Treatment strategies for Middle East respiratory syndrome coronavirus

Kayvon Modjarrad*

US Military HIV Research Program, Walter Reed Army Institute of Research, Bethesda, USA

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 1600 people, resulting in almost 600 deaths. The high case fatality rate, growing geographic distribution and vaguely defined epidemiology of this novel pathogen have created an urgent need for effective public health countermeasures, including safe and effective treatment strategies. Despite the relatively few numbers of cases to date, research and development of MERS-CoV therapeutic candidates is advancing quickly. This review surveys the landscape of these efforts and assesses their potential for use in affected populations.

Keywords: Middle East respiratory syndrome, coronavirus, therapeutics

Introduction

Respiratory tract infections are the leading cause of mortality in resource-limited settings, accounting for more than 4 million deaths each year globally [1]. Epidemic- and pandemic-prone respiratory viruses are the aetiological pathogens in many cases, and have caused several of the most prominent infectious disease outbreaks of the past two decades: these include H5N1 influenza in 1997, severe acute respiratory syndrome (SARS) in 2003 and pandemic H1N1 influenza in 2009. Most recently, Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a novel cause of severe acute respiratory illness after first being identified in a Saudi Arabian patient in 2012 [2]. Although initially restricted to the Arabian Peninsula, this emerging pathogen has respectively infected and killed more than 1600 and 580 people on four continents across 26 countries [3,4]. Phylogenetically related to SARS-CoV [5], MERS-CoV has a similar clinical presentation [6–9], albeit with a higher case fatality rate (~40% versus 10%) [3–5]. Dromedary camels serve as the principal animal reservoir for this virus; and zoonotic spillover from dromedaries to humans has, thus far, driven the course of the epidemic [10–18]. Although human-to-human transmission has been documented – particularly in the context of nosocomial outbreaks [19–24] – the spread of MERS-CoV is inefficient and unsustained, as reflected in an estimated reproduction rate of no higher than 0.7 [25,26].

MERS-CoV is an enveloped, single-stranded, positive-sense RNA virus that comprises a 30-kilobase genome that codes for four structural proteins and an RNA polymerase [27], typical of the Coronaviridae family (Figure 1). The most immunogenic of these proteins is the virus' only surface glycoprotein, Spike (S)[28–30] that mediates viral attachment and fusion via the host cognate receptor, dipeptidyl peptidase 4 (DPP4) [31]. Although the broad principles of the virus' life cycle and its mechanisms of pathogenesis are beginning to be understood, this knowledge has not yet translated to a licensed therapy or vaccine. Much of the work to develop safe and effective MERS-CoV countermeasures has centred on vaccines, but the relatively low prevalence of the disease, the sporadic nature of the case clusters and the dearth of detailed knowledge on chains of transmission highlight the need for greater investments into the discovery of effective therapeutic and secondary prophylactic regimens for infected and exposed individuals.
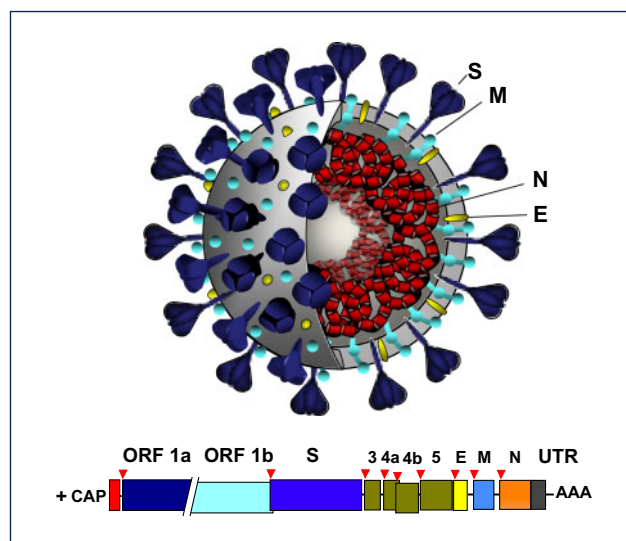
*Corresponding author: Kayvon Modjarrad, Military HIV Research Program, Walter Reed Army Institute of Research, 6720A Rockledge Drive, Suite 400, Bethesda, MD 20817, USA
Email: kmodjarrad@hivresearch.org

