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TITLE:
Coronaviruses — drug discovery and therapeutic options

ABSTRACT:
In humans, infections with the human coronavirus (HCoV) strains HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1 usually result in mild, self-limiting upper respiratory tract infections, such as the common cold. By contrast, the CoVs responsible for severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), which were discovered in Hong Kong, China, in 2003, and in Saudi Arabia in 2012, respectively, have received global attention over the past 12 years owing to their ability to cause community and health-care-associated outbreaks of severe infections in human populations. These two viruses pose major challenges to clinical management because there are no specific antiviral drugs available. In this Review, we summarize the epidemiology, virology, clinical features and current treatment strategies of SARS and MERS, and discuss the discovery and development of new virus-based and host-based therapeutic options for CoV infections. SUPPLEMENTARY INFORMATION: The online version of this article (doi:10.1038/nrd.2015.37) contains supplementary material, which is available to authorized users.

Main:
Coronaviruses (CoVs; subfamily Coronavirinae, family Coronaviridae, order Nidovirales) are a group of highly diverse, enveloped, positive-sense, single-stranded RNA viruses that cause respiratory, enteric, hepatic and neurological diseases of varying severity in a broad range of animal species, including humans1,2,3. CoVs are subdivided into four genera: Alphacoronavirus, Betacoronavirus (βCoV), Gammacoronavirus and Deltacoronavirus2,3,4,5,6,7. Over the past 12 years, two novel βCoVs, severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV), have emerged, and these viruses can cause severe human diseases8,9. The lack of effective drug treatment and associated high morbidity and mortality rates of these two CoVs as well as their potential to cause epidemics highlight the need for novel drug discovery for the treatment of CoV infections.

Epidemiology of SARS and MERS:
SARS. SARS-CoV emerged first in southern China and rapidly spread around the globe in 2002–2003 (Refs 8,10,11). In November 2002, an unusual epidemic of atypical pneumonia with a high rate of nosocomial transmission to health-care workers occurred in Foshan, Guangdong, China12,13. In March 2003, a novel CoV was confirmed to be the causative agent for SARS, and was thus named SARS-CoV14,15,16,17. A 64-year-old nephrologist who travelled from southern China to Hong Kong on 21 February 2003 became the index case of subsequent large community and health-care-associated outbreaks of SARS in Hong Kong and other regions10,11,18,19,20,21. The high infectivity of SARS was highlighted by the super-spreading event at a major teaching hospital in Hong Kong in which 138 people, including many previously healthy health-care workers, were infected within 2 weeks of exposure to an index patient who was being managed in a general medical ward for community-acquired pneumonia10,22. Through international air travel, SARS-CoV was spread to 29 countries and regions with a total of 8,098 cases and 774 fatalities (9.6% of cases) by the end of the epidemic in July 2003 (Ref. 23) (see Supplementary information S1 (figure, parts a,b)).
A retrospective serological survey suggested that cross-species transmission of SARS-CoV or its variants from animal species to humans might have occurred frequently in the wet market, where high seroprevalence was detected among asymptomatic animal handlers24. A close variant of SARS-CoV was isolated in palm civets in Dongmen market, Shenzhen, China, in 2003 (Ref. 25). During the small-scale SARS outbreaks in late 2003 and early 2004, three of the four patients had direct or indirect contact with palm civets26,27. However, viral genetic sequence analysis demonstrated that the SARS-CoV-like virus had not been circulating among masked palm civets in markets for a long time, and a serological study showed that only caged market civets and not wild civets were infected with the SARS-CoV-like virus28. CoVs that are highly similar to SARS-CoV have been isolated from Chinese horseshoe bats since 2005 (Refs 29,30,31,32). These SARS-like CoVs from bats share 88–95% sequence homology with human or civet CoV isolates, which suggests that bats were probably the natural reservoir of a close ancestor of SARS-CoV4,33,34.

MERS. The isolation of a novel βCoV from a patient in Jeddah, Saudi Arabia, who died of severe pneumonia and multi-organ failure in June 2012, was first reported in September 2012 (Ref. 35). Initially named 'human coronavirus Erasmus Medical Center', the virus was later renamed MERS-CoV by international consensus, and the disease was called Middle East respiratory syndrome (MERS)36. Retrospective analysis of a cluster of nosocomial cases in April 2012 in Jordan confirmed that MERS-CoV was also responsible for that outbreak37. Over the past 3 years, MERS-CoV has continued to spread within and beyond the Middle East, and there are ongoing reports of sporadic cases and community and health-care-associated clusters of infected individuals in the Middle East, especially in Saudi Arabia and the United Arab Emirates9,38. Travel-related cases and clusters have also been increasingly reported on other continents9. As of 9 October 2015, 1,593 laboratory-confirmed cases of MERS, including 568 deaths, have been reported to the World Health Organization39 (see Supplementary information S1 (figure, parts c,d)).

MERS-CoV is considered primarily to be a zoonotic virus that has the capability of non-sustained person-to-person spread9. Serological and virological studies have shown that camels and bats are the most likely animal reservoirs of MERS-CoV9,40,41,42,43,44,45,46,47. Although not all primary cases of MERS were individuals who had direct contact with camels, such exposure is considered to be an important factor for the spread of MERS-CoV, as evidenced by the substantially increased seroprevalence of anti-MERS-CoV antibodies among individuals with occupational exposure to camels, such as camel shepherds and slaughterhouse workers, relative to the general population in Saudi Arabia48,49. Person-to-person transmission of MERS-CoV has occurred in health-care facilities and family clusters50,51,52,53. The recent, large health-care-associated outbreaks in Jeddah and South Korea have been attributed to poor compliance with infection control measures54,55. Further studies are needed to fully understand the exact mode of transmission and other potential sources of MERS-CoV for optimization of treatment and prevention strategies for MERS56,57.

