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TITLE:
Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome: Current Therapeutic Options and Potential Targets for Novel Therapies

ABSTRACT:
No specific antivirals are currently available for two emerging infectious diseases, Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). A literature search covering pathogenesis, clinical features and therapeutics, clinically developed drugs for repurposing and novel drug targets was performed. This review presents current knowledge on the epidemiology, pathogenesis and clinical features of the SARS and MERS coronaviruses. The rationale for and outcomes with treatments used for SARS and MERS is discussed. The main focus of the review is on drug development and the potential that drugs approved for other indications provide for repurposing. The drugs we discuss belong to a wide range of different drug classes, such as cancer therapeutics, antipsychotics, and antimalarials. In addition to their activity against MERS and SARS coronaviruses, many of these approved drugs have broad-spectrum potential and have already been in clinical use for treating other viral infections. A wealth of knowledge is available for these drugs. However, the information in this review is not meant to guide clinical decisions, and any therapeutic described here should only be used in context of a clinical trial. Potential targets for novel antivirals and antibodies are discussed as well as lessons learned from treatment development for other RNA viruses. The article concludes with a discussion of the gaps in our knowledge and areas for future research on emerging coronaviruses.

Key Points:

Epidemiology of MERS and SARS ::: Introduction:
Since 2003, two human coronaviruses, SARS-CoV and MERS-CoV, emerged as global public health threats. SARS-CoV was first identified in February 2003 in Guangdong Province, Peoples Republic of China and was transmitted to humans from infected civets, likely infected from bats [1, 2]. SARS-CoV spread to 29 additional countries and was associated with high morbidity in humans (e.g. atypical pneumonia). Ultimately, SARS was contained in 2004 following a highly effective public health response but resulted in 8098 confirmed cases and 774 deaths (Fig. 1a) [3]. In 2012, MERS emerged in The Kingdom of Saudi Arabia and presented as a severe respiratory disease, with frequent gastrointestinal and renal complications. MERS-CoV, the causative agent of MERS, was later identified as a coronavirus. MERS-CoV has subsequently spread to 27 additional countries (Fig. 1B) [4]. As of September 12, 2017, 2080 confirmed cases of MERS and 722 deaths were reported [5].

Coronaviruses are enveloped, single-stranded, positive-sense RNA viruses (Fig. 2). They are members of the Coronavirinae subfamily of viruses and together with the Torovirinae subfamily comprise the Coronaviridae virus family (order Nidovirales). Coronavirinae is divided into four genera: alpha coronavirus, beta coronavirus, gamma coronavirus, and delta coronavirus. The coronaviruses share a similar genome organization. The open reading frame 1a and 1b comprise nearly 2/3 of the genome and encode the nonstructural proteins. The multiple structural proteins, including spike, nucleocapsid, envelope, and membrane proteins are encoded by downstream open reading frames (Fig. 2) [6–8]. SARS-CoV and MERS-CoV belong to the beta coronavirus genus. However, SARS-CoV belongs to lineage B, and MERS-CoV belongs to lineage C along with bat coronaviruses HKU4 and HKU5. As MERS-CoV and bat coronaviruses are part of lineage C and MERS-CoV RNA was found in a bat sample in The Kingdom of Saudi Arabia, researchers hypothesize that bats may be a natural reservoir for MERS-CoV [9, 10]. Results from a recent study support that bats may be a reservoir for MERS-CoV; however, camels and goats are thought to be intermediate hosts [11]. In this study, MERS-CoV was isolated from nasal secretions of MERS-CoV-infected dromedary camels that had a short, mild disease progression.

The suspected reservoir for SARS-CoV is the Chinese horseshoe bat [2]. However, the mechanism of emergence and adaptation to make the virus zoonotic is still not definitely

understood [2]. SARS-CoV-like isolates from these bats have up to 95% sequence similarity to human and civet SARS-CoV. During the initial outbreak, SARS-CoV was originally isolated from palm civets found in a Chinese market; but, SARS-CoV was not found in the wild palm civet population [12]. Bats harbor many coronaviruses and are considered the main reservoir for later infections in an intermediate host, such as civets or camels, which spread the disease to humans [2]. Human-to-human transmission has been most commonly associated with health-care workers and those with close, unprotected contact with infected patients [13, 14].

Clinical Features ::: Introduction:
The clinical features of MERS and SARS are similar and can range from asymptomatic or mild disease to severe pneumonia with acute respiratory distress syndrome (ARDS) and multi-organ failure [15]. Although MERS and SARS are clinically similar, the MERS mortality rate is 40% and SARS's mortality rate is 10% [16]. Approximately 75% of MERS cases were associated with underlying comorbidities with a 60% mortality rate in this subgroup (including cardiopulmonary abnormalities, obesity, and diabetes). In contrast, 10–30% of patients with SARS have comorbidities with a mortality rate of 46% within this subgroup [15, 16].
The development of symptomatic MERS and SARS mostly occurs in adults (median age of 50 years; 40 years for SARS). MERS and SARS symptoms typically follow a mean incubation time of ~ 5 days (range 2–13 and 2–14 days, respectively) and include fever, chills, cough (some associated with blood), shortness of breath, myalgia, headache, nausea, vomiting, diarrhea, sore throat, and malaise [15–17]. Progression from mild to severe disease is more rapid with MERS as compared to SARS with means of 7 and 11 days, respectively [15]. Secondary bacterial infections have occurred in patients with severe MERS; however, the role of these coinfections in MERS pathogenesis has yet to be determined [18–20]. Laboratory abnormalities associated with MERS and SARS patients include elevated lactate dehydrogenase, elevated liver enzymes; thrombocytopenia; lymphopenia and leukopenia [21–23].
Radiographic abnormalities consistent with viral pneumonitis and ARDS are common in MERS and SARS. Radiographic progression in the lower lobes has been reported to be more rapid for MERS than SARS [21–23]. For SARS, disease in the lower lobes mimics pneumonia, radiographic progression includes ground-glass opacification and lobe thickening [17]. MERS-CoV (intact virus or viral genome) is found at higher concentrations in the lower respiratory tract than in the upper respiratory tract in MERS patients and this may account for inefficient inter-human transmission [15, 24]. Currently, no approved therapeutics for patients with MERS or SARS are available, and clinical management has relied primarily on supportive care.

