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MINIREVIEW

Compounds with Therapeutic Potential against Novel Respiratory 2019 Coronavirus

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ABSTRACT Currently, the expansion of the novel human respiratory coronavirus (known as SARS-CoV-2 [severe acute respiratory syndrome coronavirus 2], COVID-2019 [coronavirus disease 2019], or 2019-nCoV [2019 novel coronavirus]) has stressed the need for therapeutic alternatives to alleviate and stop this new epidemic. The previous epidemics of infections by high-morbidity human coronaviruses, such as SARS-CoV in 2003 and the Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, prompted the characterization of compounds that could be potentially active against the currently emerging novel coronavirus, SARS-CoV-2. The most promising compound is remdesivir (GS-5734), a nucleotide analog prodrug currently in clinical trials for treating Ebola virus infections. Remdesivir inhibited the replication of SARS-CoV and MERS-CoV in tissue cultures, and it displayed efficacy in nonhuman animal models. In addition, a combination of the human immunodeficiency virus type 1 (HIV-1) protease inhibitors lopinavir/ritonavir and interferon beta (LPV/RTV–IFN-β) was shown to be effective in patients infected with SARS-CoV. LPV/RTV–IFN-β also improved clinical parameters in marmosets and mice infected with MERS-CoV. Remarkably, the therapeutic efficacy of remdesivir appeared to be superior to that of LPV/RTV–IFN-β against MERS-CoV in a transgenic humanized mouse model. The relatively high mortality rates associated with these three novel human coronavirus infections, SARS-CoV, MERS-CoV, and SARS-CoV-2, have suggested that proinflammatory responses might play a role in the pathogenesis. It remains unknown whether the generated inflammatory state should be targeted. Therapeutics that target the coronavirus alone might not be able to reverse highly pathogenic infections. This minireview aims to provide a summary of therapeutic compounds that have shown potential in fighting SARS-CoV-2 infections.

KEYWORDS SARS-CoV-2, antiviral agents, coronavirus

On 30 December 2019, a cluster of 27 pneumonia cases (including 7 severe cases) of unknown origin emerged in Wuhan (Hubei, China) and were reported to the National Health Commission of China (1). In the early stages of this pneumonia, patients developed severe acute respiratory infection symptoms, and some patients rapidly developed acute respiratory distress syndrome (2). Real-time PCR (RT-PCR) and deep sequencing analysis of lower respiratory tract samples identified a novel human coronavirus (HCoV), now called SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (3–5). By the end of January 2020, nearly 50,000 confirmed cases were reported in China, and the first confirmed cases were reported in Thailand, Nepal, Republic of Korea, the United States, Singapore, France, Vietnam, Canada, Australia, Malaysia, Germany, The United Arab Emirates (UAE), Finland, Italy, Cambodia, Sri Lanka, the Russian Federation, Spain, Sweden, India, and the Philippines. Among the patients with confirmed cases, most (80%) were aged 30 to 80 years and had mild infections. The fatality rate was around 2% (6).

Coronaviruses can cause different types of infections in diverse animals. In humans,

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they mainly produce respiratory tract infections, as observed with SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) (7, 8). Sequencing and phylogenetic analyses have shown that the novel SARS-CoV-2 strain is closely related to a group of human SARS-like coronaviruses and bat SARS-related coronaviruses (9–11). The origin of SARS-CoV-2 remains unclear; it is unknown how it was first transmitted to humans. The high prevalence of SARS-related coronaviruses in bats has suggested that a bat coronavirus might have jumped into a civet or some other mammal, and from there to humans, which started the former 2003 SARS (2003-SARS) epidemic. Initial confirmed cases of SARS-CoV-2 were associated with Huanan seafood and live-animal markets. However, no animal source has been identified to date, and spillover events may continue to occur. Although bats might be the source of SARS-CoV-2, it is critical to identify the intermediate species to stop the current spread and to prevent future human SARS-related coronavirus epidemics.