Clinical features of SARS and MERS. Patients with SARS or MERS present with various clinical features, ranging from asymptomatic or mild respiratory illness to fulminant severe acute respiratory disease with extrapulmonary manifestations8,9. Both diseases have predominantly respiratory manifestations, but extrapulmonary features may occur in severe cases56 (see Supplementary information S2 (table)). Notably, early treatment is especially important for patients with severe MERS because this disease progresses to respiratory distress, renal failure and death much more rapidly than SARS does. The three- to four-fold higher case-fatality rate of MERS relative to SARS may be related to the higher median age and prevalence of comorbidities in patients with MERS as well as the different pathogenesis of the two diseases9,58,59,60,61. Comorbidities associated with severe MERS include obesity, diabetes mellitus, systemic immunocompromising conditions and chronic cardiac and pulmonary diseases9,60,62,63. Although the rate of secondary transmission among household contacts of index MERS patients (which is approximately 4%) and the estimated pandemic potential of MERS-CoV are lower than those of SARS-CoV, the rapidly progressive clinical course and high fatality of MERS continues to pose a major threat to at-risk populations64,65,66,67,68,69,70,71 (see Supplementary information S2 (table)).

Current management strategies for SARS and MERS. Supportive care — including organ support and prevention of complications, especially acute respiratory distress syndrome, organ failure and secondary nosocomial infections — remains the most important management strategy for SARS and MERS, as there is currently no specific antiviral treatment that has been proven to be effective in randomized controlled trials8,9,56,72,73,74,75. Numerous compounds have been found to inhibit the entry and/or replication of SARS-CoV and MERS-CoV in cell culture or in animal models, but activity in vitro and even in animal experiments does not necessarily translate into efficacy in humans8,9. Owing to the high morbidity and mortality rates of SARS and MERS, some of these antiviral drugs and immunomodulators have been used empirically or evaluated in uncontrolled trials8,10,21,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90 (Table 1). Substantial efforts are underway to discover new therapeutic agents for CoV infections and these investigations are based on our understanding of the basic virology of CoVs. Importantly, treatment with these investigational therapies requires application of standard research treatment protocols and systematic clinical and virological data collection in controlled research trials, with the approval of the local ethics committee.

Development of anti-CoV therapeutics:

Key CoV targets for new drug development. Despite their high species diversity, CoVs share key genomic elements that are essential for the design of therapeutic targets (Fig. 1). The large replicase polyprotein 1a (pp1a) and pp1ab, which are encoded by the 5′-terminal open reading frame 1a/b (ORF1a/b), are cleaved by two viral proteases, the papain-like protease (PLpro) and the 3C-like protease (3CLpro), to produce non-structural proteins (NSPs) such as RNA-dependent RNA polymerase (RdRp) and helicase, which are involved in the transcription and replication of the virus9,91 (Fig. 2). Numerous enzyme inhibitors targeting these proteins have shown anti-CoV activity in vitro.

The surface structural spike glycoprotein (S) is of particular interest for antiviral development because of its critical role in the virus–cell receptor interaction. S is composed of the amino-terminal receptor-binding S1 and carboxy-terminal membrane fusion S2 subunits. Cleavage at the protease site at the S1–S2 junction is required to activate membrane fusion, virus entry and syncytium formation9. Binding of the S1 subunit receptor-binding domain (RBD) to the host receptor triggers conformational changes in the S2 subunit (the stalk region of S) to bring the viral and cell membranes into close proximity and enable fusion92. Monoclonal antibodies (mAbs) against the S1 subunit RBD and fusion inhibitors targeting the S2 subunit have potent anti-CoV activity in vitro and/or in vivo92,93,94,95,96,97,98,99,100. The key functional host receptors utilized by human pathogenic CoVs include angiotensin-converting enzyme 2 (ACE2; used by SARS-CoV and human CoV (HCoV)-NL63), dipeptidyl peptidase 4 (DPP4; used by MERS-CoV), aminopeptidase N (used by HCoV-229E), and O-acetylated sialic acid (used by HCoV-OC43 and HCoV-HKU1)101,102,103,104,105,106. The host receptor is important in determining the pathogenicity, tissue tropism and host range of the virus. mAbs or agents that target the host receptor are potential anti-CoV agents so long as they do not induce immunopathological effects in animal models.

CoVs enter the host cell using the endosomal pathway and/or the cell surface non-endosomal pathway9 (Fig. 2). Low pH and the pH-dependent endosomal cysteine protease cathepsins help to overcome the energetically unfavourable membrane fusion reaction and facilitate endosomal cell entry of CoVs107,108. Other host proteases, such as transmembrane protease serine 2 (TMPRSS2) and TMPRSS11D (also known as airway trypsin-like protease), cleave S into the S1 and S2 subunits to activate S for cell surface non-endosomal virus entry at the plasma membrane109. Inhibitors of these proteases can abrogate this proteolytic cleavage and partially block cell entry109. MERS-CoV S is also activated by furin, a serine endoprotease that has been implicated in the processing of fusion proteins and cell entry of other RNA viruses, including HIV, avian influenza A/H5N1 virus, Ebola virus, Marburg virus and flaviviruses110. Furin is also involved in MERS-CoV S1/S2 cleavage during egress from the infected cell110. Monotherapy and/or combinatorial treatment with inhibitors of host proteases involved in the various cell entry pathways have potent anti-CoV activity in vitro and should be further evaluated in animal studies109,111.