Figure 1. Figure 1. MERS-CoV structure and genomic organisation. Coronaviruses, such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) coronaviruses (CoV), are enveloped viruses that contain a single-stranded, positive-sense RNA genome. In the case of MERS-CoV the virion particle is approximately 120–160 nm in diameter and contains a genome of 30 kilobases in length that codes for four structural proteins (S: Spike, M: Matrix, N: Nucleocapsid, E: Envelope, ORF: Open reading frame, UTR: Untranslated region) and 16 non-structural proteins and two viral proteases (not shown here). (Adapted with permission from Luis Enjuanes, National Center of Biotechnology, Campus Universidad Autónoma de Madrid)

Efforts to research and develop treatment strategies for MERS-CoV are accelerating but remain limited in their scope and stage of advancement. There are few novel compounds being studied that are specific for MERS-CoV molecular targets, as most treatment options, investigational and licensed, are being repurposed from their use for other RNA viruses or other non-infectious diseases. The current landscape of MERS-CoV therapies, therefore, is dominated by an armamentarium of repositioned drugs with *in vitro* activity against MERS-CoV replication, but is also speckled with agents that are directed towards and derived from host immunity. The current review surveys the landscape of therapeutic products in each category and assesses their potential for advanced testing and development.

Host-directed/-derived therapies

Despite past efforts to develop coronavirus countermeasures, there are still no licensed therapies of proven efficacy for MERS-CoV or any other coronavirus infection. Supportive measures remain the mainstay of MERS-CoV treatment strategies and include

respiratory and circulatory support, preservation of renal, hepatic and neurological function, and prevention of secondary infections. Beyond implementing basic principles of critical care medicine, immune-based therapies have been used most commonly during both the SARS-CoV pandemic of 2003 and the current MERS-CoV epidemic, each time yielding equivocal results. There have been some promising animal data where combination treatment with ribavirin and interferon (IFN)-α2b improved clinical outcomes in MERS-CoV-infected non-human primates (NHPs). However, treatment was initiated very soon after viral challenge (~8 hours), a window that is unlikely to be replicated in a real-world clinical setting [32].

Various IFN regimens, in combination with ribavirin, have been intermittently administered to severely ill patients, although typically in an *ad hoc* manner and in the absence of systematic evaluation [33–37]. Individual case reports and uncontrolled case series not only limit determination of whether an intervention works but if it is safe as well. Ribavirin, for example, is a potent nucleoside analogue that has been used with varying measures of success against a range of RNA viruses [38]. However, patients can experience significant toxicities when given the drug alone or in combination with an interferon, including but not limited to haemolytic anaemia and metabolic abnormalities. Interferons also can elicit systemic adverse effects, psychiatric disturbances and neutropenia [39]. Thus, without the benefit of randomised controlled trial data, it becomes difficult to assess whether the treatment is worse than the disease. Certain strategies, however, have been shown to worsen clinical outcomes in the setting of a coronavirus infection. For example, studies during the SARS pandemic showed that corticosteroids, when used early on SARS-CoV infected patients, significantly increased viral load, ICU admission and mortality [40,41]. The role for interferon therapies has been less clear in the current MERS-CoV epidemic, as some data show a positive impact on proximate outcomes, such as oxygenation and inflammation, but no effect on more significant outcomes like hospital stay and long-term survival [35,36,42].

Rapidly scaled treatments based on naturally occurring neutralising antibodies such as convalescent plasma or hyperimmune globulin, on the other hand, have been shown to be relatively safe and potentially effective for reducing mortality from several infections such as SARS-CoV and influenza [43–45], and may hold promise for MERS-CoV as well. This strategy, however, relies on the rapid identification of cases and contacts and immediate deployment of products to have maximal impact. One study found that convalescent plasma decreased mortality in SARS-CoV patients only if administered within 14 days of illness [44]. A network for the use of convalescent plasma for case clusters of MERS-CoV

is currently being assembled [43] to test its safety, efficacy and feasibility. However, actualisation of this plan is limited by logistical challenges, local technical capacity and donor supply. Unfortunately, no host-derived experimental interventions have yet demonstrated appreciable benefit in acutely ill, MERS-CoV-infected patients in a consistent or controlled manner. This reality, although, has not slowed down the discovery and advancement of passive prophylactic products derived from vaccinated and infected animals and humans.