A key issue is whether the current SARS-CoV-2 epidemic is similar to other SARS outbreaks or whether it shows different features. The epidemiological and clinical characteristics of SARS-CoV-2 indicate that this new outbreak is different from the 2003-SARS outbreak. SARS-CoV-2 displays higher transmissibility and lower mortality than 2003-SARS (1, 3, 4). SARS-CoV-2 has shown efficient intrafamilial spread (4). The asymptomatic period of SARS-CoV-2 infections oscillates between 2 and 14 days, and some individuals probably transmit the virus without developing any disease symptoms. It remains to be elucidated whether this virus replicates more readily in the upper airway than SARS-CoV and MERS-CoV and whether it is similar to other human coronaviruses (HCoVs) that cause colds but not pneumonia. It will be necessary to identify molecular determinants that mediate transmission from animal to human and from human to human. Of note, in the novel SARS-CoV-2 strain, the nucleotide sequence of the external ectodomain in the spike protein receptor-binding domain is different from that of the 2003 SARS-CoV. When individual bat coronavirus spike genes were introduced into SARS-CoV infectious clones, the SARS-CoV/bat-CoV spike viruses could bind to the human, bat, or civet angiotensin converting enzyme 2 (ACE2) cellular receptor (12). Understanding the interaction between this novel SARS-CoV-2 spike protein and the host ACE2 receptor might reveal how this virus overcame the species barrier between animals and humans. As discussed below, this information might promote the design of effective antivirals.

To predict new zoonotic coronavirus jumps across species and to understand the rate of virus spread among people, it is crucial to determine whether SARS-CoV-2 is mutating to improve its binding to human receptors for infection. As an RNA virus, SARS-CoV-2 has intrinsic genetic variability, which results in a high mutation rate. Moreover, coronaviruses have the largest genomes (~30 kb) among RNA viruses. However, part of their sequence encodes a proofreading 3′ exonuclease that can increase replication fidelity (13). It has been suggested that any adaptation in the SARS-CoV-2 sequence that might make it more efficient at transmitting from person to person might also increase its virulence (14). However, this mechanism could lead to a genetic bottleneck, known as Muller's ratchet, which could significantly decrease viral fitness (15). Muller's ratchet predicts that, when mutation rates are high and a significant proportion of mutations are deleterious, a type of irreversible ratchet mechanism gradually reduces the mean fitness of small populations of asexual organisms. Because genetic bottlenecks for RNA viruses often occur during respiratory droplet transmissions, the SARS-CoV-2 is expected to become less virulent through human-to-human transmissions (16).

From the public health perspective, we urgently need to develop an effective vaccine and antiviral therapeutics to stop the SARS-CoV-2 epidemic. Moreover, social and economic issues generated by this epidemic also call for rapid interventions. This review focuses on the potential of repurposing preexisting compounds that might provide new opportunities for treating people infected with SARS-CoV-2. Previous work with SARS-CoV and MERS-CoV has provided an opportunity to accelerate the identification of meaningful therapies for fighting the novel SARS-CoV-2 epidemic. Neverthe-

less, we must be aware that, currently, no compound that targets SARS-CoV or MERS-CoV has moved beyond phase 1 trials.

The most promising antiviral for fighting SARS-CoV-2 is remdesivir (GS-5734). Remdesivir is an adenosine nucleotide analogue prodrug with broad-spectrum antiviral activity against filoviruses, paramyxoviruses, pneumoviruses, and pathogenic coronaviruses, like SARS-CoV and MERS-CoV (17). Pharmacokinetic studies have been completed and clinical trials are ongoing for testing remdesivir efficacy in treating Ebola virus (18). Previous studies have indicated that nucleotide analogues generally show low efficacy against coronaviruses, due to the presence of the virus exonuclease proofreading enzyme. Nevertheless, remdesivir was found to be effective against SARS-CoV, MERS-CoV, and bat CoV strains (17). In tissue cultures, remdesivir displayed half-maximal effective concentrations (EC_{50}s) of 0.069 μM for SARS-CoV and 0.074 μM for MERS-CoV. Of note, tissue culture studies have shown that remdesivir is also active in the submicromolar EC_{50} range against a number of highly divergent coronaviruses, including the endemic human CoVs OC43 (HCoV-OC43) and 229E (HCoV-229E). Thus, remdesivir has broad-spectrum anti-CoV activity (19). In a mouse model of SARS-CoV pathogenesis, prophylactic and early therapeutic administration of remdesivir significantly reduced the lung viral load. Viral titers were reduced by $>$2 orders of magnitude on day 4 or 5 postinfection. Remdesivir improved the clinical signs of disease and respiratory function compared to untreated control animals (17). Comparable results were obtained with MERS-CoV in prophylactic studies carried out with a MERS-CoV mouse transgenic model. In that model, a humanized MERS-CoV receptor (humanized dipeptidyl peptidase 4 [hDPP4]) was expressed and carboxylesterase 1c (Ces1c) was deleted to improve the pharmacokinetics of nucleotide prodrugs (20). Remdesivir specificity for coronavirus was demonstrated by propagating the virus in tissue culture. After 23 passages in the presence of drug, two mutations were identified (F276L and V553L) in the viral RNA-dependent RNA polymerase gene. These mutations increased the replication capacity of the virus in the presence of remdesivir (21). However, these amino acid changes decreased the viral fitness and attenuated SARS-CoV pathogenesis in mice (21). The efficacy of prophylactic and therapeutic remdesivir treatment was recently tested in a nonhuman primate (rhesus macaque) model of MERS-CoV infection (22). When prophylactic remdesivir treatment was initiated 24 h prior to inoculation, MERS-CoV was prevented from inducing clinical disease and inhibited from replicating in respiratory tissues, which prevented the formation of lung lesions. Similar results were obtained when therapeutic remdesivir treatment was initiated at 12 h after virus inoculation (22). Human safety data are available for remdesivir (18); thus, human trials can be initiated for testing the efficacy of this compound against novel coronaviruses.