CoVs disassemble inside the host cell and release the nucleocapsid and viral RNA into the cytoplasm, after which ORF1a/b is translated into pp1a and pp1ab, and the genomic RNA is replicated91. The numerous NSPs produced by the cleavage of pp1a and pp1ab form the replication–transcription complex. Attachment of the hydrophobic domains of the CoV replication–transcription complex to the limiting membrane derived from the endoplasmic reticulum produces typical CoV replication structures including double-membrane vesicles (DMVs) and convoluted membranes112,113. Novel agents, such as K22, that target membrane-bound CoV RNA synthesis inhibit DMV formation of a broad range of human and animal CoVs112. The full-length positive strand of genomic RNA is transcribed to form a full-length negative-strand template for the synthesis of new genomic RNAs and overlapping subgenomic negative-strand templates9,91. Subgenomic mRNAs are then synthesized and translated to produce the structural and accessory proteins9,91. The helical nucleocapsid, formed by the assembly of nucleocapsid protein (N) and genomic RNA, then interacts with S, envelope protein (E), and membrane protein (M) to form the assembled virion9. The virion is released into the extracellular compartment by exocytosis and the viral replication cycle is repeated9. Small interfering RNAs (siRNAs) targeting these structural genes could be useful in the treatment of CoV infections, and further optimization of the in vivo delivery of siRNAs may enable their clinical use.

Approaches to anti-CoV drug screening. The only two human-pathogenic CoVs known before the SARS epidemic were HCoV-229E and HCoV-OC43, which usually cause self-limiting upper respiratory tract infections2. Therefore, researchers and research facilities, especially those involved in antiviral development, were underprepared when SARS-CoV suddenly emerged in 2003. Subsequently, three general approaches were used to discover potential anti-CoV treatment

options for human-pathogenic CoVs — especially SARS-CoV and the emerging MERS-CoV — that are associated with more severe disease than the other HCoVs are9,114,115.

The first approach to drug discovery is to test existing broad-spectrum antiviral drugs that have been used to treat other viral infections by using standard assays that measure the effects of these drugs on the cytopathicity, virus yield and plaque formation of live and/or pseudotyped CoVs. Examples of drugs identified using this approach include interferon alfa, interferon beta, interferon gamma, ribavirin and inhibitors of cyclophilin8,74,116,117,118,119,120,121,122. These drugs have the obvious advantage of being readily available with known pharmacokinetic and pharmacodynamic properties, side effects and dosing regimens. However, they do not have specific anti-CoV effects and may be associated with severe adverse effects.

The second anti-CoV drug discovery approach involves the screening of chemical libraries comprising large numbers of existing compounds or databases that contain information on transcriptional signatures in different cell lines122,123,124,125,126,127. This approach provides rapid, high-throughput screening of many readily available compounds that can then be further evaluated by antiviral assays. Various classes of drugs have been identified in these drug repurposing programmes, including many that have important physiological and/or immunological effects such as those that affect neurotransmitter regulation, the oestrogen receptor, kinase signalling, lipid or sterol metabolism, protein processing and DNA synthesis or repair122,123,124,125,126,127. The major disadvantage of this approach is that although many of the identified drugs exhibit anti-CoV activities in vitro, most are not clinically useful because they are either associated with immunosuppressive effects or they have anti-CoV half-maximal effective concentration (EC50) values that markedly exceed the peak serum concentration (Cmax) levels that are achievable at therapeutic dosages. A notable exception, which was found to be effective in a non-human primate model and in non-randomized clinical trials, is the anti-HIV protease inhibitor lopinavir–ritonavir76,77,128 (Table 1).

The third approach for anti-CoV drug discovery involves the de novo development of novel, specific agents based on the genomic and biophysical understanding of the individual CoVs. Examples include siRNA molecules or inhibitors that target specific viral enzymes involved in the viral replication cycle, mAbs that target the host receptor, inhibitors of host cellular proteases, inhibitors of virus endocytosis by the host cell, human or humanized mAbs that target the S1 subunit RBD and antiviral peptides that target the S2 subunit (Fig. 2). Although most of these drugs have potent in vitro and/or in vivo anti-CoV activity, their pharmacokinetic and pharmacodynamic properties and side-effect profiles have yet to be evaluated in animal and human trials. Furthermore, the development of these candidate drugs into clinically useful therapeutic options with reliable delivery modes for patients usually takes years.

Overall, these three drug discovery approaches are often used together during emerging CoV outbreaks to identify candidate drug compounds that can be broadly classified into virus-based and host-based treatment options.