Treatment of SARS ::: Clinical Usage ::: Therapeutic Agents:
Effectiveness of antiviral treatments used during the SARS epidemic has been mainly based on case studies and retrospective analysis of patient cohorts. Few randomized, blinded, clinical trials of anti-SARS treatments were performed, which adds complexity when interpreting the available data (Table 1). Ribavirin, a nucleoside analog that prevents RNA and DNA virus replication, was initially used in the treatment of SARS due to its broad-spectrum efficacy. For example, in a Taiwanese study, 51 SARS patients were treated daily with fluoroquinolone antibiotics, [levofloxacin (500 mg) or moxifloxacin (400 mg)] following diagnosis. Out of 51 patients, 44 SARS patients were also treated intravenously (IV) with 2000 mg of ribavirin then orally daily with 1200 mg while 7 SARS patients did not receive ribavirin. Corticosteroids, IV methylprednisolone, or oral prednisolone were administered as needed to treat worsening lung infiltrates and fever [25]. Ribavirin treatment led to hypoxia and anemia and increased risk for death in SARS patients. In a retrospective analysis, a cohort of 229 patients from Hong Kong, Singapore, and Toronto were treated with ribavirin in conjunction with corticosteroids, immunoglobulins, and/or antibiotics [26]; ribavirin did not demonstrate efficacy. Patients in Hong Kong and Singapore were treated with ribavirin at 1200 mg orally at diagnosis, followed by oral treatments with 2400 mg daily, or continual IV ribavirin therapy [8 mg/kg every 8 h (h)]. In Toronto, patients received ribavirin IV treatment with 2000 mg, followed by 1000 mg every 6 h for 4 days, and 300 mg every 8 h for 3 days. Unfortunately, fatality rates were similar between the ribavirin-treated and control groups. Later, researchers demonstrated that the ribavirin dosage required to be effective against SARS-CoV in vitro was not clinically achievable [27]. Ribavirin treatment also resulted in adverse effects including anemia, hypoxemia and decreased hemoglobin levels, and did not improve patient outcome [26]. Due to the increasing adverse effects and lack of efficacy, Health Canada stopped permitting the use of ribavirin for SARS [25].

Additional studies tested the efficacy of ribavirin in conjunction with lopinavir, an anti-retroviral agent. Lopinavir demonstrated in vitro activity against SARS-CoV [28]. In a non-randomized, open-enrollment trial of 152 suspected SARS patients [27], all patients were treated with ribavirin and corticosteroids similar to the previously described studies. In addition, 41 of the confirmed SARS patients were also treated with a combination of lopinavir (400 mg) and ritonavir (100 mg). Mean viral loads in nasopharyngeal swabs within this treatment group decreased to undetectable levels by day 10. Overall, SARS-related symptoms subsided, disease progression was milder, and no adverse effects were reported as compared to the historical control group.

In an open-label, non-randomized study of 22 SARS patients, 9 patients who received subcutaneous (SC) injections of interferon (IFN)α, alfacon-1, for 10 days at an initial dose of 9 µg/day for 2 days increasing to 15 µg/day with disease progression. All 9 patients survived with minor adverse effects [29].

Treatment of MERS ::: Clinical Usage ::: Therapeutic Agents:
The evaluation of treatments in MERS patients has been hampered as high-quality clinical data from randomized clinical trials are limited. Ribavirin (with or without IFN, or corticosteroids) was the primary treatment during the MERS outbreak. In a retrospective analysis, a cohort of 20 patients was treated with oral ribavirin and SC pegylated IFN-α2a at a dose of 180 µg/week for 2 weeks (Table 2) [30]. The initial dose of ribavirin was 2000 mg, followed by a 200–1200 mg dose depending on creatinine clearance. A group of 24 patients that received supportive care and corticosteroids were considered the control group. At 14 days after confirmed diagnosis of MERS, survival was increased in the treated group (70%) compared to the control group (29%). By 28 days post-diagnosis, 30% of treated subjects survived versus 17% of the control group [30]. In an additional case study, a 69-year-old Greek patient who contracted MERS in Jeddah was treated with oral lopinavir/ritonavir (400/100 mg twice daily), pegylated IFN (180 µg SC once per week for 12 weeks), and ribavirin (2000 mg initial dose; 1200 mg every 8 h for 8 days, initiated on day 13 post-diagnosis). Two days after treatment initiation, viremia could not be detected; however, viral RNA was detected in several patient samples (feces, respiratory secretions, and serum) up to 14 weeks post-diagnosis. Despite prolonged survival, the patient succumbed from septic shock 2 months post-diagnosis [31]. An ongoing randomized clinical trial in Saudi Arabia is evaluating treatment of MERS patients with IFN-β1b in combination with lopinavir/ritonavir [32].

Antidiarrheal Agents ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
Loperamide, an approved anti-diarrheal agent, is on the World Health Organization (WHO) Model List of Essential Medicines and is available in many countries. The drug acts on the opioid receptor and reduces intestinal motility [34]. Results from pharmacokinetic (PK) studies show that oral loperamide is well absorbed from the gut with less than 1% of the drug entering systemic circulation [35]. Loperamide demonstrated anti-MERS-CoV, anti-SARS, and anti-HCoV229E activity in an in vitro screen of approved drugs [36], although the mechanism of action is unknown. Interestingly, loperamide was suggested for limiting gastrointestinal fluid and electrolyte losses in patients with Ebola virus disease (EVD) [37].

Antimalarial Agents ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
The antimalarial agents, chloroquine (CQ), amodiaquine, and mefloquine have activity against SARS-CoV and MERS-CoV in vitro [36, 38, 39]. CQ is a U.S. Food and Drug Administration (FDA)-approved antimalarial agent that is also used to treat autoimmune disease such as rheumatoid arthritis due to its anti-inflammatory effects [40]. CQ has activity against a number of viruses in vitro and in vivo including flaviviruses [dengue virus (DENV)], Togaviruses [chikungunya virus (CHIKV)], paramyxoviruses (Hendra, Nipah virus), influenza viruses, HIV, and filoviruses [Ebola virus (EBOV)] [41–47].

Several mechanisms of action have been identified for the antiviral effect of CQ and suggest that the drug acts nonspecifically at virus entry or at the later stages of virus production. CQ accumulates within acidic organelles such as endosomes, Golgi vesicles, and lysosomes, where the drug is protonated resulting in increased pH within the vesicle [48]. Viruses depend on these acidic organelles for entry, viral replication, and maturation of virus progeny. Similarly, MERS-CoV entry into cells depends on several proteases. Dipeptidyl peptidase 4 (DPP4) acts as functional virus receptor [49], and cellular proteases [e.g. type II transmembrane serine protease (TMPRSS2)

and members of the cathepsin family] activate the viral spike (S) glycoprotein [50]. CQ may have an effect on any of these proteases. CQ also affects the glycosylation step within the Golgi that directs trafficking and maturation of viral proteins [51–53]. For SARS-CoV, the antiviral activity of CQ has also been attributed to a deficit in glycosylation of the receptor angiotensin-converting enzyme 2 (ACE2) [54].

The broad-spectrum antiviral activity makes CQ an attractive antiviral for repurposing and treating coronaviral and other emerging viral infections. In vivo activity of CQ in MERS or SARS animal models has not yet been reported. However, the antiviral activity of the drug has been evaluated against other viruses in preclinical and clinical studies with mixed results. CQ plasma steady state concentrations in mice are similar to those reported for humans (10 μM) and are within range of the EC50 values determined for MERS-CoV (3.6 μM) and SARS-CoV (2.3 μM) [43, 55]. Preclinical studies with CQ in mice against other viruses have shown survival benefits for influenza and EBOV infections.

In clinical studies, CQ was effective at reducing viral loads in asymptomatic HIV patients [56, 57], but results of CQ treatment of CHIKV and DENV infections were mixed [58, 59].

