isolated in September 2012 from a patient in Saudi Arabia who presented two months earlier with severe acute respiratory infection and acute renal failure (1). Retrospective testing of samples in Jordan identified earlier cases from a nosocomial outbreak in April 2012 (2). Although the majority of MERS-CoV cases (~85%) have occurred in Saudi Arabia, 25 other countries have confirmed imported or autochthonous cases (Figure 1) (3, 4). The most recent and largest outbreak outside of Saudi Arabia occurred in South Korea in May 2015 (5), raising concern for an eruption of regional outbreaks or accelerated global spread, similar to the phylogenetically related severe acute respiratory syndrome coronavirus (SARS-CoV) that killed nearly a thousand people a decade earlier (6). Although the definitive host for MERS-CoV has not yet been established, closely related coronaviruses have been isolated from bats across wide geographic areas (7-9). Mounting evidence has implicated dromedary camels as the intermediate animal reservoir, as serological surveys throughout the Middle East and North Africa have demonstrated them to have a high prevalence of MERS-CoV binding or neutralizing antibodies (10-13). Additionally, outbreak investigations have suggested, but not definitively confirmed, epidemiologic linkage between farm camels and human cases (14).

MERS-CoV is a spherical, enveloped, single-stranded, positive sense RNA beta-coronavirus (1, 15). Its genome contains a replicase locus at the 5’ end and codes for structural proteins toward the 3’ end. The most immunogenic of the viral proteins is Spike (S), a trimeric, envelope-anchored, type I fusion glycoprotein that interfaces with
its human host cognate receptor, dipeptidyl peptidase 4 (DPP4), to mediate viral entry (16, 17). S comprises two subunits: S1, which contains the receptor-binding domain and determines cell tropism; and S2, the location of the cell fusion machinery. Although DPP4 has a broad tissue distribution, most of the clinical manifestations of MERS-CoV can be attributed to its localization to the lower respiratory tract (18, 19). However, much like other coronaviruses, MERS-CoV can cause significant dysfunction of the gastrointestinal, cardiovascular, renal and neurologic systems. MERS-CoV is distinct, though, in its tendency to cause greatest harm to older individuals with concurrent comorbidities of one or more of these organ-systems (20, 21).

Figure 1. Geographic Distribution of Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) Confirmed Cases, 2012-2015

Shading corresponds to the last year a case was confirmed in that country. Red circles only indicate the number of cases confirmed in 2015. The number of cases before 2015 are not reflected in this map.

Despite past efforts to develop coronavirus countermeasures in response to the SARS-CoV pandemic, there are currently no prophylactic or therapeutic interventions of
proven efficacy for MERS-CoV or any other coronavirus infection. Although combination treatment with ribavirin and interferons were shown to improve clinical outcomes in MERS-CoV-infected non-human primates (NHPs), treatment was initiated very soon after viral challenge (~8 hours) and results have not been replicated in humans (22). In fact, no experimental interventions have demonstrated appreciable benefit in acutely ill patients in a consistent or controlled manner. Rapidly scaled treatments based on naturally occurring neutralizing antibodies such as convalescent plasma or hyperimmune globulin, on the other hand, have demonstrated mortality reductions for other respiratory infections and may hold promise for MERS-CoV as well (23). Their development, however, is limited by logistical challenges, local technical capacity and donor supply. A growing collection of humanized or human monoclonal antibodies are being investigated and developed for potential use as pre- or post-exposure prophylaxis in the setting of outbreaks; however, no product has advanced beyond pre-clinical testing. Likewise, nearly a dozen vaccine candidates have been demonstrated as safe and immunogenic in animal models but none have yet been evaluated in humans. Despite these ongoing efforts to develop MERS-CoV countermeasures, best infection control practices and supportive management—adapted from guidelines for other diseases—have remained the mainstay of MERS-CoV prevention and treatment.

**Diagnostics**

When a new or re-emergent pathogen causes a major outbreak, rapid and accurate diagnosis of cases becomes critical to developing an effective public health response. Without a timely assessment of case burden and distribution, informed decisions about
appropriate responses cannot be made, particularly those that relate to fielding new interventions or adapting existing ones. Currently, the standard case definition for MERS is based on laboratory confirmation (24), as the clinical presentation is not specific enough to be a reliable surrogate for documentation of viral infection. Although several serological and nucleic tests have been used over the course of the epidemic, there are no licensed commercial assays for the diagnosis of MERS-CoV infection. The US Centers for Disease Control and Prevention (CDC) has developed a real-time reverse transcription polymerase chain reaction (rRT-PCR) assay (RealStar®) that targets the nucleocapsid gene and has provided the platform to a number of governmental and non-governmental laboratories under emergency use authorization (25). Additional rRT-PCR assays have been developed that can be used in conjunction with the CDC test. The first and most widely used assays targeted the upstream E protein (upE) gene and open reading frame 1a (ORF1a) and 1b (ORF1b). Recently, the first external quality assessment of these different assays was performed, finding that ORF1b is less sensitive than the others and should not be used for primary screening (26). Additional nucleic acid amplification assays have since been developed that target other regions of the MERS-CoV genome (27, 28), but not have been compared to others in a monitored quality assessment program.