Virus-based anti-CoV treatment options:

Viral nucleosides, nucleotides and nucleic acids. Nucleosides and nucleotides are the building blocks of viral nucleic acids (Fig. 2). Drugs that target nucleosides or nucleotides and/or viral nucleic acids generally have broad-spectrum activity against a wide range of CoVs and other viruses (Table 2). Mycophenolate mofetil is an anti-rejection drug that inhibits inosine monophosphate dehydrogenase and the synthesis of guanine monophosphate122. The active compound, mycophenolic acid, exhibits antiviral activity in vitro against various viruses, including hepatitis B virus (HBV), hepatitis C virus (HCV) and arboviruses122. Mycophenolic acid was identified as a potential anti-MERS-CoV drug using high-throughput screening and has potent anti-MERS-CoV activity in vitro122. However, a subsequent study in a non-human primate model showed that MERS-CoV-infected common marmosets treated with mycophenolate mofetil had a worse outcome with more severe disease and higher viral loads in necropsied lung and extrapulmonary tissues than untreated animals did128. Renal transplant recipients who were on maintenance mycophenolate mofetil therapy also developed severe or fatal MERS129,130. Thus, the usual dosage of mycophenolate mofetil monotherapy is unlikely to be useful for prophylaxis or treatment of CoV infections.

Ribozymes (also known as catalytic RNA or RNA enzymes) are RNA molecules that catalyse specific biochemical reactions. A chimeric DNA–RNA hammerhead ribozyme that specifically recognizes the base sequence GUC, which is present in the loop region of SARS-CoV mRNA, substantially reduces the expression of recombinant SARS-CoV RNA in vitro131. However,

ribozymes are rapidly degraded in vivo and delivery methods would have to be optimized in humans before ribozymes could become clinically useful.

Agents targeting the specific host cell membrane-bound CoV replication complex have also been investigated. One such compound, K22, inhibits membrane-bound CoV RNA synthesis and is active against a broad range of CoVs in vitro112. In cell culture, K22 exerts potent anti-CoV activity during an early step of the viral replication cycle and impairs formation of DMVs112. HCoV-229E escape mutants that are resistant to K22 have substitutions in the potential membrane-spanning domains in nsp6, a membrane-spanning integral component of the CoV replication complex that is involved in DMV formation, including nsp6H121L and nsp6M159V (Ref. 112). The emergence of K22 resistance should be monitored in subsequent in vivo studies.

Recently, a new class of broad-spectrum antivirals that targets long viral double-stranded RNA (dsRNA) has been reported. For example, dsRNA-activated caspase oligomerizer (DRACO) is a chimeric protein with a viral dsRNA-binding domain and a pro-apoptotic domain that selectively induces apoptosis in cells that contain viral dsRNA but spares uninfected host cells132. DRACO is active against many RNA viruses in vitro and/or in vivo132. If an effective mode of DRACO delivery can be achieved, a broad-spectrum anti-CoV drug that targets highly conserved CoV RNA sequences might become a reality.

Viral enzymes. All of the major enzymes and proteins of CoVs that are involved in viral replication are potentially druggable targets (Table 2). The SARS-CoV and MERS-CoV PLpro enzymes exhibit proteolytic, deubiquitylating and deISGylating activities133,134,135. Crystallography has facilitated the characterization of these PLpro enzymes and the identification of PLpro inhibitors136. Numerous SARS-CoV PLpro inhibitors belonging to different classes have been identified, including small-molecule inhibitors, thiopurine compounds, natural products, zinc ion and zinc conjugate inhibitors and naphthalene inhibitors137. However, some of these drugs only inhibit the enzymatic activities of PLpro without inhibiting viral replication, or vice versa137,138,139. None has been validated in animal or human studies137,138. Furthermore, most PLpro inhibitors have narrow-spectrum activity because of the structural differences among the PLpro enzymes of different CoVs140,141. For example, most SARS-CoV PLpro inhibitors are inactive against MERS-CoV because of the structurally different, flexible blocking loop 2 (BL2) domains in the PLpro enzymes of SARS-CoV and MERS-CoV140.

3CLpro is the other major CoV protease that cleaves the large replicase polyproteins during viral replication. SARS-CoV 3CLpro can be targeted by numerous classes of protease inhibitors, including zinc or mercury conjugates, C2-symmetric diols, peptidomimetic-α,β-unsaturated esters, anilides, benzotriazole, N-phenyl-2-acetamide, biphenyl sulfone, glutamic acid and glutamine peptides with a trifluoromethylketone group, pyrimidinone and pyrazole analogues142. Some of these 3CLpro inhibitors demonstrate broad-spectrum in vitro activities against CoVs with highly similar key residues for substrate recognition at their 3CLpro enzymes143,144. Among these 3CLpro inhibitors, the most readily available one is lopinavir, a protease inhibitor used to treat HIV infections that is usually marketed as a ritonavir-boosted form (lopinavir–ritonavir). Lopinavir and/or lopinavir–ritonavir have anti-CoV activity in vitro, as well as in MERS-CoV-infected non-human primates and in non-randomized trials of SARS patients76,77,123,128,145. It is postulated that the 3CLpro-inhibiting activity of lopinavir–ritonavir contributes at least partially to its anti-CoV effects146. It remains to be seen if resistance emerges, as it has in patients with HIV infection, when lopinavir–ritonavir is routinely used to treat CoV infections.