Therapies that are approved by the Food and Drug Administration (FDA) have been evaluated for antiviral activity against SARS-CoV and MERS-CoV. For example, lopinavir (LPV), a human immunodeficiency virus 1 (HIV-1) protease inhibitor, was combined with ritonavir (RTV) to increase the LPV half-life. The combination of LPV and RTV (LPV/RTV) was shown to be effective against SARS-CoV in patients and in tissue culture. The estimated EC_{50} in fetal rhesus kidney-4 cells was 4 μg/ml (23). LPV/RTV also reduced weight loss, clinical scores, viral titers, and disease progression in marmosets infected with MERS-CoV (24). Nevertheless, the antiviral activity of LPV against MERS-CoV in tissue culture remains controversial. No optimal EC_{50} was found in Vero cells (25), but an EC_{50} of 8 μM was reported in Huh7 cells (26).

Clinical observations in animals and humans showed that MERS-CoV infections were mediated by both virus replication and host inflammatory responses. Those findings led to explorations of combination therapies that included type I interferon (IFN-I) and IFN-II. Interferon beta (IFN-β) displayed the best efficacy, with EC_{50}s of 1.37 to 17 IU/ml, for reducing MERS-CoV replication in tissue culture (25, 27). Similarly to LPV/RTV, clinical improvements with IFN-β were observed in common marmosets infected with MERS-CoV (24). In the Kingdom of South Arabia, an ongoing randomized control trial (the MIRACLE Trial) was initiated to determine whether the combination of LPV/RTV and IFN-β could improve clinical outcomes in MERS-CoV infections (28). Importantly, an-

other controlled trial was launched in China to test the efficacy of LPV/RTV and IFN-α 2b in hospitalized patients with SARS-CoV-2 infections (ClinicalTrials registration no. ChiCTR2000029308).

The prophylactic and therapeutic properties of remdesivir and LPV/RTV–IFN-β were previously compared in a humanized transgenic mouse MERS-CoV infection model (29). Remdesivir improved pulmonary function, reduced lung viral loads, and ameliorated severe lung pathology. In contrast, prophylactic LPV/RTV–IFN-β reduced viral loads only slightly and did not impact other disease parameters, and therapeutic LPV/RTV–IFN-β improved pulmonary function but did not reduce virus replication or severe lung pathology (29). Overall, these results indicated that remdesivir showed more potential than LPV/RTV–IFN-β for treating MERS-CoV infections.

Ribavirin, a guanosine analogue, is an antiviral compound used to treat several virus infections, including respiratory syncytial virus, hepatitis C virus, and some viral hemorrhagic fevers. In most cases, ribavirin is combined with IFN. Ribavirin was first marketed in 1980 for the treatment of respiratory syncytial virus in children. Although promising results were obtained previously with ribavirin and IFN-α 2b in a MERS-CoV rhesus macaque model (30), data have been conflicting on patients with MERS-CoV infections that were treated with a combination of ribavirin and IFN (either IFN-α 2a or IFN-β1) (31). However, ribavirin reduces hemoglobin concentrations, an undesirable side effect in patients with respiratory disorders. This feature reduces its potential as an antiviral against SARS-CoV-2.