Monoclonal antibodies (mAbs)

Despite intensive efforts to develop a MERS-CoV vaccine, the prevalence and transmissibility of this emerging pathogen are both relatively low [3,26], making it difficult to define a target population for vaccination. mAbs, on the other hand, can be administered in the setting of an outbreak without the need to discriminate who might be at greatest risk for infection. They can be used to treat cases early in their natural history and for post-exposure prophylaxis of case contacts. mAbs also carry the benefits of higher potency, greater specificity, more extensive pre-licensing evaluation and consequently a more vetted safety profile. Additionally, mAbs can help define immunogenic epitopes through crystallographic analysis, thereby providing atomic-level detail for the design of better immunogens. They also have been proven as effective therapies in the areas of cancer treatment and autoimmune disease management. Although there is only one pathogen, respiratory syncytial virus, for which a mAb is licensed for use, there are a number of other infectious disease indications—such as Ebola virus disease treatment and human immunodeficiency virus primary and secondary prevention—for which mAbs are being tested in advanced phase clinical trials (www.clinicaltrials.gov). Despite all of these advantages, the timelines and costs of mAb research and development (R&D) are respectively longer and higher than that for polyclonal antibody preparations.

In spite of the requirements for greater upfront investments and a more rigorous testing and approval process, several groups have identified highly potent MERS-CoV mAbs and are advancing them through preclinical stages of development (Table 1). Some have been isolated from immunised animals (mice/humanised mice/NHPs) [46–54], while others have been identified from either an antibody human phage library [55] or memory B cells of infected and recovered human survivors [56]. Almost all of the published mAbs and all of those in development target the S receptor-binding domain (RBD), which contains the most immunogenic epitopes on the virus. Many bind to the RBD, expressed both on a recombinant S and on the surface of live virus, with picomolar affinity and neutralise MERS-CoV at a half maximal inhibitory concentration (IC_{50}) of 10 ng/μL or less. Additionally, several groups have demonstrated

Table 1. Middle East Respiratory Syndrome Coronavirus (MERS-CoV) monoclonal antibodies (mAbs) in various stages of research and development (R&D). Several groups have identified monoclonal antibodies that have at least shown potent neutralisation against MERS-CoV and in some cases have protected transgenic mice and non-human primates (NHPs) from MERS-CoV disease after viral challenge

Institution	Name	Source	Target	R&D	Reference
Chinese Academy of Sciences	4C2, 2E6	RBD immunised mice	RBD	Mouse efficacy	[48]
Dana-Farber Cancer Institute and AbViro LLC	3B11 (AV-3)	Human antibody library	RBD	NHP efficacy	[55]
HUMABS BioMed	LCA60	Human survivor	RBD	Mouse/NHP efficacy	[56]
New York Blood Center	Mersmab1	S1 imunised mouse	RBD	*In vitro*	[46]
NIH National Cancer Institute	M336, m337, m338	Human antibody library	RBD	*In vitro*	[52]
NIH NIAID	D12, F11, G2, G4	S/S1 immunised mouse	RBD, S1, S2	NHP efficacy	[51]
Regeneron	REGN3048/REGN3051	Humanised mouse	RBD	Mouse/NHP efficacy	[49]
Tsinghua University	MERS-4, MERS-27	Human antibody library	RBD	*In vitro*	[47]

RBD: receptor binding domain; S: Spike glycoprotein; S1: Spike domain containing RBD; S2: Spike domain containing fusion machinery.

protective efficacy in pre- and post-exposure prophylaxis animal models. Because most of the antibodies target the RBD, there is a potential for viral escape from any one mAb. Thus, there may be a need to develop antibodies against other vulnerable sites on S or to investigate the use of combination mAbs to overcome the potential emergence of therapeutic resistance. It is likely that mAbs directed at other sites on the S glycoprotein have already been recovered but are not as potent neutralisers, as is the case in one report [51]. A more efficient search for potent neutralising antibodies that target epitopes outside the RBD could be facilitated by a more detailed understanding of the atomic-level structure of the entire S glycoprotein, as has already been resolved for the RBD. The successes thus far in isolating potent and protective mAbs, although significant, are likely to be tempered by the challenges of advancing these products to licensing and full-scale production at affordable costs for as of yet undefined populations in a relatively short timeframe. Thus, mAbs should be advanced along a development pipeline in parallel with a program of rational drug design and discovery.