RdRp is an essential part of the CoV replication–transcription complex and is involved in the production of genomic and subgenomic RNAs. Ribavirin is a guanosine analogue with broad-spectrum antiviral activity and has been used in the treatment of severe respiratory syncytial virus infection, HCV infection and viral haemorrhagic fevers. Its exact mechanism of action is undetermined, but inhibition of mRNA capping and induction of mutations in RNA-dependent viral replication are considered to be important for RNA viruses, including CoVs147. High-dose ribavirin has been used to treat SARS patients, but the benefits were unclear8,10,21,72,74,75,117. It exhibits moderate anti-MERS-CoV activity at high doses in vitro and in MERS-CoV-infected rhesus macaques, but there was no obvious survival benefit in small cohorts of MERS patients86,87,88,89,121,148. Moreover, the severe side effects associated with the use of high-dose ribavirin limit its clinical application in patients with severe CoV infections8,74. Recently, a novel adenosine analogue, BCX4430 (Immucillin-A), was developed149. It acts as a non-obligate RNA chain terminator to inhibit viral RNA polymerases of a wide range of RNA viruses, including CoVs such as SARS-CoV and MERS-CoV as well as filoviruses such as Ebola and Marburg viruses149. Its development for human use has been fast-tracked to increase the number of treatment options for the recent Ebola virus epidemic in West

Africa. Existing nucleoside analogues, such as acyclovir, could be modified by incorporating fleximers, which have increased binding affinity and can overcome resistance caused by point mutations in biologically important binding sites150. These acyclic fleximer nucleoside analogues inhibit MERS-CoV and HCoV-NL63 in vitro at micromolar concentrations150. Notably, resistance to nucleoside analogues due to mutations in RdRp has been reported for other RNA viruses, and should be monitored when these agents are used to treat CoV infections. In addition to nucleoside analogues, siRNA molecules targeting SARS-CoV RdRp have been used to inhibit SARS-CoV in vitro151,152.

Helicase catalyses the unwinding of duplex oligonucleotides into single strands in an ATP-dependent reaction during the CoV replication cycle. Helicase inhibitors are attractive anti-CoV treatment options because the helicases of different CoVs are highly homologous. Based on their mechanisms of action, CoV helicase inhibitors can be broadly categorized into two groups. The first group includes bananins and 5-hydroxychromone derivatives, which inhibit the unwinding and ATPase activity of SARS-CoV helicase, resulting in inhibition of viral replication in vitro153,154. However, the toxicity resulting from the inhibition of cellular ATPases or kinases by these compounds has limited their development for human use. The second group of CoV helicase inhibitors includes compounds that selectively inhibit the unwinding activity but not the ATPase activity of CoV helicase. An example is SSYA10-001, a triazole that inhibits a broad range of CoVs, including SARS-CoV, MERS-CoV and mouse hepatitis virus155,156. The toxicity of SSYA10-001 should be evaluated in animal models.

Viral spike glycoprotein. The membrane-anchored glycoprotein, S, is a major immunogenic antigen and is essential for the interaction between the virus and the host cell receptor (Fig. 2). Adoptive transfer of sera containing anti-MERS-CoV-S antibodies blocked virus attachment and accelerated viral clearance from the lungs of MERS-CoV infected BALB/c mice that were recently transduced by adenoviral vectors expressing human DPP4 (Ref. 157) (Table 2). Small cohorts of SARS patients who received convalescent-phase plasma containing neutralizing antibodies that probably targeted CoV S had significantly higher discharge rates by 3 weeks after symptom onset and a lower mortality rate83,84. However, the use of convalescent-phase plasma therapy during emerging CoV outbreaks is limited by the good will of convalescent patients with high serum neutralizing antibody titres. Disease worsening associated with immune enhancement that results from treatment with products containing low antibody titres has been reported in cell line and animal studies158,159. To overcome these problems, mAbs targeting different regions of SARS-CoV S have been generated by immunization of human immunoglobulin transgenic mice, cloning of small chain variable regions from naive and convalescent patients as well as from immortalization of convalescent S-specific B cells160. Most of these mAbs target specific epitopes on the S1 subunit RBD to inhibit virus–cell receptor binding, whereas others bind to the S2 subunit to interrupt virus–cell fusion160. Regardless of their binding sites and mechanisms, these mAbs exhibit neutralizing activities and reduced viral titres in vitro and/or in small animal models. Similarly, several mAbs targeting different epitopes on the S1 subunit RBD of MERS-CoV S have been developed94,95,96,97,100. These monoclonal antibodies bind to the RBD with 10-fold to >450-fold higher affinity than does human DPP4, resulting in broader and higher neutralizing activity in vitro. Importantly, combination therapy with two or more synergistically acting humanized or human mAbs targeting non-cross-resistant epitopes or different regions of S may help to reduce the frequency with which viruses mutate to escape antibody-mediated neutralization94. Treatment with these mAbs showed protective effects in MERS-CoV-infected human DPP4-transgenic mice and mice transduced by adenoviral vectors expressing human DPP4 (Refs 100,161,162). Their safety profiles and treatment effects in patients should be further evaluated.

Antiviral peptides targeting different regions of S are another promising therapeutic strategy. The S2 subunits or stalk regions of both SARS-CoV and MERS-CoV S are class I viral fusion proteins that each contain an N-terminal fusion peptide, heptad repeat 1 (HR1) and HR2 domains, a transmembrane domain and a cytoplasmic domain92. Antiviral peptides analogous to the N terminus, pre-transmembrane domain or the loop region separating the HR1 and HR2 domains of SARS-CoV inhibited virus plaque formation by 40–80% at micromolar concentrations163,164. Similarly, antiviral peptides spanning the HR2 domain of MERS-CoV inhibit S-mediated cell–cell fusion and viral entry into cells in vitro92,93. A peptide called HP2P-M2 that is derived from the HR2 domain, if administered intranasally before or after viral challenge, protected C57BL/6 mice and mice deficient for V(D)J recombination-activating protein 1 (RAG1) that were recently transduced by adenoviral vectors expressing human DPP4 from MERS-CoV infection with 10-fold to >1,000-fold reduction in viral titres in the lung; this protection was enhanced by combining this