Nucleic acid tests are likely to have the highest yield if performed on lower respiratory tract specimens collected either by sputum induction, tracheal aspirate or bronchoalveolar lavage. Nasopharyngeal and oropharyngeal specimens can also yield positive results, though at viral loads in these compartments tend to be lower. PCR assays can also be carried out on serum samples, although viremia in MERS-CoV infection is uncommon and typically at low levels. Serum is best used, instead, for the detection of IgM and IgG antibodies by enzyme linked immunosorbent assay (ELISA), immunofluorescence assay
(IFA) or microneutralization assay. Antigen-capture assays are also being developed (29, 30), but none of these assays for MERS-CoV have yet been validated on a large scale. This is partly due to the absence of antibody reagents developed from infected survivors. The lack of validated serologic assays complicates the execution and interpretation of epidemiologic studies needed to define the reservoirs, transmission dynamics and correlates of protection and recovery from the virus.

**Vaccines**

The global will to develop a coronavirus vaccine faded in the aftermath of SARS-CoV pandemic but has since gained renewed momentum in the face of the current MERS-CoV outbreak. Previous approaches to the development of coronavirus vaccines were broad and included whole-inactivated and live-attenuated viruses, recombinant vectors and protein subunits, as well as DNA and RNA based platforms (6). Most developers based their immunogen designs on the S surface glycoprotein, the primary target for neutralizing antibodies during a natural coronavirus infection. A number of preclinical and clinical studies showed that the SARS-CoV S1 protein subunit, and specifically the RBD at its core, could serve as a dominant target for neutralizing antibodies in mice, nonhuman primates, and humans (31). S1, therefore, became the basis for a number of promising SARS-CoV vaccine candidates.
Figure 2. Active Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Vaccine Candidates in Development

Currently there are five general vaccine platforms in development for MERS-CoV. At the time of this report, all candidates are still in preclinical stages of development. S- Spike glycoprotein; Fc-crystallizable fraction of a human antibody; RBD- receptor binding domain of the Spike glycoprotein; Ad5- adenovirus serotype 5; Ad41- adenovirus serotype 41, MVA- modified vaccinia Ankara virus.

The S1 protein subunit and RBD have also been the basis for several MERS-CoV vaccine candidates (32-36) (Figure 2). Resolution of RBD crystal structures alone or in complex with the DPP4 receptor (32, 37, 38) have informed the design of immunogens that have been expressed either as recombinant protein fragments or conjugates to the fragment crystallizable (Fc) region of human antibodies. Both types of constructs, in formulation with aluminum salt or oil-in-water adjuvants, have elicited neutralizing antibodies of high potency across multiple viral strains. Despite their demonstrated immunogenicity in animal models and anticipated safety in humans, RBD or S1-subunit based vaccine candidates are limited in their epitope breadth. Although the coronavirus genomes are not as variable as other RNA viruses, the RBD is the most mutable region, containing mutation sites that define antibody escape variants (32, 39). Thus, vaccine
candidates that elicit a larger antibody repertoire as well as a robust cellular immune response may offer the advantage of broader and more durable protection.

Full-length S used as an immunogen could at least increase the breadth of the antibody response; however, it has been difficult to express stably. Some groups have tried to overcome this problem through the development of S micellar nanoparticles that are expressed stably and have demonstrated immunogenicity in murine models (40). Vaccines that mimic natural infection, such as live-attenuated viruses or recombinant viral vectors, may elicit even more robust immunity. Live attenuated viruses have historically been among the most immunogenic platforms available, as they have the capacity to present multiple antigens across the viral life cycle in their native conformations. Although a live-attenuated MERS-CoV has yet to be tested, one has been successfully constructed and has the potential be protective (41). Live attenuated viruses carry the hazard of reverting to wild type and causing disseminated disease, particularly in immunocompromised hosts. Given that moderately immunocompromised adults with co-morbidities such as diabetes mellitus and chronic kidney disease have suffered the most severe MERS-CoV disease, these individuals may comprise a target population for immunization, thus making a live attenuated virus vaccine a less viable option. Replication competent viral vectors could pose a similar threat for disseminated disease in the immunosuppressed. Replication deficient vectors, however, avoid that risk while maintaining the advantages of native antigen presentation, elicitation of T cell immunity and the ability to express multiple antigens. To date, two recombinant vectors—modified vaccinia virus Ankara (MVA) and adenovirus vectors—have been
used to express MERS-CoV S glycoprotein. Both were immunogenic and one of the MVA candidates was protective in a mouse model transduced with human DPP4 (42-45).