In summary, CQ has broad-spectrum potential and the information gained from studies on other viruses can be used to plan the most appropriate strategies for evaluating its specific clinical value for treating for MERS-CoV and SARS-CoV infections. CQ has several advantages including rapid absorption from gastrointestinal tract, low cost, and very effective biodistribution. CQ may be an excellent candidate for combinatorial treatments with other antivirals. However, considerable challenges remain for the treatment of viral infections including increased understanding of the pharmacodynamics of CQ, achievement of sufficient plasma concentrations in patients, and toxicity concerns [60]. Importantly, hydroxychloroquine, a CQ derivate, may provide an alternative due to lower toxicity and similar pharmacology profile [55].

A related antimalarial drug, amodiaquine, also has activity in vitro against MERS-CoV and SARS-CoV [38]. Previous investigations have demonstrated that amodiaquine inhibits filovirus replication, and the mechanism of action is hypothesized to be similar to that of CQ [43]. Amodiaquine is well tolerated and is commonly used for malaria treatments in many countries. Further, amodiaquine in combination with artesunate was administered to EVD patients during the 2013–2016 epidemic, and the resulting decrease in fatality rates may have been associated with the use of amodiaquine as an antimalarial agent [61]. Nonhuman primate (NHP) studies are currently underway to investigate the effect of amodiaquine treatment on EVD [62].

Mefloquine, a synthetic analog of quinine, is another antimalarial drug with activity against MERS-CoV and SARS-CoV [38]. It belongs to the WHO Model List of Essential Medicines. Mefloquine is known to penetrate the blood-brain barrier and was found to inhibit JC virus infection and replication at concentrations generally achieved in the brains of patients given mefloquine for malaria [63] leading to the clinical evaluation of this drug for the treatment of progressive multifocal leukoencephalopathy [64, 65]. In 2013, the FDA added a boxed warning to the US label of mefloquine regarding the potential for neuropsychiatric side effects. Additional investigations are warranted to determine if amodiaquine or mefloquine have value for repurposing for treatment of MERS or SARS.

Cyclophilin Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
Cyclophilins are ubiquitous host proteins believed to have multiple roles in trafficking, protein folding and T cell activation [66]. Cyclosporine A (CysA), forms a complex with cyclophilin A, thereby blocking T cell activation. CysA is licensed for use in organ transplantation to suppress the immune response. CysA has also been shown to inhibit coronaviruses including SARS-CoV and MERS-CoV effectively in cell culture [67, 68]; however, the mechanism has yet to be determined. There is increasing evidence that cyclophilins are involved in viral replication of RNA viruses such as hepatitis C virus (HCV) and West Nile virus, and this may also apply to coronaviruses [69]. Although the immunosuppressive properties of CysA are considered a risk for treating viral infections in patients, nonimmunosuppressive analogs of CysA that bind to cyclophilins with higher affinity have been developed and some are in clinical trials as HCV therapeutics [70–72].

Interferons ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
Interferons (IFNs) are approved by the FDA for other indications such as hepatitis C. Although IFN-α reduced SARS-CoV replication in mice and NHPs [73, 74], efficacy of IFN-α treatment in

SARS patients was mixed (see Sect. 2.1.1). From in vitro studies, another type I interferon, IFN-β1a, may be more effective than IFN-α either alone or in combination with IFN-γ [75–77]. Combinations of IFN-β and -γ were synergistic against SARS-CoV in vitro [77].

With regards to MERS, in vitro and in vivo preclinical studies have indicated that IFN-α2b alone or in combination with ribavirin, may have a therapeutic effect if given early in disease [78, 79]. In clinical trials, however, IFN-α2b (given in combination with other treatments) did not lead to a significant benefit to patients (see Sect. 2.1.2). IFN-β1a (EC50 = 1.37 IU/mL) was superior in activity against MERS-CoV infection in vitro compared to IFN-α2a, IFN-α2b, and IFN-γ; these IFNs had EC50 values of 160.8, 21.4, and 56.5 IU/mL, respectively [80]. IFN-β1b is currently under evaluation for MERS-CoV in a randomized clinical trial (in combination with lopinavir/ritonavir) [32]. Investigating the IFN-β subtypes (1a and 1b) in combination with other antivirals may be worthwhile as potential synergistic combinations could reduce the effective drug dosage and IFN-associated adverse effects.

Kinase Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:

Many cellular processes are regulated independently of changes in transcription or translation through kinase-mediated cell signaling pathways. As a testament to the biological importance of kinases, there have been over 500 kinases identified along with more than 900 genes encoding proteins with kinase activity [81, 82]. As of April 2015, 28 kinase inhibitors have been granted approval by the US FDA with over half gaining approval from 2012–2015. Further, kinases are the most frequently targeted gene class in cancer therapy, second only to the G protein-coupled receptors as therapeutic targets [83, 84].

The therapeutic potential for host-targeted immunomodulatory agents in viral infections has received considerable attention [85–87]. Recently, Dyall et al. identified two Abelson (Abl) kinase inhibitors (imatinib and dasatinib) that inhibited MERS-CoV and SARS-CoV infection through a cell-screening assay [38]. Both compounds significantly inhibited MERS-CoV and SARS-CoV with micromolar EC50 values and low cytotoxicity. Abl2 has been identified as critical for MERS-CoV and SARS-CoV virus entry, and may be the target that imatinib inhibits to block entry of both viruses [88]. A recent systems kinome analysis investigation of in vitro MERS-CoV infection suggested that ERK/MAPK and PI3 K/Akt/mTOR signaling pathways were specifically modulated during infection [89]. Subsequent analysis of licensed kinase inhibitors targeting these pathways demonstrated that kinase inhibitors targeting the ERK/MAPK signal pathway (selumetinib and trametinib) inhibited MERS-CoV infection by ≥ 95% when added pre- or post-infection [89]. Further, trametinib demonstrated significantly stronger inhibitory activity against MERS-CoV than selumetinib suggesting that specific intermediates of the ERK/MAPK signaling pathway may represent crucial foci during early (viral entry) and late (viral replication) events in the viral life cycle. In contrast, sorafenib, an inhibitor of Raf-1 and B-Raf, components of the ERK/MAPK signaling pathway, and vascular endothelial growth factor receptor 2 (VEGFR2), inhibited MERS-CoV infection by > 90% when added to cells prior to infection; however, the inhibitory activity was reduced to < 30% when added post-infection suggesting Raf kinases were primarily involved in early viral life cycle events. In addition, the inhibitory activity dabrafenib, a Raf kinase inhibitor, was also largely ablated when added post-infection. Miltefosine, an alkyl phospholipid, considered to be an inhibitor of protein kinase B (Akt), garnered FDA approval for infectious disease-related treatments (cutaneous or mucosal leishmaniasis) [90]. In 2013, miltefosine became directly available from the US Centers for Diseases Control and Prevention for the treatment of free-living amoeba infections [91]. Pre-treatment of cells with miltefosine reduced MERS-CoV infection by 28%, but had no effect when added post-infection [89]. In contrast, inhibition of mTOR with sirolimus or everolimus reduced MERS-CoV infection by ~ 60% when added prior pre- or post-infection suggesting a critical role for mTOR in MERS-CoV infection. A recent clinical investigation by Wang et al. [92] evaluated sirolimus and corticosteroids in addition to standard antiviral treatment in a randomized controlled trial in patients with severe H1N1 pneumonia and acute respiratory failure [92]. Importantly, the addition of sirolimus was associated with improved patient outcomes including decreased hypoxia and multi-organ dysfunction, reduced mean times for liberation from mechanical ventilation, and increased clearance of virus. Thus, it may be prudent to extend the study of repurposed kinase inhibitors beyond stand-alone therapeutic investigations and also consider their potential as adjunctive therapies.

