1   **Compounds with therapeutic potential against novel respiratory 2019 coronavirus**

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14 **Abstract**

15 Currently, the expansion of the novel human respiratory coronavirus (known as: SARS-CoV-2,

16 COVID-2019, or 2019-nCoV) has stressed the need for therapeutic alternatives to alleviate and

17 stop this new epidemic. The previous epidemics of high-morbidity human coronaviruses, such

18 as the acute respiratory syndrome coronavirus (SARS-CoV) in 2003, and the Middle East

19 respiratory syndrome corona virus (MERS-CoV) in 2012, prompted the characterization of

20 compounds that could be potentially active against the currently emerging novel coronavirus

21 SARS-CoV-2. The most promising compound is remdesivir (GS-5734), a nucleotide analog

22 prodrug currently in clinical trials for treating Ebola virus infections. Remdesivir inhibited the

23 replication of SARS-CoV and MERS-CoV in tissue cultures, and it displayed efficacy in non-

24 human animal models. In addition, a combination of the human immunodeficiency virus type 1

25 (HIV-1) protease inhibitors, lopinavir/ritonavir, and interferon beta (LPV/RTV-INFb) were shown

26 to be effective in patients infected with SARS-CoV. LPV/RTV-INFb also improved clinical

27 parameters in marmosets and mice infected with MERS-CoV. Remarkably, the therapeutic

28 efficacy of remdesivir appeared to be superior to that of LPV/RTV-INFb against MERS-CoV in a

29 transgenic humanized mice model. The relatively high mortality rates associated with these

30 three novel human coronavirus infections, SARS-CoV, MERS-CoV, and SARS-CoV-2, has

31 suggested that pro-inflammatory responses might play a role in the pathogenesis. It remains

32 unknown whether the generated inflammatory state should be targeted. Therapeutics that

33 target the coronavirus alone might not be able to reverse highly pathogenic infections. This

34 minireview aimed to provide a summary of therapeutic compounds that showed potential in

35 fighting SARS-CoV-2 infections.

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36   On December 30, 2019, a cluster of 27 pneumonia cases (including 7 severe cases) of unknown

37   origin emerged in Wuhan (Hubei, China) and were reported to the National Health Commission

38   of China (1). In the early stages of this pneumonia, patients developed severe acute respiratory

39   infection symptoms, and some patients rapidly developed acute respiratory distress syndrome

40   (2). Real time RT-PCR and deep sequencing analysis from lower respiratory tract samples

41   identified a novel human coronavirus, now called SARS-CoV-2 (3–5). By the end of January,

42   2020, nearly 50,000 confirmed cases were reported in China, and the first confirmed cases were

43   reported in Thailand, Nepal, Republic of Korea, USA, Singapore, France, Viet Nam, Canada,

44   Australia, Malaysia, Germany, UAE, Finland, Italy, Cambodia, Sri Lanka, the Russian Federation,

45   Spain, Sweden, India, and the Philippines. Among the patients with confirmed cases, most were

46   aged 30–80 years and had mild infections (80%). The fatality rate was around 2% (6).

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

49   produce respiratory tract infections, as observed with SARS-CoV and MERS-Cov (7, 8).

50   Sequencing and phylogenetic analyses have shown that the novel SARSCoV-2 virus is closely

51   related to a group of human SARS-like coronaviruses and bat SARS-related coronaviruses (9–

52   11). The origin of SARSCoV-2 remains unclear; it is unknown how it was first transmitted to

53   humans. The high prevalence of SARS-related coronaviruses in bats has suggested that a bat

54   coronavirus might have jumped into a civet or some other mammal, and from there to humans,

55   which started the former 2003 SARS epidemic. Initial confirmed cases of SARSCoV-2 were

56   associated with Huanan seafood and live animal markets. However, no animal source has been

57   identified to date, and spillover events may continue to occur. Although bats might be the

3

58   source of SARSCoV-2, it is critical to identify the intermediate species to stop the current spread

59   and to prevent future human SARS-related coronavirus epidemics.