Although replication deficient vectors are relatively safe and immunogenic, their ability to deliver genetic material for expression could be impeded by pre-existing or developing immunity to the vector itself. One way to overcome this limitation is by administering different vectors in a so-called prime-boost immunization regimen. As this strategy has been effective for other pathogens, it is likely that the same success could be recapitulated for MERS-CoV. The use of more than one type of platform or antigen in a single vaccine also increases the likelihood of inducing a broad repertoire of antibodies with diverse mechanisms of viral neutralization. One heterologous vaccine regimen is based on full-length S DNA and a truncated S1 subunit glycoprotein and has elicited neutralizing antibodies in mice directed at both the S1—within and outside the RBD—and S2 subunits. Immunization with these constructs also protected NHPs from severe lung disease after intra-tracheal challenge with MERS-CoV(32). A DNA-only vaccine, expressing multiple antigens, has also been shown to be immunogenic in different animals and protective in non-human primates (46).

**Monoclonal antibodies (mAbs)**

Although vaccine candidates are being developed for prophylactic use, the total number of cases (~1500)(3, 4) and reproductive rate (~0.7) of MERS-CoV are both relatively low (47); thus it will be difficult to define target populations for vaccination. Human mAbs, on the other hand, could be used without as much discrimination in an outbreak setting for
post-exposure prophylaxis and early treatment. The advantages of mAbs over polyclonal antibodies (administered through convalescent plasma or hyperimmune globulin) are their higher potency and greater specificity. Additionally, mAbs can help define immunogenic epitopes through crystallographic analysis, thereby providing atomic level detail for the design of better immunogens. However, the timeline and costs for mAb development are respectively longer and higher.

Despite requirements for greater upfront investment, several groups have developed highly potent mAbs that are currently being advanced through pre-clinical stages of testing (Table 1). Some have been isolated from immunized animals (mice/humanized mice/NHPs) (32, 48), while others have been identified from either an antibody human phage library (49-52) or memory B cells of infected and recovered human survivors (53) (B. Graham, personal communication). Almost all of the mAbs that have been reported target the Spike RBD. It is likely that mAbs directed at other sites on the Spike glycoprotein have been recovered but are not as potent neutralizers. A number of those that have been published bind to recombinant Spike with picomolar affinity and neutralize MERS-CoV pseudovirus at a half maximal inhibitory concentration (IC$_{50}$) of 10ng/µl or less. Additionally, some have demonstrated protective efficacy in pre- and post-exposure prophylaxis animal models (52, 53). The successes thus far in isolating potent and protective mAbs, however, is likely to be tempered by the challenges in advancing these products to licensing and full scale production at affordable costs for as of yet undefined populations.
<table>
<thead>
<tr>
<th>Product Name</th>
<th>Origin</th>
<th>Antigen Target</th>
<th>Potency (Pseudovirus)</th>
<th>Potency (Live virus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REGN3051</td>
<td>humanized mouse</td>
<td>RBD</td>
<td>0.0097</td>
<td>0.069</td>
</tr>
<tr>
<td>REGN3048</td>
<td>humanized mouse</td>
<td>RBD</td>
<td>0.0105</td>
<td>0.027</td>
</tr>
<tr>
<td>MERS-4</td>
<td>human antibody library</td>
<td>RBD</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>MERS-27</td>
<td>human antibody library</td>
<td>RBD</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>3B12</td>
<td>human antibody library</td>
<td>RBD</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>MERS-4</td>
<td>human antibody library</td>
<td>RBD</td>
<td>0.13</td>
<td>3.3nM</td>
</tr>
<tr>
<td>MERS-27</td>
<td>human antibody library</td>
<td>RBD</td>
<td>64nM</td>
<td>13.3nM</td>
</tr>
<tr>
<td>3B12</td>
<td>human antibody library</td>
<td>RBD</td>
<td>&gt; 1.25</td>
<td>----</td>
</tr>
<tr>
<td>m336</td>
<td>human antibody library</td>
<td>RBD</td>
<td>0.005</td>
<td>0.07</td>
</tr>
<tr>
<td>Mersmab1</td>
<td>S1 immunized mouse</td>
<td>RBD</td>
<td>0.12</td>
<td>----</td>
</tr>
<tr>
<td>F11</td>
<td>S/S1 immunized mouse</td>
<td>RBD</td>
<td>0.008</td>
<td>0.052</td>
</tr>
<tr>
<td>D12</td>
<td>S/S1 immunized mouse</td>
<td>RBD</td>
<td>0.013</td>
<td>0.04</td>
</tr>
<tr>
<td>G2</td>
<td>S/S1 immunized mouse</td>
<td>S1</td>
<td>0.013</td>
<td>0.35</td>
</tr>
<tr>
<td>G4</td>
<td>S/S1 immunized mouse</td>
<td>S2</td>
<td>0.133</td>
<td>0.806</td>
</tr>
<tr>
<td>LCA60</td>
<td>human survivor</td>
<td>RBD</td>
<td>0.10</td>
<td>----</td>
</tr>
</tbody>
</table>