Neurotransmitter Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:

Numerous neurotransmitter receptors inhibitors showed activity against MERS-CoV and SARS-CoV infection [38]. These drugs were initially developed as antipsychotics, antihistamines, and sedatives. Five neurotransmitter receptor antagonists belong to the chemical class of phenothiazines: chlorpromazine, triflupromazine, thiethylperazine, promethazine and fluphenazine. Phenothiazines were breakthrough medications developed in the 1950s for treating mental health patients and reduced episodes of bizarre behavior, hallucinations, and irrational thoughts [93]. Although phenothiazines primarily block dopamine receptors, they also have anticholinergic, antihistamine, and antiemetic effects.

The phenothiazines, chlorpromazine and triflupromazine, are approved antipsychotics. Chlorpromazine has been used off-label for short-term treatment of nausea and migraines. Triflupromazine is used to treat severe emesis, but the drug has more serious side effects than chlorpromazine including akathisia and tardive dyskinesia. The antiviral effect of chlorpromazine has been extensively studied, and the drug interferes with clathrin-mediated endocytosis, a process that many viruses exploit for host cell entry. Chlorpromazine inhibits entry of Junin virus [94], West Nile virus [95], EBOV [96], HCV [97], and Japanese encephalitis virus [98] suggesting broad-spectrum activity that could be exploited early during a novel virus outbreak. Chlorpromazine may have similar effects on coronaviruses as the drug effectively inhibits MERS-CoV, SARS-CoV, and human coronavirus 229E expressing green fluorescent protein [36, 38]. However, time-of-addition studies indicate that the inhibitory activity against MERS-CoV is retained whether added pre- or post-infection suggesting that there are additional effects to clathrin-mediated entry impairment [36].

Thiethylperazine is an approved antiemetic. Both chlorpromazine and thiethylperazine have been shown to inhibit alphaviruses, Semliki forest virus (SFV) and chikungunya virus (CHIKV) [45, 99]. As these drugs cross the brain-blood barrier, use of these drugs could be beneficial in the treatment of CHIKV, including common neurologic complications. Promethazine is an antihistamine used as a sedative in many countries under different brand names, but also acts as a weak anti-psychotic activity. Fluphenazine is a common antipsychotic used to treat chronic psychoses (primarily schizophrenia) and belongs to the WHO Model List of Essential Medicines. Promethazine and fluphenazine have shown in vitro activity against MERS-CoV and SARS-CoV and may have value as candidates for repurposing for coronaviral infections [38].

Benztropine mesylate, an approved anticholinergic used to treat Parkinson's, had activity against MERS-CoV and SARS-CoV [38]. Benztropine was also identified in other screens of clinically approved drugs for antiviral activities against HCV and EBOV [100–102]. Although the detailed mechanism of action is unknown, HCV studies indicate that benztropine inhibits at a virus entry step, while not interfering with viral genome replication, transcription or production of viral progeny or virus production of viral progeny [102]. It has been argued that a virus entry inhibitor may have value in decreasing the incidence of relapse in chronic HCV patients that receive liver transplants [102]. However, the peak plasma concentrations of benztropine may be too low to be effective for treating an acute infection [103]. Benztropine was also independently identified in two drug screens for EBOV antivirals [100, 101]. A recent report suggests that a step after virus attachment, but prior to viral/cell membrane fusion is targeted by benztropine [104].

Clomipramine, a tricyclic antidepressant, and thiothixene, a thioxanthene antipsychotic, have also been shown to inhibit MERS-CoV and SARS-CoV infection in vitro [38]. In addition, they were found to inhibit EVD VLP entry [101]. Both are approved clinically, and clomipramine belongs to the WHO Model List of Essential Medicines.

Several other neurotransmitter inhibitors, astemizole, promethazine, chlorphenoxamine, and fluspirilene, were active against MERS-CoV and SARS-CoV in cell culture, but we were not able to find reports on activity against other viruses [38]. Astemizole is an H1-histamine receptor antagonist for treating allergic rhinitis that was withdrawn from the market. Cardiac adverse events due to drug overdose have been reported, but are extremely rare [105]. Recently, astemizole has gained renewed interest as an anticancer and antimalarial drug [106, 107]. Chlorphenoxamine is an antihistamine and anticholinergic that is currently in preclinical trials for malaria. Fluspirilene is an approved antipsychotic for treatment of schizophrenia. It is a known autophagy inducer [108]. Autophagy is a cellular degradative pathway that viruses exploit for their propagation [109]. Modulators of autophagy may perturb MERS-CoV or SARS-CoV infection, and investigation of their broad-spectrum potential for the treatment of coronaviral infections would be interesting [110].

Nucleic Acid Synthesis Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:

Several RNA/DNA synthesis inhibitors have broad-spectrum activity against viruses including SARS-CoV and MERS-CoV [38, 80, 111–114]. Inosine monophosphate dehydrogenase (IMPDH) inhibitors such as ribavirin, mycophenolic acid, and mizoribine inhibit an important step in de novo synthesis of nucleic acids although the potency of these drugs against viruses varies. Ribavirin has been used in combination with IFN in the clinic for treatment of viral infections such as hepatitis C. Treatment regimens with ribavirin are well characterized and have been used in SARS and MERS patients with mixed results (see 2.1). Ribavirin weakly inhibits MERS-CoV in vitro, and conflicting data have been reported for the activity of ribavirin against SARS-CoV [80, 111, 115]. Many of the studies on ribavirin were performed in Vero cells that reportedly have a defect in ribonucleoside uptake, which could explain lack of activity for ribavirin in these cells [116]. Another coronavirus, mouse hepatitis virus (MHV), becomes sensitive to ribavirin when its exoribouclease activity is inactivated. In presence of exoribonuclease, ribavirin does not inhibit MHV replication [117]. The MHV exoribonuclease has been suggested to function as a 'proofreading' viral enzyme that is necessary for high-fidelity replication of MHV. Similarly, the exoribonuclease activity of MERS-CoV and SARS-CoV could possibly counteract inhibitory activity of ribavirin.

Mycophenolic acid (MPA), an immunosuppressant used to prevent organ rejection, has broad-spectrum antiviral activities, and antifungal, antibacterial, anticancer, and antipsoriatic properties [45, 118, 119]. Although MPA has weak inhibitory activity against SARS-CoV in vitro, it has promising activity against MERS-CoV [80, 112]. A potential alternative to MPA, the prodrug mycophenolate mofetil, has improved oral bioavailability [120]. Mycophenolate mofetil evaluated in the common marmoset model of MERS did not reduce disease manifestations compared to that observed in control subjects [121]. However, the MERS marmoset model does not recapitulate human disease due to its rapid onset and pathology associated with exposure methods [122]. Mizoribine, an approved immunosuppressant in organ transplantation with limited adverse side effects, has shown in vitro activity against HCV and bovine viral diarrhea virus (BVDV), and was considered as an alternative to ribavirin/IFN combinations for treatment of HCV infections [123]. In vivo analysis of ribavirin and other IMPDH inhibitors in SARS-CoV-infected mice have suggested that these agents would be of limited benefit [111].