Work with influenza virus has shown that monoclonal and polyclonal antibodies can be useful prophylactic and therapeutic tools. Several antibodies have been shown previously to bind influenza virus hemagglutinin and inhibit virus replication (12). For example, human immunoglobulin G1 (IgG1) monoclonal antibody (MHAA4549A) binds to a highly conserved epitope on the stalk of influenza A virus hemagglutinin. In a phase 2 human influenza A virus challenge study, MHAA4549A significantly reduced the clinical symptoms and viral burden relative to placebo (32). Another example is VIS410, a monoclonal antibody engineered to target all known influenza A virus strains. A phase 2a trial showed that VIS410 had some clinical benefits (33). Current development efforts in monoclonal and polyclonal antibodies against coronaviruses are mainly targeting MERS-CoV. In a phase 1 clinical trial, a human polyclonal antibody, SAB-301, which is generated in *trans*-chromosomic cattle, was found to be safe and well tolerated in healthy participants. (34). However, therapeutic treatment with human monoclonal antibodies did not protect against the severe disease or the loss of lung function induced by MERS-CoV in animal models (20). The lack of viral sequence homology among different human coronaviruses suggests that current investigational antibody-based therapeutics will not be effective against novel virus variants. Nevertheless, in considering future treatments for novel coronaviruses, immune-based therapies should be not discarded.

Another potential treatment option could be the use of novel coronavirus sera prepared from the blood of patients in convalescence (convalescent-phase sera). Passive immunization is well established for viral infection prophylaxis. Polyclonal antibody products have been licensed that target cytomegalovirus, hepatitis B virus, and varicella-zoster virus. A meta-analysis of reports on the 1918 influenza A virus (H1N1) epidemic concluded that early administration of convalescent blood products reduced the absolute risk of pneumonia-related death from 37% to 16% (35). Nevertheless, the appropriate titer of convalescent-phase sera antibody that is required for therapeutic efficacy against SARS-CoV-2 remains to be determined. Moreover, additional studies performed with influenza virus have produced controversial results regarding the clinical benefit of administering high titers of anti-influenza immunoglobulins (36). Finally, it remains unclear whether methods based on the use of a sufficient pool of potential donors are feasible. Work carried out with MERS-CoV showed that sera from patients recovering from infections did not contain sufficient antibody titers for therapeutic use (37).

Another interesting therapeutic alternative that was previously explored with influ-

enza virus was that of targeting cellular components involved in the host inflammatory response to the infection. For example, the activation of the inflammatory response to an infection can induce a cytokine outburst that results in an acute lung injury. An example of a therapy for this type of infection has been targeting of cellular Toll-like receptor 4 (TLR4) with specific antibodies. TLR4 is a transmembrane protein that belongs to the pattern recognition receptor (PRR) family. The prototype pathogen-associated molecular pattern (PAMP) that TLR4 recognizes is that corresponding to the Gram-negative bacterium endotoxin lipopolysaccharide (LPS). TLR4 has been implicated in pathology associated with other infections and with tissue damage caused by noninfectious insults. TLR4 activation leads to activation of the NF-κB intracellular signaling pathway and to inflammatory cytokine production, which in turn activate the innate immune system. Interestingly, TLR4-null mice were highly resistant to infection by the mouse-adapted influenza A virus (38). Thus, protection against influenza virus infections was achieved by targeting TLR4 with small-molecule antagonists, like TAK-242, or with anti-TLR4-specific antibodies (39, 40). Indeed, targeting a cellular protein would overcome the drawbacks associated with virus or coronavirus genetic heterogeneity.

The high mortality rates observed in some emerging respiratory diseases induced by viruses like MERS-CoV, SARS-CoV, and novel influenza A virus strains (H5N1) have given rise to the hypothesis that the proinflammatory response might be involved in the disease pathogenesis. Consequently, immunosuppressants (e.g., corticosteroids) might be used as an adjunct for treating severe forms of the disease. However, the therapeutic use of immunosuppressants is not free of controversy. To date, no conclusive results have been found for the effects of immunosuppressants in severe influenza virus infections (12). Furthermore, the use of corticosteroids to treat influenza virus has been associated with an increased risk of superinfection, prolonged viral replication, and an increased risk of death (41). In contrast, corticosteroid treatment for MERS-CoV infections was not significantly associated with mortality, although a delay in MERS-CoV RNA clearance was observed (42). Further studies should be performed to clarify the potential clinical benefit of prescribing immunosuppressants for coronavirus infections.