**Table 1. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) monoclonal antibodies (mAbs) in development**

*MERS-CoV neutralizing mAbs isolated from different sources are presented. 50% and 80% maximal inhibitory concentrations (IC$_{50}$ & IC$_{80}$) are presented when available, as measured by a pseudovirus or live virus neutralization assay. mAbs were isolated either from immunized mice, humanized mice, naive human antibody phage display libraries, or in one case from the blood of a MERS-CoV survivor.*
Antiviral Drugs

Although intensive, supportive care serves as the primary treatment option for MERS, antiviral therapies are being actively investigated for use in severely ill patients. For non-immune based therapies, there are two main pathways of drug discovery: 1) repurposing of licensed medications; 2) *de novo* development. As the life cycle and genetic sequence of this new coronavirus has become better elucidated, the rational design and development of novel and approved agents with potent anti-viral activity have become possible. The same was true for SARS-CoV, at least initially. As the SARS-CoV pandemic waned, however, large scale toxicity and efficacy screens for clinical advancement were abandoned in favor of other competing priorities. The impact of this decline in investments in coronavirus research could be seen in the repeat use of ribavirin, steroid, and interferon (α2a, α2b, β1b) therapies early in the MERS-CoV epidemic, even though they had been shown to be of little benefit against SARS-CoV.

Although *in vitro* and animal data have demonstrated some improvement in activity against MERS-CoV compared to SARS-CoV, the clinical efficacy of these regimens—inferred from observational studies—has been equivocal (54, 55).

The advent of high-throughput screens of licensed compounds and small molecules has allowed researchers to efficiently assess large libraries of drugs for their *in vitro* anti-viral activity against novel targets (56). Using slightly different screening technologies, different groups have converged on some common classes of compounds, including nucleoside analogs, antibacterial protein synthesis inhibitors, anti-metabolites and anti-protozoal agents (57-59) Kinase signaling, which has been shown to influence the life
cycle of several virus classes, has also proven from drug screens to be important for coronavirus replication. Mycophenolic acid, an inhibitor of both T an B lymphocytes, has also been found to have strong activity against MERS-CoV, as it does against other RNA viruses such as West Nile, hepatitis C and dengue. To date, several dozen licensed compounds have been reported to inhibit MERS-CoV replication; however, none have yet been tested in humans (57-60).

There are fewer new antivirals for MERS-CoV, but they may be further along in development. One in particular, GS-5734 a nucleotide analog that is being developed by Gilead Sciences, Inc., has shown survival benefit in NHPs inoculated with Ebola virus and has claimed to have *in vitro* activity against MERS-CoV as well (61). Similarly, BCX4430 is a nucleoside analog that is being developed by Biocryst Pharmaceuticals, Inc. for potential treatment of filoviruses, coronaviruses and other RNA viruses (62). Additionally, small interfering RNA molecules and peptide inhibitors are being investigated for their ability to disrupt MERS-CoV replication, though these products are still in very early phases of investigation. Given some of the common pathways of pathogenesis for RNA viruses, antivirals may be developed to have activity against more than one class of virus.