peptide with interferon beta99. Combining antiviral peptides targeting different regions of the S2 subunit may be synergistic in vitro and overcome the theoretical risk of drug resistance165. Importantly, an analogous fusion inhibitor, enfuvirtide, which binds to glycoprotein 41 of HIV to block membrane fusion and HIV cell entry, has been successfully marketed for treatment of HIV-1 infection166. Primary resistance to enfuvirtide is rare and can be overcome by modifying the drug such that it contains secondary compensatory mutations167,168. This example of successful drug development includes measures to counteract drug resistance and therefore favours antiviral peptides over anti-CoV S siRNAs for further evaluation in vivo; siRNAs have remained in preclinical development despite their reported antiviral activities in vitro and in SARS-CoV-infected rhesus macaques owing to the lack of reliable drug delivery methods in humans169,170,171,172.
Another class of anti-CoV agents that target S to inhibit CoV entry is the carbohydrate-binding agents. Griffithsin is an antiviral protein originally isolated from the red alga Griffithsia spp.173. It binds specifically to oligosaccharides on viral surface glycoproteins such as S and HIV glycoprotein 120. It inhibits a broad range of CoVs, including SARS-CoV, HCoV-229E, HCoV-OC43 and HCoV-NL63 in vitro and in SARS-CoV-infected mice173,174. The optimal delivery modes and safety profiles of these agents in humans should be further evaluated.
Viral envelope, membrane, nucleocapsid and accessory proteins. E, M and N and some of the accessory proteins are not only essential for virion assembly but may also have additional functions that suppress the host immune response to facilitate viral replication. For example, the accessory proteins 4a and 4b, and possibly also M and accessory protein 5 of MERS-CoV, exhibit interferon antagonist activities, and SARS-CoV N acts as a viral suppressor of RNA silencing and suppresses RNA interference triggered by either short hairpin RNAs or siRNAs175,176,177,178 (Table 2). siRNAs targeting E, M, N, ORF3a, ORF7a or ORF7b of SARS-CoV inhibited viral replication in vitro179,180. However, similar to anti-CoV S siRNAs, none of these siRNAs is ready for human use until better delivery methods become available.
Alternatively, an increasing number of agents that target specific binding sites or functions of these proteins are being generated through crystallography and functional assays. Examples include the viroporin inhibitor hexamethylene amiloride, which reduces the ion channel activity of E in SARS-CoV and HCoV-229E, and PJ34, which binds to a distinct ribonucleotide-binding pocket at the N-terminal domain of N in HCoV-OC43 (Refs 181,182,183). However, these agents are likely to be narrow-spectrum as the binding sites and functions of these proteins are unique to individual CoVs.
Novel lipophilic thiazolidine derivatives, such as LJ001 and JL103, are membrane-binding photosensitizers that produce singlet oxygen molecules to induce changes in the properties of lipid membranes and prevent fusion between viral and target cell membranes. They exhibit broad-spectrum activities against numerous enveloped viruses and may be active against CoVs184,185,186,187.

Host-based anti-CoV treatment options:
Broad-spectrum host innate immune response. The host innate interferon response is crucial for the control of viral replication after infection188. Although CoVs are able to suppress the interferon response for immune evasion, they remain susceptible to interferon treatment in vitro189,190. The interferon response can be augmented by the administration of recombinant interferons or interferon inducers (Table 3). Recombinant interferon alfa and interferon beta inhibit the replication of both SARS-CoV and MERS-CoV in vitro and in animal models8,99,116,121,122,128,148,191,192. Various combinations of interferon alfa or interferon beta with other antivirals such as ribavirin and/or lopinavir–ritonavir have been used to treat patients with SARS or MERS. Overall, combination treatments consisting of interferons and ribavirin did not consistently improve outcomes8,9,74,86,87,89. The apparent discrepancy between in vitro findings and in vivo outcomes may be related to the high EC50/Cmax ratios of these drugs and the delay between symptom onset and drug administration8,121,122. This delay is especially relevant for MERS patients, as they have a much shorter median time interval between symptom onset and death than do SARS patients9,58. The use of recombinant interferon beta-1b, which has the lowest EC50/Cmax ratio against MERS-CoV among tested preparations of recombinant interferons, should be evaluated in combination with other effective antivirals in clinical trials at early stages of the infection122,128.
Polyinosinic:polycytidylic acid (poly(I:C)) is a synthetic analogue of dsRNA that strongly induces type I interferons. It substantially reduced the MERS-CoV load in BALB/c mice that were transduced by adenoviral vectors expressing human DPP4 shortly before poly(I:C) administration, although its effects in standard cell culture protection assays are not published157. Intramuscular

injection of poly(I:C) stabilized with poly-L-lysine and carboxymethylcellulose seemed to be well tolerated by patients with malignant gliomas in Phase II clinical trials193,194. Nitazoxanide is another potent type I interferon inducer that has been used in humans for parasitic infections195. It is a synthetic nitrothiazolyl–salicylamide derivative that exhibits broad-spectrum antiviral activities against both RNA and DNA viruses including canine CoV, influenza viruses, HBV, HCV, HIV, rotavirus, norovirus and flaviviruses195. It has been evaluated in Phase II and Phase III clinical trials for the treatment of HCV infection and influenza and has a good safety profile195,196,197. Other innate immunomodulators that have anti-SARS-CoV effects in animal models include the antimicrobial peptide rhesus θ-defensin 1 and protein cage nanoparticles that elicit a host immune response in inducible bronchus-associated lymphoid tissue198,199. The combined use of interferon inducers and innate immunomodulators with effective antiviral agents may be synergistic and should be evaluated in animal models.