To end this minireview, I discuss an interesting potential antiviral strategy. The spike protein of SARS-CoV mediates viral entry into target cells. Intriguingly, cleavage and activation of the SARS-CoV spike protein by a host cell protease are essential for infectious viral entry (43). This host protease could be a type II transmembrane serine protease, TMPRSS2, which was shown to cleave and activate SARS-CoV spike protein in cell cultures. Therefore, TMPRSS2 is a potential a target for antiviral interventions. For example, the serine protease inhibitor camostat mesylate inhibits the enzymatic activity of TMPRSS2 (44). Recently, administration of K11777, a cysteine protease inhibitor, in the subnanomolar range was shown to inhibit SARS-CoV and MERS-CoV replication in tissue cultures (45). Moreover, the clinically proven serine protease inhibitor camostat mesylate, which is active against TMPRSS2, partially blocked SARS-CoV-2 spike-driven entry into Caco-2 and Vero-TMPRSS2 cells (46). Future tissue culture and animal model studies should be conducted to clarify the potential antiviral activity of targeting TMPRSS2.

By the end of February 2020, 2 months after the first cases of SARS-CoV-2 were reported in China, several hundreds of new infection cases had been registered, mainly in other Asian regions and Europe. This news has strongly suggested that we are in the thick of a SARS-CoV-2 pandemic. Social alarm and health authorities have called for the development of therapeutic alternatives for fighting the current and, possibly, new coronavirus epidemics. Animal models and clinical studies are urgently needed for evaluating the effectiveness and safety of promising antiviral compounds that target the virus and/or the immunopathology involved in the host responses. The identification and characterization of novel compounds and therapeutic alternatives will be required to better control this probable pandemic outbreak.

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I declare that I have no conflict of interest.