**Target populations**

The vaguely defined epidemiology of MERS-CoV has complicated the design and implementation of appropriate public health countermeasures. Most transmission events have occurred either in the setting of household clusters or nosocomial outbreaks (63-
It is also likely that virus has been introduced into human populations from a large zoonotic reservoir, i.e. dromedary camels. Given the broad distribution and ownership of camels in the Arabian peninsula where most cases have occurred, a targeted vaccine campaign may prove difficult. As the outbreak in the Republic of Korea revealed, patients and workers in the same healthcare facility as an infected patient are at high risk for secondary acquisition. An optimal strategy may be to use vaccines, monoclonal antibodies and antivirals in conjunction with stringent infection control practices in hospitals where MERS-CoV cases are being treated.

The possible epidemiologic link of MERS-CoV between bats, camels and humans presents an opportunity for a veterinary approach to interrupt the transmission cycle. A successful precedent for this so-called “OneHealth” approach toward mitigating human disease with a veterinary vaccine exists in the example of Equivac®, a Hendra virus vaccine developed solely for horses (69). Although Hendra virus is even rarer than MERS-CoV it is highly fatal with no treatment other than intensive supportive management. In 2012, a protein subunit vaccine was licensed and rolled-out in Australia, where all outbreaks of the virus have occurred. Since that time, the incidence in horses has fallen precipitously and no human cases have been detected (70). A similar strategy may be applicable to MERS-CoV; however, a veterinary vaccination in this context would be deployed solely for the sake of protecting humans, as the virus causes only mild upper respiratory illness in camels. Safety and reduction in viral shedding would have to be demonstrated in immunization, challenge and transmission
studies of camel or camelid populations, two of which are under way, but none yet published.

**Knowledge gaps**

One of the primary challenges to developing countermeasures to MERS-CoV is the lack of an appropriate animal model that recapitulates the natural history of severe human disease. Much of the difficulty originates from the absence of the virus’s cognate DPP4 receptor. One group has approached this problem by successfully transducing mice with an adenoviral vector expressing human DPP4 (71). Although more relevant than a standard murine model, transient transduction of the desired protein is potentially limited by inconsistent tissue expression. Agrawal et al. made an important advance with the development of a transgenic mouse model that demonstrated productive, disseminated MERS-CoV infection (72). Although rhesus macaques do not manifest full clinical disease they develop a transient lower respiratory infection that can be quantified and evaluated by computed tomography. Investigators at the NIH Rocky Mountain Laboratories (RML) are also developing a potentially lethal marmoset model that could be used for the evaluation of vaccines, mAbs and therapeutics (V. Munster, personal communication).

As MERS-CoV vaccines—both active and passive—are developed and tested, not only will more relevant animal models be required, but there will also be a need for a more detailed understanding of the epidemiology, immunology and pathogenesis of the virus. Although MERS-CoV still causes relatively few cases in a limited geographic
distribution, its high case fatality and sudden outbreak in Korea have proven it to be a pathogen of public health concern. The concentration of the epidemic to the Saudi Arabia also raises the specter of international spread every year during Hajj, one of the largest mass gathering events in the world. Ultimately, the development of a safe and effective vaccine for MERS-CoV may not yield its greatest benefit for the current epidemic but for the knowledge gained in creating a platform for combating coronaviruses as a whole.

**Summary**

The 2014-2015 Ebola epidemic in West Africa revealed both great potential and pernicious deficiencies within existing mechanisms for rapid medical product development. In the aftermath of the epidemic, the global health community coalesced around the realization that a multi-faceted plan was required to quickly and efficiently respond to the next outbreak. The World Health Organization is currently developing a blueprint by which that preparation and response can follow, with MERS-CoV highlighted as a case study. Although global coordination has resulted in the clinical advancement of some urgently needed, novel countermeasures for MERS-CoV, they will have to be developed along faster timelines than before, with greater investments earlier in the preclinical development pipeline that can generate products for more timely efficacy testing in affected populations. As the global community takes lessons from the most recent outbreak and prepares for the potential of another regional epidemic or broader pandemic, stakeholders in the area of medical countermeasure
research and development must set out a sound strategy now for where to best target their investments in anticipation of future outbreaks.

Acknowledgments

I thank Mr. Han-Jun Kim for assistance in preparing and formatting the tables and references; Dr. Linghsu Wang at the NIH Vaccine Research Center for providing the template from which Table 2 was adapted; Dr. Vincent Munster from the NIH Rocky Mountain Laboratories for helpful discussions; and Dr. Barney Graham for helpful valuable inputs and edits. I also thank the World Health Organization for the invitation to develop this summary and the US Military HIV Research Program and Walter Reed Army Institute for Research for its material and financial support.

References

60. DHHS, "Middle East Respiratory Syndrome Coronavirus: Status Update and Findings from April 2015 Medical Countermeasures Assessment," (2015).