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61   A key question is whether the current SARSCoV-2 epidemic is similar to other SARS outbreaks or

62   whether it shows different features. The epidemiological and clinical characteristics of

63   SARSCoV-2 indicate that this new outbreak is different from the 2003-SARS. SARSCoV-2 displays

64   higher transmissibility and lower mortality compared to the 2003-SARS (1, 3, 4). SARSCoV-2 has

65   shown efficient intra-familial spread (4). The asymptomatic period of SARSCoV-2 infections

66   oscillates between 2 and 14 days, and some individuals probably transmit the virus without

67   developing any disease symptoms. It remains to be elucidated whether this virus replicates

68   more readily in the upper airway than SARS-CoV and MEERS-CoV and whether it is similar to

69   other human coronaviruses (HCoV) that cause colds, but not pneumonia. It will be necessary to

70   identify molecular determinants that mediate transmission from animal to human, and from

71   human to human. Of note, in the novel SARS-CoV-2, the nucleotide sequence of the external

72   ectodomain in the spike protein receptor-binding domain is different from that of the 2003

73   SARS-CoV. When individual bat coronavirus spike genes were introduced into SARS-CoV

74   infectious clones, the SARS-CoV/bat-CoV spike viruses could bind to the human, bat, or civet

75   angiotensin converting enzyme 2 (ACE2) cellular receptor (12). Understanding the interaction

76   between this novel SARS-CoV-2 spike protein and the host ACE2 receptor might reveal how this

77   virus overcame the species barrier between animals and humans. As discussed below, this

78   information might promote the design of effective antivirals.

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4

80   To predict new zoonotic coronavirus jumps across species and to understand the rate of virus

81   spread among people, it is crucial to determine whether SARSCoV-2 is mutating to improve its

82   binding to human receptors for infection. As an RNA virus, SARS-CoV-2 has intrinsic genetic

83   variability, which results in a high mutation rate. Moreover, coronaviruses have the largest

84   genomes (∼30 kb) among RNA viruses. However, part of their sequence encodes a

85   proofreading 3' exonuclease that can increase replication fidelity (13). It has been suggested

86   that any adaptation in the SARS-CoV-2 sequence that might make it more efficient at

87   transmitting from person to person might also increase its virulence (14). However, this

88   mechanism could lead to a genetic bottleneck, known as Muller's ratchet, which could

89   significantly decrease viral fitness, (15). Muller's ratchet predicts that, when mutation rates are

90   high and a significant proportion of mutations are deleterious, a type of irreversible ratchet

91   mechanism will gradually reduce the mean fitness of small populations of asexual organisms.

92   Because genetic bottlenecks for RNA viruses often occur during respiratory droplet

93   transmissions, the SARS-CoV-2 is expected to become less virulent through human to human

94   transmissions (16).

95

96   From the public health perspective, we urgently need to develop an effective vaccine and

97   antiviral therapeutics to stop the SARS-CoV-2 epidemic. Moreover, social and economic issues

98   generated by this epidemic also call for rapid interventions. This review focuses on the

99   potential of repurposing preexisting compounds that might provide new opportunities for

100   treating people infected with SARS-CoV-2. Previous work with SARS-CoV and MERS-CoV has

101   provided an opportunity to accelerate the identification of meaningful therapies for fighting the

102   novel SARS-CoV-2 epidemic. Nevertheless, we must be aware that, currently, no compound

103   that targets SARS-CoV or MERS-CoV has moved beyond phase 1 trials.

104

105   The most promising antiviral for fighting SARS-CoV-2 is remdesivir (GS-5734). Remdesivir is an

106   adenosine nucleotide analogue prodrug with broad-spectrum antiviral activity against

107   filoviruses, paramyxoviruses, pneumoviruses, and pathogenic coronaviruses, like SARS-CoV and

108   MERS-CoV (17). Pharmacokinetic studies have been completed and clinical trials are ongoing

109   for testing remdesivir efficacy in treating Ebola virus (18). Previous studies have indicated that

110   nucleotide analogues generally show low efficacy against coronaviruses, due to the virus

111   exonuclease proofreading enzyme. Nevertheless, remdesivir was effective against SARS-CoV,

112   MERS-CoV, and bat-CoV strains (17). In tissue cultures, remdesivir displayed half-maximum

113   effective concentrations (EC50s) of 0.069 for SARS-CoV and 0.074 µM for MERS-CoV. Of note,

114   tissue culture studies have shown that remdesivir is also active in the submicromolar EC50

115   range against a number of highly divergent coronaviruses, including the endemic human CoVs,

116   OC43 (HCoV-OC43) and 229E (HCoV-229E). Thus, remdesivir has broad-spectrum anti-CoV

117   activity (19). In a mouse model of SARS-CoV pathogenesis, prophylactic and early therapeutic