REFERENCES

1. WHO. 2020. Novel coronavirus (2019-nCoV) situation reports. World Health Organization. http://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/.
2. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
3. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Xia J, Yu T, Zhang X, Zhang L. 2020. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 395:507–513. https://doi.org/10.1016/S0140-6736(20)30211-7.
4. Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, Xing F, Liu J, Yip CCY, Poon RWS, Tsoi HW, Lo SKF, Chan KH, Poon VKM, Chan WM, Ip JD, Cai JP, Cheng VCC, Chen H, Hui CKM, Yuen KY. 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395:514–523. https://doi.org/10.1016/S0140-6736(20)30154-9.
5. Gorbalenya AE. 2020. Severe acute respiratory syndrome-related coronavirus: the species and its viruses—a statement of the Coronavirus Study Group. bioRxiv https://doi.org/10.1101/2020.02.07.937862.
6. Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. 17 February 2020, posting date. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19)—China, 2020. Zhonghua Liu Xing Bing Xue Za Zhi (In Chinese.) https://doi.org/10.3760/cma.j.issn.0254-6450.2020.02.003.
7. Zaki AM, Van Boheemen S, Bestebroer TM, Osterhaus A, Fouchier R. 2012. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med 367:1814–1820. https://doi.org/10.1056/NEJMoa1211721.
8. Drosten C, Günther S, Preiser W, Van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RAM, Berger A, Burguière AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Müller S, Rickerts V, Stürmer M, Vieth S, Klenk HD, Osterhaus A, Schmitz H, Doerr HW. 2003. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med 348:1967–1976. https://doi.org/10.1056/NEJMoa030747.
9. Zhou P, Yang X-L, Wang X-G, Hu B, Zhang L, Zhang W, Si H-R, Zhu Y, Li B, Huang C-L, Chen H-D, Chen J, Luo Y, Guo H, Jiang R-D, Liu M-Q, Chen Y, Shen X-R, Wang X, Zheng X-S, Zhao K, Chen Q-J, Deng F, Liu L-L, Yan B, Zhan F-X, Wang Y-Y, Xiao G-F, Shi Z-L. 3 February 2020, posting date. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature https://doi.org/10.1038/s41586-020-2012-7.
10. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395:565–574. https://doi.org/10.1016/S0140-6736(20)30251-8.
11. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. 2020. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 382:727–733. https://doi.org/10.1056/NEJMoa2001017.
12. Beigel JH, Nam HH, Adams PL, Krafft A, Ince WL, El-Kamary SS, Sims AC. 2019. Advances in respiratory virus therapeutics – a meeting report from the 6th ISIRV Antiviral Group conference. Antiviral Res 167:45–67. https://doi.org/10.1016/j.antiviral.2019.04.006.
13. Bradwell K, Combe M, Domingo-Calap P, Sanjuán R. 2013. Correlation between mutation rate and genome size in riboviruses: mutation rate of bacteriophage Qβ. Genetics 195:243–251. https://doi.org/10.1534/genetics.113.154963.
14. Wang C, Horby PW, Hayden FG, Gao GF. 2020. A novel coronavirus outbreak of global health concern. Lancet 395:470–473. https://doi.org/10.1016/S0140-6736(20)30185-9.
15. Chao L. 1990. Fitness of RNA virus decreased by Muller's ratchet. Nature 348:454–455. https://doi.org/10.1038/348454a0.
16. Duarte E, Clarke D, Moya A, Domingo E, Holland J. 1992. Rapid fitness losses in mammalian RNA virus clones due to Muller's ratchet. Proc Natl Acad Sci U S A 89:6015–6019. https://doi.org/10.1073/pnas.89.13.6015.
17. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pyrc K, Feng JY, Trantcheva I, Bannister R, Park Y, Babusis D, Clarke MO, MacKman RL, Spahn JE, Palmiotti CA, Siegel D, Ray AS, Cihlar T, Jordan R, Denison MR, Baric RS. 28 June 2017, posting date. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med https://doi.org/10.1126/scitranslmed.aal3653.
18. Mulangu S, Dodd LE, Davey RT, Mbaya OT, Proschan M, Mukadi D, Manzo ML, Nzolo D, Oloma AT, Ibanda A, Ali R, Coulibaly S, Levine AC, Grais R, Diaz J, Clifford Lane H, Muyembe-Tamfum JJ, Sivahera B, Camara M, Kojan R, Walker R, Dighero-Kemp B, Cao H, Mukumbayi P, Mbala-Kingebeni P, Ahuka S, Albert S, Bonnett T, Crozier I, Duvenhage M, Proffitt C, Teitelbaum M, Moench T, Aboulhab J, Barrett K, Cahill K, Cone K, Eckes R, Hensley L, Herpin B, Higgs E, Ledgerwood J, Pierson J, Smolskis M, Sow Y, Tierney J, Sivapalasingam S, Holman W, Gettinger N, Vallée D, et al. 2019. A randomized, controlled trial of Ebola virus disease therapeutics. N Engl J Med 381:2293–2303. https://doi.org/10.1056/NEJMoa1910993.
19. Brown AJ, Won JJ, Graham RL, Dinnon KH, Sims AC, Feng JY, Cihlar T, Denison MR, Baric RS, Sheahan TP. 2019. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. Antiviral Res 169:104541. https://doi.org/10.1016/j.antiviral.2019.104541.
20. Cockrell AS, Yount BL, Scobey T, Jensen K, Douglas M, Beall A, Tang XC, Marasco WA, Heise MT, Baric RS. 2016. A mouse model for MERS coronavirus-induced acute respiratory distress syndrome. Nat Microbiol 2:16226. https://doi.org/10.1038/nmicrobiol.2016.226.
21. Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Smith EC, Case JB, Feng JY, Jordan R, Ray AS, Cihlar T, Siegel D, Mackman RL, Clarke MO, Baric RS, Denison MR. 2018. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. mBio 9:e00221-18. https://doi.org/10.1128/mBio.00221-18.
22. de Wit E, Feldmann F, Cronin J, Jordan R, Okumura A, Thomas T, Scott D, Cihlar T, Feldmann H. 13 February 2020, posting date. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection. Proc Natl Acad Sci U S A https://doi.org/10.1073/pnas.1922083117.
23. Chu CM, Cheng VCC, Hung IFN, Wong MML, Chan KH, Chan KS, Kao RYT, Poon LLM, Wong CLP, Guan Y, Peiris JSM, Yuen KY; HKU/UCH SARS Study Group. 2004. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 59:252–256. https://doi.org/10.1136/thorax.2003.012658.
24. Chan JFW, Yao Y, Yeung ML, Deng W, Bao L, Jia L, Li F, Xiao C, Gao H, Yu P, Cai JP, Chu H, Zhou J, Chen H, Qin C, Yuen KY. 2015. Treatment with lopinavir/ritonavir or interferon-β1b improves outcome of MERS-CoV infection in a nonhuman primate model of common marmoset. J Infect Dis 212:1904–1913. https://doi.org/10.1093/infdis/jiv392.
25. Chan JFW, Chan KH, Kao RYT, To KKW, Zheng BJ, Li CPY, Li PTW, Dai J, Mok FKY, Chen H, Hayden FG, Yuen KY. 2013. Broad-spectrum antivirals for the emerging Middle East respiratory syndrome coronavirus. J Infect 67:606–616. https://doi.org/10.1016/j.jinf.2013.09.029.
26. De Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, Van Nieuwkoop S, Bestebroer TM, Van Den Hoogen BG, Neyts J, Snijder EJ. 2014. Screening of an FDA-approved compound library identifies four