Other host signalling pathways involved in viral replication. In addition to direct potentiation of the interferon response, other cell signalling pathways have been identified as potential anti-CoV treatment targets (Table 3). Cyclophilins interact with SARS-CoV nsp1 to modulate the calcineurin pathway, which is important in the T cell-mediated adaptive immune response120. The calcineurin inhibitor cyclosporine inhibits a broad range of CoVs in vitro118,119,120. However, its clinical application is limited by its immunosuppressive effects and high EC50/Cmax ratio at standard therapeutic dosages. The antiviral activities of newer, non-immunosuppressive calcineurin inhibitors, which are active against HCoV-NL63, should be evaluated for SARS-CoV and MERS-CoV200. Similarly, agents that modulate other cellular signalling pathways, such as kinase signalling pathway inhibitors, also exhibit anti-CoV activities and are commercially available124,125. However, their toxicities may limit their use in patients with severe CoV infections.

Host factors utilized by CoVs for viral replication. CoVs utilize specific host factors for virus entry and replication (Fig. 2). The host receptor can be targeted by specific monoclonal or polyclonal antibodies, peptides or functional inhibitors (Table 3). For example, anti-DPP4 mAbs inhibit MERS-CoV cell entry in vitro201. YS110 is a recombinant humanized IgG1 anti-DPP4 mAb that seems to be well tolerated in patients with advanced solid tumours201. For the treatment of SARS-CoV, small-molecule entry inhibitors such as N-(2-aminoethyl)-1-aziridine-ethanamine (NAAE) inhibit the catalytic activity of ACE2 and SARS-CoV S-mediated cell–cell fusion in vitro202. Synthetic peptides analogous to critical segments of ACE2 also have anti-SARS-CoV activity at micromolar concentrations in vitro203. However, none of these receptor-directed compounds has yet been tested in patients with CoV infections. Their anti-CoV activity is likely to be narrow-spectrum, as different CoVs utilize different host cell receptors. Furthermore, the risks of immunopathology must be assessed, especially given the multiple essential biological and immunological functions of these receptors.

The entry of CoVs into host cells via the endosomal and/or cell surface pathways is facilitated by host proteases that cleave and activate S. Cathepsins are cysteine proteases that are involved in the endosomal pathway and can be inhibited by cathepsin inhibitors such as K11777 and its related vinylsulfone analogues111. These compounds seem to be safe and effective against various parasitic infections in animal models, and have broad-spectrum activities against enveloped RNA viruses such as CoVs (SARS-CoV, MERS-CoV, HCoV-229E and HCoV-NL63), filoviruses (Ebola and Marburg viruses) and paramyxoviruses111,204,205,206. TMPRSS2 is a serine protease that mediates the cell surface entry pathway; camostat mesylate is a synthetic low-molecular-weight serine protease inhibitor that has been used to treat chronic pancreatitis in humans with minimal side effects207,208. This molecule inhibits SARS-CoV and MERS-CoV in vitro and improves survival of SARS-CoV-infected mice109,111. Furin, another ubiquitously expressed host protease, is also important in MERS-CoV S-mediated entry. Blocking furin with decanoyl-Arg-Val-Lys-Arg-chloromethylketone inhibits MERS-CoV entry and cell–cell fusion in vitro110.

Another group of candidate anti-CoV drugs target the endocytosis of CoV during cell entry. Chlorpromazine is an antipsychotic drug used in the treatment of schizophrenia that also affects the assembly of clathrin-coated pits at the plasma membrane. It is active against HCV, alphaviruses and numerous CoVs, including SARS-CoV and MERS-CoV, in vitro123. Cardiotonic steroids that bind sodium/potassium-transporting ATPase subunit α1, such as ouabain and bufalin, also inhibit clathrin-mediated endocytosis of MERS-CoV at nanomolar concentrations209. However, the use of these clathrin-mediated endocytosis inhibitors in patients with CoV infections is limited by either very high EC50/Cmax ratios or toxicity. Alternatively, endocytosis can also be suppressed by a high pH. Chloroquine is an anti-malarial drug that sequesters protons into

lysosomes to increase the intracellular pH. It has broad-spectrum antiviral activities against numerous CoVs (SARS-CoV, MERS-CoV, HCoV-229E and HCoV-OC43) and other RNA viruses in vitro123,210,211,212,213,214. However, it did not substantially reduce viral replication in SARS-CoV-infected mice, possibly because the cell surface pathway was not simultaneously blocked215. The anti-CoV effects, pharmacokinetic and pharmacodynamic profiles and toxicity of the combinations of different protease and endocytosis inhibitors that target these different cell entry pathways should be further evaluated in vivo.