118   administration of remdesivir significantly reduced the lung viral load. Viral titers were reduced

119   by >2 orders of magnitude on day 4 or 5 post infection. Remdesivir improved the clinical signs

120   of disease and respiratory function compared to untreated control animals (17). Comparable

121   results were obtained with MERS-CoV in prophylactic studies carried out with a MERS-CoV

122   mouse transgenic model. In that model, a humanized MERS-CoV receptor (dipeptidyl peptidase

123   4, hDPP4) was expressed and carboxylesterase 1c (Ces1c) was deleted to improve the

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124   pharmacokinetics of nucleotide prodrugs (20). Remdesivir specificity for coronavirus was

125   demonstrated by propagating the virus in tissue culture. After 23 passages in the presence of

126   drug, two mutations were identified (F276L and V553L) in the viral RNA-dependent RNA

127   polymerase gene. These mutations increased the replication capacity of the virus in the

128   presence of remdesivir (21). However, these amino acid changes decreased the viral fitness and

129   attenuated SARS-CoV pathogenesis in mice (21). The efficacy of prophylactic and therapeutic

130   remdesivir treatment was recently tested in a nonhuman primate (rhesus macaque) model of

131   MERS-CoV infection (22). When prophylactic remdesivir treatment was initiated 24 h prior to

132   inoculation, MERS-CoV was prevented from inducing clinical disease and inhibited from

133   replicating in respiratory tissues, which prevented the formation of lung lesions. Similar results

134   were obtained when therapeutic remdesivir treatment was initiated at 12 h after virus

135   inoculation (22). Human safety data are available for remdesivir (18); thus, human trials can be

136   initiated for testing the efficacy of this compound against novel coronaviruses.

137

138   Therapies that are approved by the Food and Drug Administration (FDA) have been evaluated

139   for antiviral activity against SARS-CoV and MERS-CoV. For example, lopinavir (LPV), a human

140   immunodeficiency virus 1 (HIV-1) protease inhibitor, was combined with ritonavir (RTV) to

141   increase the LPV half-life. LPV/RTV was shown to be effective against SARS-CoV in patients and

142   in tissue culture. The estimated EC50 in fetal rhesus kidney-4 cells was 4 μg/ml (23). LPV/RTV

143   also reduced weight loss, clinical scores, viral titers, and disease progression in marmosets

144   infected with MERS-CoV (24). Nevertheless, the antiviral activity of LPV against MERS-CoV in

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145   tissue culture remains controversial. No optimal EC50 was found in Vero cells (25), but an EC50

146   of 8 µM was reported in Huh7 cells (26).

147

148   Clinical observations in animals and humans showed that MERS-CoV infections were mediated

149   by both virus replication and host inflammatory responses. Those findings led to explorations of

150   combination therapies that included types I and II interferons (IFN I and II). Interferon beta

151   (IFNb) displayed the best efficacy, with EC50s of 1.37-17 IU/ml, for reducing MERS-CoV

152   replication in tissue culture (25, 27). Similar to LPV/RTV, clinical improvements with IFNb were

153   observed in common marmosets infected with MERS-CoV (24). In the Kingdom of South Arabia,

154   an ongoing randomized control trial (MIRACLE Trial) was initiated to determine whether the

155   combination of LPV/RTV and IFNb could improve clinical outcomes in MERS-CoV infections (28).

156   Importantly, another controlled trial was launched in China to test the efficacy of LPV/RTV and

157   IFNα-2b in hospitalized patients with SARS-CoV-2 infections (ChiCTR2000029308).

158

159   The prophylactic and therapeutic properties of remdesivir and LPV/RTV-IFNb were compared in

160   a humanized transgenic mouse MERS-CoV infection model (29). Remdesivir improved

161   pulmonary function, reduced lung viral loads, and ameliorated severe lung pathology. In

162   contrast, prophylactic LPV/RTV-IFNb only slightly reduced viral loads and did not impact other

163   disease parameters, and therapeutic LPV/RTV-IFNb improved pulmonary function, but did not

164   reduce virus replication or severe lung pathology (29). Overall, these results indicated that

165   remdesivir showed more potential than LPV/RTV-IFNb for treating MERS-CoV infections.