The chemotherapeutic gemcitabine, has shown in vitro activity against MERS-CoV and SARS-CoV [38]. The drug's anti-cancer mechanism is attributed to its ability to inhibit ribonucleotide reductase essential for de novo pyrimidine biosynthesis. Gemcitabine has been shown to suppress influenza virus RNA transcription and replication by targeting ribonucleotide reductase and showed anti-retroviral activity in vivo in the mouse model for murine leukemia virus [124, 125].

Protease Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:

MERS-CoV and SARS-CoV require activation of their envelope glycoproteins by host proteases for cell entry by the endosomal or the non-endosomal pathways. Inhibitors of host cell proteases are being investigated as possible antivirals [126]. The serine protease TMPRRSS2 mediates entry via the non-endosomal pathway for both MERS-CoV and SARS-CoV [50, 127–129]. Camostat mesylate, which has been used in the treatment of chronic pancreatitis, inhibits TMPSSR2-mediated glycoprotein activation of MERS-CoV, SARS-CoV, and influenza virus [126, 130–132]. K11777, a cysteine protease inhibitor, is in clinical development for treating parasitic infections. K11777 has broad-spectrum activity against coronaviruses (MERS-CoV, SARS-CoV, HCoV-229E), filoviruses (EBOV, Marburg virus), and paramyxoviruses (Nipah virus) [133]. Interestingly, Zhou et al. [133] demonstrated that Camostat and K11777 had inhibitory activity against SARS-CoV whereas EBOV was only inhibited by K11777, suggesting differential host protease requirements for these viruses [133]. E-64-D, an inhibitor of an endosomal cysteine protease currently in Phase III trials for the treatment of muscular dystrophy, inhibits both MERS-CoV and SARS-CoV in vitro [38]. E-64-D also inhibits filovirus cell entry [134, 135]. The dependency of viruses for specific serine or cysteine host proteases must be considered in the selection of protease inhibitors for antiviral therapeutic applications. Therefore, an increased understanding of the relationship between host proteases and viral pathogenesis will determine the most effective treatment options for viral infections.

Lopinavir was identified as an inhibitor of MERS-CoV and SARS-CoV in vitro, and time-of-addition experiments indicate that the drug acts at an early stage of viral entry [36]. Lopinavir, an inhibitor of the HIV protease, is used clinically for the treatment of HIV infections [136]. It is given in combination with ritonavir, an inhibitor of cytochrome P450 3A4, to increase blood concentrations because of the low bioavailability of lopinavir [136]. Lopinavir also inhibits human papilloma virus

and is currently under development for the topical treatment of cervical cancer [137]. Treatment with lopinavir/ritonavir resulted in reduced mortality in a NHP model of MERS [121]. Lopinavir has been shown to target the main protease (Mpro) of SARS-CoV [138]. However, lopinavir has also been shown to act on other intracellular processes that are involved in coronavirus replication. Additional studies are needed to fully understand the mechanism of action of lopinavir involving cellular proteases. During the 2003 SARS outbreak, patients in open clinical trials were treated with lopinavir/ritonavir in combination with ribavirin had a milder disease course and reduction in fatality rate compared to that observed with historical controls [27, 28].

Protein Synthesis Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
Three protein synthesis inhibitors with activity against coronaviruses were identified, emetine, anisomycin and omacetaxine mepesuccinate [38, 139]. Emetine, a natural plant alkaloid, and anisomycin, an antibiotic, both inhibit protein elongation and were identified as anti-protozoals [140, 141]. While emetine is approved for amoebiasis treatment, anisomycin did not move beyond clinical trials [141, 142]. Dehydroemetine, a synthetic emetine derivative, has fewer side effects and is available as an investigational new drug [143, 144]. Anisomycin was originally discovered as a peptidyl transferase inhibitor, but also activates the MAP kinase signaling pathway [145]. In addition to activity against MERS-CoV and SARS-CoV, emetine and anisomycin inhibit the animal picornavirus encephalomyocarditis virus [146]. Anisomycin has in vitro activity against poliovirus [147]. Omacetaxine mepesuccinate, a plant-derived alkaloid, is an anticancer therapeutic that received FDA approval in 2012 for the treatment of chronic myeloid leukemia. Omacetaxine inhibits MERS-CoV, bovine coronavirus, human enteric coronavirus and hepatitis B virus [139, 148]. In spite of omacetaxine broad spectrum anti-coronavirus activity, the drug had no activity against SARS-CoV [38]. Drugs that inhibit coronaviruses by targeting protein synthesis may have potential in the development of combination therapies with drugs that target other antiviral pathways.

Selective Estrogen Receptor Modulators ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
Recent investigations have demonstrated the potential of estrogen receptor (ER) antagonists for repurposing as anti-coronavirus compounds [38]. For example, toremifene citrate and tamoxifen citrate with activity against SARS-CoV and MERS-CoV were developed and approved as anticancer therapeutics. Both drugs have shown activity against HCV replication in vitro [149, 150]. Mechanistic studies revealed that the ER is functionally associated with HCV replication [150]. ER promotes the interaction between the HCV replication complex and the HCV polymerase NS5B. ER–mediated regulation of HCV genome replication is abrogated by tamoxifen. Toremifene and tamoxifen also effectively inhibit EBOV infection in vitro [151]. However, in contrast to HCV, mechanistic studies have shown that toremifene-mediated EBOV inhibition is independent of the ER pathway as toremifene was still active against EBOV in cells that did not express ER [151, 152]. Toremifene acts at a late step of virus entry after internalization of EBOV and may prevent fusion between the viral and endosomal membranes [151–153]. Based on the chemical structure, toremifene is a cationic amphiphilic drug (CAD) that is known to be lysosomotropic and could affect endosomal processes during virus entry [151, 154]. Treatment with toremifene led to 50% survival of EBOV–infected mice confirming that this drug has an effect in vivo as well [151].
In terms of clinical application, toremifene and tamoxifen have good bioavailability, safety and tolerability profiles combined with a long history of use in the clinic. However, prolongation of the QT interval has been noted for toremifene and should not be prescribed to patients with congenital or acquired long QT syndrome, uncorrected hypokalemia or uncorrected hypomagnesemia [155]. Tamoxifen can increase uterine malignancies, stroke and pulmonary embolism in women with ductal carcinoma in situ or at high risk for breast cancer [156]. Despite these side effects, the drugs may have substantial value for short-term treatment of acute coronaviral infections. Advanced patient studies and careful evaluation of the pharmacokinetic profiles may facilitate dosing strategies that limit the risk of adverse events.