Antivirals

Although intensive, supportive care still serves as the primary treatment option for MERS-CoV and mAbs are the focus of the most advanced R&D efforts, antiviral therapies are being actively investigated for use in severely ill patients. There are two main pathways for drug discovery: *de novo* development and repurposing licensed medications. There are few new antivirals for MERS-CoV; however, the Ebola epidemic has had an unanticipated consequence of facilitating their development. One in particular, GS-5734 developed by Gilead Sciences, is an adenine analogue that is incorporated into viral RNA to disrupt replication [57]. It has shown survival benefit in NHPs inoculated with Ebola virus and is now advancing through a Phase I dose escalation trial. It has been claimed to have *in vitro* activity against MERS-CoV as well, but publication of these data is pending. Similarly, BCX4430 is a nucleoside analogue that is being developed by Biocryst Pharmaceuticals for potential treatment of filoviruses, coronaviruses and other RNA viruses [58]. Additionally, small interfering RNA molecules and peptide inhibitors are being investigated for their ability to disrupt MERS-CoV replication, although these products are still in very early phases of investigation [59,60].

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 antiviral activity have become possible. The advent of high-throughput screens of licensed compounds and small molecules has also allowed researchers to efficiently evaluate large libraries of drugs for their *in vitro* antiviral activity against novel targets [61–66]. To date, several dozen licensed drugs have been reported to inhibit MERS-CoV replication. Using slightly different screening technologies, different groups have converged on some common classes of compounds, including nucleoside analogues, antibacterial protein synthesis inhibitors, kinase signalling modifiers, antimetabolites and antiprotozoal agents.

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 [63]. Only one of the drugs to show *in vitro* activity against MERS-CoV, lopinavir, however, has been tested in an animal model. MERS-CoV-challenged marmosets that were treated with this protease inhibitor had better clinical, pathological, virological and radiological outcomes than controls or those treated with mycophenolate mofetil [67]. Additionally, two peptides, HR1P and HR2P are being developed as potential fusion inhibitors [59]. By acting on the six-helix bundle core of the MERS-CoV S protein

to prevent protein-mediated cell-to-cell fusion, this class of compounds may hold potential beyond MERS-CoV towards a long-term objective of a pan-coronavirus antiviral. Given some of the common life cycles and pathways of pathogenesis for RNA viruses and homologies in protein structures across different coronaviruses, there may be economies of effort and investment in developing antivirals that have activity against more than one virus or family of viruses. Irrespective of the breadth of these novel or repurposed compounds, treatment studies should be carried out prospectively according to protocols that plan for the collection of quality controlled data and serial biological sampling to assess viral evolution and biomarkers of favourable clinical outcomes.

Summary

Recent infectious disease outbreaks such as the 2009 H1N1 influenza pandemic, the H7N9 influenza epidemic in China, the Ebola crisis in West Africa and now the MERS-CoV outbreak have highlighted the need for better R&D preparedness and improved coordination of clinical testing in the face of the accelerating number of emerging and re-emerging infectious diseases. The ability to have an armamentarium of countermeasures and clinical trial infrastructure in the early phases of an outbreak is critical for mounting an effective public health campaign. For example, the SARS-CoV pandemic caused more than 8000 cases of severe acute respiratory illness and nearly 900 deaths but few prospective, controlled studies were undertaken to determine the optimal management of the disease. Consequently, treatment options for SARS-CoV were never defined clearly and thus difficult to adapt for MERS-CoV. Although global coordination has resulted in the 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 MERS-CoV R&D must set out a sound strategy now for where to best target their investments in anticipation of the changing dynamics of the current and future outbreaks.

Acknowledgements

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