small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. Antimicrob Agents Chemother 58:4875–4884. https://doi.org/10.1128/AAC.03011-14.
27. Hart BJ, Dyall J, Postnikova E, Zhou H, Kindrachuk J, Johnson RF, Olinger GG, Frieman MB, Holbrook MR, Jahrling PB, Hensley L. 2014. Interferon-β and mycophenolic acid are potent inhibitors of Middle East respiratory syndrome coronavirus in cell-based assays. J Gen Virol 95:571–577. https://doi.org/10.1099/vir.0.061911-0.
28. Arabi YM, Alothman A, Balkhy HH, Al-Dawood A, AlJohani S, Al Harbi S, Kojan S, Al Jeraisy M, Deeb AM, Assiri AM, Al-Hameed F, Al Saedi A, Mandourah Y, Almekhlafi GA, Sherbeeni NM, Elzein FE, Memon J, Taha Y, Almotairi A, Maghrabi KA, Qushmaq I, Al Bshabshe A, Kharaba A, Shalhoub S, Jose J, Fowler RA, Hayden FG, Hussein MA, Martin GS, Schoenfeld DA, Walmsley SL, Carson S, Al Harbi S, Al Jeraisy M, Al Muhaidib M, Musharaf S, Al Anizi H, Dael R, Al Mazroa M, Asiri A, Memish ZA, Ghazal SS, Alfaraj SH, Al Harthy A, Al Sulaiman M, Mady A, et al. 2018. Treatment of Middle East respiratory syndrome with a combination of lopinavir-ritonavir and interferon-β1b (MIRACLE trial): study protocol for a randomized controlled trial. Trials 19:81. https://doi.org/10.1186/s13063-017-2427-0.
29. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Montgomery SA, Hogg A, Babusis D, Clarke MO, Spahn JE, Bauer L, Sellers S, Porter D, Feng JY, Cihlar T, Jordan R, Denison MR, Baric RS. 2020. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 11:222. https://doi.org/10.1038/s41467-019-13940-6.
30. Falzarano D, De Wit E, Rasmussen AL, Feldmann F, Okumura A, Scott DP, Brining D, Bushmaker T, Martellaro C, Baseler L, Benecke AG, Katze MG, Munster VJ, Feldmann H. 2013. Treatment with interferon-α2b and ribavirin improves outcome in MERS-CoV-infected rhesus macaques. Nat Med 19:1313–1317. https://doi.org/10.1038/nm.3362.
31. Arabi YM, Shalhoub S, Omari AA, Mandourah Y, Al-Hameed F, Sindi A, Alraddadi B, Motairi AA, Khatib KA, Mommin AA, Qushmaq IA, Mady A, Solaiman O, Aithan AA, Balkhy HH, Al-Raddadi R, Rajab A, Mekhlafi G, Harthy AA, Kharaba A, Al-Jabbary A, Pinto R, Sadat M, Al Mutairi H, Al Qasim E, Jose J, Deeb AM, Merson L, Hayden FG, Fowler R, Aldawood A. 2017. Effect of ribavirin and interferon on the outcome of critically ill patients with MERS. Am J Respir Crit Care Med 195:A6067. https://doi.org/10.1164/rccm.201706-1172OC.
32. McBride JM, Lim JJ, Burgess T, Deng R, Derby MA, Maia M, Horn P, Siddiqui O, Sheinson D, Chen-Harris H, Newton EM, Fillos D, Nazzal D, Rosenberger CM, Ohlson MB, Lambkin-Williams R, Fathi H, Harris JM, Tavela JA. 2017. Phase 2 randomized trial of the safety and efficacy of MHAA4549A, a broadly neutralizing monoclonal antibody, in a human influenza a virus challenge model. Antimicrob Agents Chemother 61:e01154-17. https://doi.org/10.1128/AAC.01154-17.
33. Hershberger E, Sloan S, Narayan K, Hay CA, Smith P, Engler F, Jeeninga R, Smits S, Trevejo J, Shriver Z, Oldach D. 2019. Safety and efficacy of monoclonal antibody VIS410 in adults with uncomplicated influenza A infection: results from a randomized, double-blind, phase-2, placebo-controlled study. EBioMedicine 40:574–582. https://doi.org/10.1016/j.ebiom.2018.12.051.
34. Beigel JH, Voell J, Kumar P, Raviprakash K, Wu H, Jiao JA, Sullivan E, Luke T, Davey RT. 2018. Safety and tolerability of a novel, polyclonal human anti-MERS coronavirus antibody produced from transchromosomic cattle: a phase 1 randomised, double-blind, single-dose-escalation study. Lancet Infect Dis 18:410–418. https://doi.org/10.1016/S1473-3099(18)30002-1.