Sterol Metabolism Inhibitors ::: Drugs with Repurposing Potential for Treatment of Coronaviral Infections ::: Therapeutic Agents:
Two sterol synthesis inhibitors, terconazole and triparanol, have shown activity against MERS-CoV and SARS-CoV [38]. Studies with virus-like particles (VLPs) have demonstrated that

terconazole inhibits coronavirus cell entry, including MERS-CoV and SARS-CoV. The sterol synthesis pathway has been shown to be required for infection by several viruses including HCV [157, 158]. Terconazole, approved for vaginal yeast infections, can be administered orally, topically or by suppository. Triparanol was approved for lowering plasma cholesterol, but was withdrawn due to numerous side effects. Both are CADs that induce accumulation of cholesterol in late endosomes and have been shown to inhibit EBOV entry [152].

Potential Targets for Inhibition of MERS-CoV and SARS-CoV ::: Drugs in Development ::: Therapeutic Agents:
In addition to drug repurposing, development of novel antiviral countermeasures is needed for emerging coronaviruses. To this end, design or development strategies have targeted the viral replication cycle and host pathways essential for viral replication (Table 4). Two nucleoside inhibitors of viral RNA-dependent RNA polymerases, GS-5734 and BCX4430, have potential as broad-spectrum antivirals [159, 160]. Both drugs are active against MERS-CoV and SARS-CoV in cell culture, but in vivo efficacy remains to be investigated. In addition, a new class of nucleosides with a flexible purine base has anti-coronaviral activity, and further optimization could generate potent inhibitors of the coronaviral polymerase [161]. The surface glycoprotein (S) of SARS-CoV and MERS-CoV and other coronaviruses consists of two domains: S1, containing the receptor-binding domain (RBD) needed for extracellular binding; and S2, containing the fusion peptide needed for membrane fusion and release. Endocytosis of SARS-CoV is facilitated by the binding of RBD with the angiotensin converting enzyme 2 (ACE2) receptor on host cells. Membrane-bound cathepsin L cleaves the S protein revealing the S2 fusion protein, which fuses with the membrane and releases the viral RNA. Inhibitors of cathepsin L, the ACE2–SARS–S1 complex, or the S2 fusion peptide could be suitable targets to inhibit SARS-CoV entry [162]. Results from recent studies have identified inhibitors of viral entry, viral proteases, and helicases that potently inhibit both MERS-CoV and SARS-CoV [162]. Proteases, such as papain-like protease and 3C-like protease, could also be useful as antiviral targets for drug development as they are required for cleaving non-structural proteins for viral maturation. Most protease inhibitors are "suicide" protease inhibitors that bind to the target irreversibly. However, reversible protease inhibitors may have greater potential as they are less toxic and better tolerated [113]. Recent studies with helicase inhibitors show that three domain targets, N-terminal metal binding domain, a hinge domain, and a NTP/helicase domain, have potential for the development of new drugs [163].

RNA Interference ::: Drugs in Development ::: Therapeutic Agents:
Directed RNA interference (RNAi) presents a powerful approach for the development of novel virus-specific therapeutics based on gene silencing [164]. Recent studies have shown that small interfering RNAs (siRNAs) or short hairpin RNAs can inhibit expression of viral genes and thereby block the replication of SARS-CoV in cultured cells [165–172]. Intranasal delivery of a combination of small interfering RNA (siSC2-5) targeting SARS-CoV open reading frame 1 and S protein decreased SARS pathogenesis in NHPs [173]. Potential RNAi candidates identified through computational modeling for MERS-CoV require further in vitro and preclinical investigation [174]. Several RNAi therapeutics for the treatment of viral infections have entered clinical trials including TKM-Ebola, a siRNA/lipid nanoparticle platform targeting EBOV [164, 175, 176]. This technology has great potential for therapeutics for emerging viruses as viral genome sequencing can now be completed in a very short time frame that is crucial in situations when an epidemic of a novel emerging viral infection unfolds. The main obstacle for RNAi strategies lies in the identification of suitable viral targets and in the delivery efficiency of nucleic acids to target cells in vivo.

Peptide Entry Inhibitors ::: Drugs in Development ::: Therapeutic Agents:
Peptides share common physicochemical properties with CADs that facilitate interaction with cell membranes and interference with the fusion of cellular and viral membranes during virus entry. Researchers are making progress in defining the mechanism of action of peptide entry inhibitors of enveloped viruses such as enfuvirtide, an approved HIV inhibitor [177]. Enfuvirtide is a 36-residue peptide derived from the amphipathic loop/C-helix heptad repeat domain of HIV gp41. A rational approach based on scanning fusion protein sequences for amphipathic sequences has led to the discovery of additional peptide inhibitors for other viruses including MERS-CoV and SARS-CoV [178–180]. Chemical modifications of peptides have increased their in vivo stability and bioavailability, improving their potential for clinical applications as novel broad-spectrum viral entry inhibitors [181–183].

Antibody Therapy ::: Therapeutic Agents:

The success of palivizumab for treatment of respiratory syncytial virus infection has reinvigorated efforts to develop monoclonal antibody-based therapeutics for infectious diseases [184]. ZMapp, a monoclonal antibody cocktail targeting EBOV, has been tested in NHPs with success and was moved to Phase I and II clinical trials in humans during the EVD epidemic [185, 186]. Similarly, a monoclonal antibody against Hendra virus has been administered to humans on a "compassionate use" basis [187–189]. These examples demonstrate the potential for antibody therapy to combat emerging/re-emerging viruses, and similar strategies have been pursued by multiple groups for development of antibodies to MERS-CoV [190–197]. Monoclonal antibodies to MERS-CoV have been sourced from humanized mice libraries or human antibody libraries [192, 194, 197, 198]. Antibodies target the S RBD, S1, or S2 subunits, and have demonstrated efficacy in animal models as reviewed in Ying et al. [199]. Monoclonal antibody therapy can impart a selective pressure for generation of resistant viruses. Although mutants escaping monoclonal antibody pressure tend to be less fit, analysis of the emergence of mutations that confer resistance to the monoclonal antibody will need to be performed.

An alternative to monoclonal antibody therapy is polyclonal antibody therapy using convalescent sera (sera sourced from a nonhuman or humanized animal). Polyclonal antibodies provide an advantage over monoclonal antibodies in that escape mutants are less likely to emerge [200, 201]. Convalescent sera have been recommended for MERS, and a Phase II interventional clinical trial is ongoing to determine efficacy [198]. However, the availability of a suitable donor subject presents a significant complication for this approach. Nonhuman animal sera has been considered, but safety concerns limit this option. Fractionation of nonhuman sera is an alternative; however, antibody-mediated clearance can be limited due to failure of the human Fc receptors to recognize the antibody heavy chain.

An alternative is de-speciating the antibodies by using only the Fab antibody fragment; however, cost and sufficient material may make mass production of Fab fragments difficult. The use of sera from humanized mice or other small laboratory animals is complicated by sample acquisition/volume restraints. Larger laboratory animals may provide a potential alternative. SAB Biotherapeutics has developed a trans-chromosomic bovine platform for the generation of human IgG antibodies [201]. Vaccination of trans-chromosomic cattle with S protein nanoparticles or inactivated, whole virus generated fully humanized polyclonal antibodies that demonstrated efficacy in the Ad-DPP4 murine model of MERS.