Outlook and challenges:

Animal models for testing anti-CoV drugs. Suitable animal models are especially important for testing anti-CoV drugs because most of these drugs have not been used in humans. In contrast to the limited number of animal models established for the mild infections caused by HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, various small animal and non-human primate models have been evaluated for studies of the pathogenesis and treatment of SARS and MERS234,235,236,237. The identification of ACE2 and DPP4 as the functional receptors for SARS-CoV and MERS-CoV, respectively, was essential to the development of animal models that are representative of severe human disease101,102. A number of different non-human primates were found to be permissive to SARS-CoV, but none of them consistently reproduced characteristics of the severe human disease, and mortality was not observed237. These models were predominantly useful to fulfil Koch's postulates238. Small animals — including young and aged BALB/c and C57BL/6 mice, knockout mice with deficiencies in T, B and/or NK cells, golden Syrian hamsters and ferrets — could be productively infected with SARS-CoV, but few of them developed clinically apparent disease237. The best available small animal models for SARS utilize transgenic mice that express human ACE2 and/or mouse-adapted SARS-CoV strains that are capable of causing lethal disease in mice239,240,241. The limited availability of these ACE2-transgenic mice and mouse-adapted virus strains remains a major obstacle to testing anti-SARS-CoV drugs.

Similar to SARS, non-human primate models were also used to fulfil Koch's postulates and investigate the pathogenesis of MERS. Rhesus macaques developed only a mild, self-limiting disease and were not optimal for the evaluation of treatments for MERS148,242,243. By contrast, MERS-CoV-infected common marmosets developed a disseminated and fatal infection that closely resembled severe human disease128,244. However, the availability of common marmosets is limited and experiments on these small primates are technically demanding. Therefore, other small animal models for MERS were evaluated. Unlike with SARS-CoV, most small animals — including BALB/c mice, golden Syrian hamsters, ferrets and rabbits — were not susceptible to MERS-CoV infection245,246,247. Intranasal inoculation of adenoviral vectors expressing human DPP4 followed by MERS-CoV inoculation was a novel method that rapidly rendered mice susceptible to MERS-CoV infection, but the disease was relatively mild and confined to the respiratory tract157. Transgenic mice expressing human DPP4 develop severe pulmonary and disseminated infection and are currently the best available small animal model for MERS248. Potential anti-MERS-CoV treatment options identified in in vitro antiviral assays should be further evaluated in these transgenic mice.

Generic challenges in the clinical development of novel anti-CoV drugs. There are a number of virological and patient-associated factors that pose major challenges in the clinical development of novel anti-CoV drugs. First, CoVs are one of the most diverse and rapidly mutating groups of viruses, and novel CoVs emerge repeatedly at unpredictable times. Therefore, most anti-CoV drugs that specifically target the replication apparatus of an existing CoV may not be effective against another novel CoV. This is especially applicable to viral enzyme inhibitors, mAbs and antiviral peptides that target S, as well as agents that target the host cell receptor. Second, there are a limited number of animal models available for infections caused by HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1. Even for SARS and MERS, experiments using suitable animal models such as mice with transgenes encoding ACE2 or DPP4, and non-human primates, are only available in a few designated research biosafety level 3 laboratories, and these experiments are technically demanding. Last and most important, the mild clinical severity of infections caused by HCoV-229E, HCoV-OC43, HCoV-NL63 and HCoV-HKU1, together with the absence of new SARS cases, have made recruitment of patients into clinical trials difficult and reduced the incentives for pharmaceutical companies to develop specific antiviral drugs for these CoV infections. The continuing threat of MERS-CoV to global health security 3 years after its first discovery presents a golden opportunity to tackle current obstacles in the development of new anti-CoV drugs. It is prudent that a well-organized, multidisciplinary, international collaborative

network consisting of clinicians, virologists and drug developers, coupled to political commitment, is formed to carry out clinical trials using anti-CoV drugs that have already been shown to be safe and effective in vitro and/or in animal models.

Prioritization of virus-based and host-based treatment options for clinical development. Despite the report of a large number of virus-based and host-based treatment options with potent in vitro activities for SARS and MERS, only a few are likely to fulfil their potential in the clinical setting in the foreseeable future. Most drugs have one or more major limitations that prevent them from proceeding beyond the in vitro stage. First, many drugs have high EC50/Cmax ratios at clinically relevant dosages. Examples of such drugs include cyclosporine, chlorpromazine and interferon alfa. Second, some have severe side effects or cause immunosuppression. For example, the use of high-dose ribavirin may be associated with haemolytic anaemia, neutropenia, teratogenicity and cardiorespiratory distress. MERS-CoV-infected common marmosets treated with mycophenolate mofetil developed a fatal infection with even higher viral loads in their lungs and extrapulmonary tissues than untreated controls did128. Agents targeting host signalling pathways or receptors may induce immunopathology. Furthermore, the lack of a reliable drug delivery method in vivo is particularly problematic for siRNAs and other agents that have not been previously used in humans.

Looking ahead, the most feasible options that should be further evaluated in clinical trials for the ongoing MERS epidemic include monotherapy or combinational therapies that include lopinavir–ritonavir, interferon beta-1b and/or mAbs and antiviral peptides targeting MERS-CoV S. These agents have protective effects against MERS in non-human primate or mouse models. Moreover, they are either marketed drugs (in the case of lopinavir–ritonavir and interferon beta-1b) or they have been successfully developed for other infections (such as palivizumab, which is used for respiratory syncytial virus infection, and enfuvirtide, which is used for HIV infection). In the long term, the development of novel, broad-spectrum, pan-CoV antiviral drugs that are active against a wide range of CoVs may become the ultimate treatment strategy for circulating and emerging CoV infections.