35. Luke TC, Kilbane EM, Jackson JL, Hoffman SL. 2006. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? Ann Intern Med 145:599–609. https://doi.org/10.7326/0003-4819-145-8-200610170-00139.
36. Hung IFN, To KKW, Lee CK, Lee KL, Yan WW, Chan K, Chan WM, Ngai CW, Law KI, Chow FL, Liu R, Lai KY, Candy CC, Liu SH, Chan KH, Lin CK, Yuen KY. 2013. Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. Chest 144:464–473. https://doi.org/10.1378/chest.12-2907.
37. Arabi Y, Balkhy H, Hajeer AH, Bouchama A, Hayden FG, Al-Omari A, Al-Hameed FM, Taha Y, Shindo N, Whitehead J, Merson L, AlJohani S, Al-Khairy K, Carson G, Luke TC, Hensley L, Al-Dawood A, Al-Qahtani S, Modjarrad K, Sadat M, Rohde G, Leport C, Fowler R. 19 November 2015, posting date. Feasibility, safety, clinical, and laboratory effects of convalescent plasma therapy for patients with Middle East respiratory syndrome coronavirus infection: a study protocol. Springerplus https://doi.org/10.1186/s40064-015-1490-9.
38. Shirey KA, Lai W, Scott AJ, Lipsky M, Mistry P, Pletneva LM, Karp CL, McAlees J, Gioannini TL, Weiss J, Chen WH, Ernst RK, Rossignol DP, Gusovsky F, Blanco JCG, Vogel SN. 2013. The TLR4 antagonist Eritoran protects mice from lethal influenza infection. Nature 497:498–502. https://doi.org/10.1038/nature12118.
39. Perrin-Cocon L, Aublin-Gex A, Sestito SE, Shirey KA, Patel MC, André P, Blanco JC, Vogel SN, Peri F, Lotteau V. 2017. TLR4 antagonist FP7 inhibits LPS-induced cytokine production and glycolytic reprogramming in dendritic cells, and protects mice from lethal influenza infection. Sci Rep 7:40791. https://doi.org/10.1038/srep40791.
40. Piao W, Shirey KA, Ru LW, Lai W, Szmacinski H, Snyder GA, Sundberg EJ, Lakowicz JR, Vogel SN, Toshchakov VY. 2015. A decoy peptide that disrupts TIRAP recruitment to TLRs is protective in a murine model of influenza. Cell Rep 11:1941–1952. https://doi.org/10.1016/j.celrep.2015.05.035.
41. Rodrigo C, Leonardi-Bee J, Nguyen-Van-Tam J, Lim WS. 2016. Corticosteroids as adjunctive therapy in the treatment of influenza. Cochrane Database Syst Rev 3:CD010406. https://doi.org/10.1002/14651858.CD010406.pub2.
42. Arabi YM, Mandourah Y, Al-Hameed F, Sindi AA, Almekhlafi GA, Hussein MA, Jose J, Pinto R, Al-Omari A, Kharaba A, Almotairi A, Al Khatib K, Alraddadi B, Shalhoub S, Abdulmomen A, Qushmaq I, Mady A, Mady O, Al-Aithan AM, Al-Raddadi R, Ragab A, Balkhy HH, Balkhy A, Deeb AM, Al Mutairi H, Al-Dawood A, Merson L, Hayden FG, Fowler RA; Saudi Critical Care Trial Group. 2018. Corticosteroid therapy for critically ill patients with Middle East respiratory syndrome. Am J Respir Crit Care Med 197:757–767. https://doi.org/10.1164/rccm.201706-1172OC.
43. Glowacka I, Bertram S, Muller MA, Allen P, Soilleux E, Pfefferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, Niemeyer D, Schneider H, Drosten C, Pohlmann S. 2011. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol 85:4122–4134. https://doi.org/10.1128/JVI.02232-10.
44. Kawase M, Shirato K, van der Hoek L, Taguchi F, Matsuyama S. 2012. Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. J Virol 86:6537–6545. https://doi.org/10.1128/JVI.00094-12.
45. Zhou Y, Vedantham P, Lu K, Agudelo J, Carrion R, Nunneley JW, Barnard D, Pöhlmann S, McKerrow JH, Renslo AR, Simmons G. 2015. Protease inhibitors targeting coronavirus and filovirus entry. Antiviral Res 116:76–84. https://doi.org/10.1016/j.antiviral.2015.01.011.
46. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. 4 March 2020. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. https://doi.org/10.1016/j.cell.2020.02.052.