AIDS ::: Lessons Learned:

Despite extensive efforts over the past 30 years, a therapeutic or prophylactic HIV vaccine has remained elusive. Antiviral agents are the only available treatments for AIDS. Over 25 antivirals belonging to 6 different drug classes targeting different stages of viral replication are available (e.g. reverse transcriptase, protease, fusion, entry, integrase) [203]. Combination treatments with 2 to 3 drugs are effective and result in a sustained virologic response [204]. Two aspects have been found to be important for avoiding resistance: (1) selecting drugs with at least two different targets, and (2) selecting drugs that belong to different chemical classes. These considerations may also apply for drug combinations with synergistic effects against MERS- and SARS-CoV.

Hepatitis C ::: Lessons Learned:

Broad-spectrum antiviral therapies can be of great value for treating emerging infections when it takes time to develop direct-acting antivirals. For treatment of chronic hepatitis C, clinicians have depended on IFN and ribavirin for a number of years [205]. Eventually, IFN and ribavirin combination was replaced by very effective fixed-combination therapies using direct-acting antivirals that target multiple steps of the HCV life cycle [206]. IFN and ribavirin contribute significantly to the treatment of viral infections for which no direct-acting antivirals exist. However, they have major side effects [207]. More options for broad-spectrum antivirals with improved safety profiles would be beneficial for use for emerging coronavirus infections.

Influenza ::: Lessons Learned:

Influenza viruses are characterized by a high mutation rate of the RNA genome. As available vaccines may not be protective against a novel pandemic strain, antiviral agents are considered an essential component for preparedness. Combinations of direct-acting antivirals are under evaluation for additive or synergistic effects and prevention of resistance [208, 209]. One triple

combination (oseltamivir, amantadine and ribavirin) is synergistic and prevents resistance in vivo [210, 211], highlighting the potential of combinatorial therapy.

Ebola Virus Disease ::: Lessons Learned:
The recent epidemic of EVD in Western Africa has renewed the urgency for development of treatments against emerging viruses. Although vaccines and direct-acting antiviral treatment are under investigation, none are approved for clinical use [212–216]. The WHO prioritized a panel of drugs approved for other indications that were considered for repurposing under FDA's Emergency Use Authorization [62]. Two of these drugs also have activity against MERS-CoV and SARS-CoV, amodiaquine (antimalarial agent) and toremifene citrate (breast cancer treatment). Additional broad-spectrum antiviral agents (including repurposed drugs) should be a top priority for future emerging infections including coronavirus infections. A panel of broad-spectrum drugs that have been carefully validated for efficacy and safety and that could be used in combination would supply a minimum of protection for patients and healthcare workers at outbreak locations. This panel of drugs could be used in situations of a known re-emerging pathogen for which specific antiviral agents and vaccines has not been approved or of an unknown novel pathogen that could arise.

Animal Models of MERS and SARS ::: Gaps in Knowledge and Future Outlook:
Effective development of countermeasures depends on developing appropriate animal models that uniformly recapitulate human disease progression and severity of pathological manifestations. As with most animal models of human disease, no one animal model fully reflects SARS or MERS, therefore researchers are faced with exploring several small animal models or choosing the "best-fit" model. To date, animal models do not fully recapitulate human disease, thus animal models of MERS and SARS need further refinement. Many small animal models have been evaluated as potential MERS and SARS models including mice, hamsters, and ferrets for MERS and Syrian hamsters, and guinea pigs for SARS [217–219]. Four murine models have been reported for MERS. The first model that demonstrated promise involved transduction of the respiratory tract with the putative MERS-CoV receptor, human DPP4 (or CD26) [220]. The major indicator of disease in this model is viral load in the lung at 4 days post-inoculation. Although clinical signs of disease, including weight loss, were limited in this model, it has been used for pathogenesis countermeasure studies [221, 222]. Lethal, disseminated MERS infection has been demonstrated in transgenic mice expressing human DPP4 [223]. Inflammatory processes were observed in the brains of these mice in contrast to human disease in which CNS involvement has not been reported. A transgenic mouse MERS model was developed in which the mouse DPP4 gene was replaced by the human DPP4 gene under control of the endogenous mouse DPP4 promoter. Using this model, MERS-CoV-infected mice developed lung pathology [194]. In addition, administration of human monoclonal antibodies against the spike protein in these transgenic mice provided protection against MERS-CoV infection [194]. Clustered regularly interspaced short palindromic repeat-CRISPR-associated protein 9 (CRISPR-CAS9) gene editing technology was used to modify the mouse DPP4 to match human DPP4 by altering amino acids at positions 288 and 330. Interestingly, wild type virus infection of these mice did not result in an improved model of MERS. However, serial passage of MERS-CoV resulted in MERS-CoV-15. Intranasal exposure of MERS-CoV-15 in 288/330, mice led to weight loss and a severe respiratory disease that included ARDS-like signs and reduced pulmonary function [224].
MERS-CoV infection of rabbits has also been evaluated as a model for MERS. Haagmans et al. [225] demonstrated that MERS-CoV infected rabbits did not develop obvious clinical signs, but infectious virus could be detected in the upper respiratory tract [225]. Furthermore, epithelial cells of the bronchioles and terminal bronchioles respiratory tract were positive for MERS-CoV by immunohistochemistry and in-situ hybridization, which reflects tissue tropism in human disease [225]. Using the rabbit model, Houser et al. [191] demonstrated that human monoclonal antibody 336 given pre-exposure reduced viral RNA lung titer at 3 days post-exposure, but not when given post-exposure [191].
Due to phylogenetic similarities with humans, NHP models of disease have long been considered as necessary for evaluation of countermeasures to infectious diseases. Rhesus monkey and common marmoset have been evaluated as potential models for MERS. Following intratracheal instillation of MERS-CoV in rhesus monkey models, lung pathology was observed [122, 226–228]. Experiments using rhesus monkeys have indicated that they develop limited systemic disease and a transient respiratory disease. Radiologic evaluations have indicated inflammatory infiltrates

that develop shortly after exposure. Analysis of lung tissues by reverse transcriptase- quantitative polymerase chain reaction indicated virus replication in the lung.

Similar to MERS, African green monkeys (AGMs), rhesus monkeys, cynomolgus monkeys, and common marmosets have been identified as potential models for SARS [229]. Smits et al. [230] compared SARS CoV infection in young AGMs to cynomolgus monkeys, they observed that neither species developed clinical signs during a 4-day experiment [230]. Gross pathology indicated multi-focal pulmonary consolidation with consolidated grey-red firm lungs. These lesions affected 30% of the lungs in one subject. By comparison, the cynomolgus monkeys developed small patchy macroscopic lesions. Similar to MERS-CoV infection of NHPs, viral load decreases from exposure day to day 4. A comparison between AGMs, rhesus monkeys, and cynomolgus monkeys further support AGMs as the best available NHP model for SARS [231]. AGMs developed the highest viral load and most disease when compared to cynomolgus and rhesus monkeys. Lethal disease was not observed in any of these species; therefore, further development of the SARS model is warranted since lethal respiratory tract disease was the hallmark of SARS.

As an alternative to Old World NHPs, many groups have employed marmosets as models of human infectious disease [232–234]. Common marmosets have been evaluated as a MERS model [121, 122, 235]. These studies have demonstrated that common marmosets develop disease following exposure to MERS-CoV as shown by histopathological analysis, radiological analysis, and RT-qPCR. However, variable results have been reported, and exposure methodology can impact disease progression. Therefore, mock-infected groups must be included to account for pathological artifacts. The virus-specific pathology could be quantified using computed tomography, and future experiments using large group sizes could be used for countermeasure evaluation. Greenough et al. [236] performed a serial euthanasia study of SARS-CoV infected marmosets [236]. Subjects were intratracheally exposed with SARS-CoV. They observed mild inconsistent clinical signs of disease. Viral loads peaked at day 4 post-infection. Histopathology indicated interstitial pneumonitis with multinucleated syncytia that were described as mild and not observed in all late time-point subjects. Overall, further research is needed to develop animal models of SARS that reflect human disease presentation.

Combinations with Synergy ::: Gaps in Knowledge and Future Outlook:

Drugs with repurposing potential discussed here (Table 3) have the advantage of easy access, availability and decreased cost of development and provide a wide array of options for combination studies. The pharmacological knowledge available for such compounds may also reduce concerns regarding adverse effects in patients. The generation of a translational database encompassing pharmacodynamics data and infectious disease biology data has been proposed and would greatly facilitate decision making to pursue new drug combinations [237]. Many of the drugs have potential for broad-spectrum antiviral activity and have already been in clinical use for treating other viral infections. As novel drugs in development move from the pre-clinical to clinical phase, they also become available for combination therapy. Care should be taken with the pharmacological evaluation of each combination to avoid possible contraindications of the drugs with regards to disease or adverse effects. Novel broad-spectrum replication inhibitors, such as GS-5734 (Gilead Sciences, in Phase I clinical trial), immunomodulators (nitazoxanide; steroids; statins) along with direct-acting antiviral agents for coronaviruses that are in development represent interesting partners for combinations. Combinations can involve broad-spectrum versus specific antiviral agents; drugs with different mechanism of action; or drugs that target different steps of the viral life cycle. Identifying one or more potent combinations with activity in an animal model would greatly increase preparedness for the next coronavirus outbreak.

Structure-Based Drug Design ::: Gaps in Knowledge and Future Outlook:

Elucidation of the crystal structure of viral proteins has led to novel approaches for rational drug design. Rational design investigations using protein structure information and in silico screening for affinity to active sites of viral proteins holds promise. HIV-1 protease inhibitors have been one of the big successes of rational drug design. Only 6 years after the publication of the HIV-1 protease structure, saquinavir was developed in record time from bench to bedside and was licensed for use against AIDS in 1995 [238–240]. In total, six antivirals against the HIV-1 protease were designed and approved between 1995 and 2000. Similarly, for HCV, computer–aided approaches based on the known crystal structure of a viral protein have successfully guided the design and synthesis of inhibitors for the HCV NS3/NS4A proteases such as the peptidomimetics, telaprevir and boceprivir [241–243].

Due to the power of computational modeling using crystal structures from known coronaviruses, the crystal structures for the viral proteases, Mpro and PLpro, of SARS-CoV and MERS-CoV were determined relatively quickly [244]. These structures have already been used for the discovery of inhibitors with high binding affinity to the active site of the proteases. The structures of additional MERS-CoV or SARS-CoV viral proteins have yet to be determined and would offer additional viral targets for drug discovery.

Structural design can help with development of inhibitors in preparing for future outbreaks of yet unknown emerging coronaviruses. Based on the potential of zoonotic transmission of coronaviruses from bats to humans, crystal structures for the main protease (Mpro) of different bat coronavirus families have been proposed for screening and identifying broad-spectrum antiviral agents [244]. Proof-of-principle was shown for the novel protease inhibitor SG85, which inhibits the bat coronavirus HKU4 [244]. In 2012, SG85 was quickly identified as an inhibitor of MERS-CoV through in silico docking studies with the Mpro of MERS-CoV and the bat coronavirus HKU4.

Cationic Amphiphilic Drugs: A Novel Class of Antiviral Agents? ::: Gaps in Knowledge and Future Outlook:

Several drugs (e.g. toremifene citrate, terconazole, Fig. 3) belong to a group of compounds termed cationic amphiphilic drugs (CADs) [100, 151, 152, 154, 245]. Phenothiazines (e.g. fluphenazine chlorpromazine, Fig. 3) are CADs that have been shown to inhibit HCV entry at virus-host cell fusion by intercalating into the cholesterol-rich domains of the host cell membrane and increasing membrane fluidity [97]. Drugs that act through this mechanism may present an interesting new class of broad-spectrum antivirals. CADs are known to be lysomotropic and accumulate in acidic compartments where their tertiary amine groups are protonated. The compounds act as mild bases and can neutralize the low pH of the acidic environment of endo/lysosomes. CADs can intercalate into membranes, alter the biophysical properties of membranes and thereby could potentially interfere with fusion of virus with the endo/lysosomal membrane. The concept of interfering with virus entry and budding through physicochemical properties of drugs is intriguing. Many viruses would be susceptible to this type of inhibition and CADs could be used as broad-spectrum antiviral agents. Detailed structure-activity relationship studies on CADs will be required to determine the chemical core structures and physicochemical properties important for this type of antiviral agent. Future investigations regarding the conservation of this mechanism of action to coronaviruses, as well as other emerging viruses, are warranted.

Analogs of Developed Drugs ::: Gaps in Knowledge and Future Outlook:

Approved drugs with activity against MERS-CoV or SARS-CoV could be used as lead compounds for further antiviral drug development. Pharmaceuticals usually have undergone multiple rounds of structure-activity relationship studies generating analogs to improve drug activity against the original indication or target. The analogs of drugs with activity against MERS-CoV and SARS-CoV may still be available and could be screened to identify other analogs with increased antiviral activity. Although drug analogs would have to go through the full licensure process, there would be little to no added initial cost associated with producing the structure-activity relationship compounds. Recycling old analogs is one approach that may have value for developing novel drugs for viral infections.

Conclusion:

A more streamlined process is needed for development of effective treatment measures for emerging and re-emerging pathogens. The availability of a panel of approved broad-spectrum drugs would clearly be beneficial as they could be used for treating disease symptoms and reducing morbidity until more specific acting antivirals and vaccines are developed.

A large number of potential drugs and therapeutics for the treatment of MERS and SARS have been discussed. The greatest challenge will be how best to down-select and evaluate the different approaches. As we have learned from drug development for AIDS and hepatitis, alleviating disease symptoms and increasing life span may be a more achievable goal rather than looking for a treatment that will provide complete recovery. Broad-spectrum antivirals, specific antivirals, and immune modulators each have an important role in treating viral infections either individually or in combination. Effective communication between the different institute partners (government, industry, academic; national, and international partners) is essential. Combining these drug discovery efforts will increase the chance of having one or more potential therapeutic agents at an advanced development stage by the time another outbreak of an emerging coronavirus occurs.