166

167   Ribavirin, a guanosine analogue, is an antiviral compound used to treat several virus infections,

168   including respiratory syncytial virus, hepatitis C virus, and some viral hemorrhagic fevers. In

169   most cases, ribavirin is combined with IFN. Ribavirin was first marketed in 1980 for the

170   treatment of respiratory syncytial virus in children. Although promising results were obtained

171   with ribavirin and IFNα-2b in a MERS-CoV rhesus macaque model (30), data have been

172   conflicting on patients with MERS-CoV infections that were treated with a combination of

173   ribavirin and IFN (either α2a or β1) (31). However, ribavirin reduces hemoglobin

174   concentrations, an undesirable side effect in patients with respiratory disorders. This feature

175   reduces its potential as an antiviral against SARS-CoV-2.

176

177   Work with influenza virus has shown that monoclonal and polyclonal antibodies can be useful

178   prophylactic and therapeutic tools. Several antibodies have been shown to bind influenza virus

179   hemagglutinin and inhibit virus replication (12). For example, human immunoglobulin G1 (IgG1)

180   monoclonal antibody (MHAA4549A) binds to a highly conserved epitope on the stalk of

181   influenza A hemagglutinin. In a phase 2 human influenza A virus challenge study, MHAA4549A

182   significantly reduced the clinical symptoms and viral burden relative to placebo (32). Another

183   example is VIS410, a monoclonal antibody engineered to target all known influenza A strains. A

184   phase 2a trial showed that VIS410 had some clinical benefits (33). Current development efforts

185   in monoclonal and polyclonal antibodies against coronaviruses are mainly targeting MERS-CoV.

186   In a phase 1 clinical trial, a human polyclonal antibody, SAB-301, which is generated in trans-

187   chromosomic cattle, was found to be safe and well tolerated in healthy participants. (34).

188   However, therapeutic treatment with human monoclonal antibodies did not protect against the

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189   severe disease or the loss of lung function induced by MERS-CoV in animal models (20). The

190   lack of viral sequence homology among different human coronaviruses suggests that current

191   investigational antibody-based therapeutics will not be effective against novel virus variants.

192   Nevertheless, immune-based therapies should be not discarded, when considering future

193   treatments for novel coronaviruses.

194

195   Another potential treatment option could be the use of novel coronavirus sera prepared from

196   the blood of patients in convalescence (convalescent sera). Passive immunization is well

197   established for viral infection prophylaxis. Polyclonal antibody products have been licensed that

198   target cytomegalovirus, hepatitis B virus, and varicella-zoster virus. A meta-analysis of reports

199   on the 1918 influenza A (H1N1) epidemic concluded that early administration of convalescent

200   blood products reduced the absolute risk of pneumonia-related death from 37% to 16% (35).

201   Nevertheless, the appropriate titer of convalescent sera antibody that is required for

202   therapeutic efficacy against SARS-CoV-2 remains to be determined. Moreover, additional

203   studies performed with influenza virus have produced controversial results regarding the

204   clinical benefit of administering high titers of anti-influenza immunoglobulins (36). Finally, it

205   remains unclear whether a sufficient pool of potential donors is feasible. Work carried out with

206   MERS-CoV showed that sera from patients recovering from infections did not contain sufficient

207   antibody titers for therapeutic use (37).

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209   Another interesting therapeutic alternative that was previously explored with influenza virus is

210   to target cellular components involved in the host inflammatory response to the infection. For

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211   example, the activation of the inflammatory response to an infection can induce a cytokine

212   outburst that results in an acute lung injury. An example of a therapy for this type of infection

213   has been to target the cellular toll-like receptor 4 (TLR4) with specific antibodies. TLR4 is a

214   transmembrane protein that belongs to the pattern recognition receptor (PRR) family. The

215   prototype pathogen-associated molecular pattern (PAMP) that TLR4 recognizes is the gram-

216   negative bacteria, endotoxin, lipopolysaccharide (LPS). TLR4 has been implicated in the

217   pathology associated with other infections and with tissue damage caused by non-infectious

218   insults. TLR4 activation leads to the NF-κB intracellular signaling pathway and inflammatory

219   cytokine production, which activate the innate immune system. Interestingly, TLR4-null mice

220   were highly resistant to infection by the mouse-adapted influenza A virus (38). Thus, protection

221   against influenza infections was achieved by targeting TLR4 with small molecule antagonists,

222   like TAK-242, or with anti-TLR4-specific antibodies (39, 40). Indeed, targeting a cellular protein

223   would overcome the drawbacks associated with virus or coronavirus genetic heterogeneity.

224

225   The high mortally rates observed in some emerging respiratory diseases induced by viruses like

226   MERS-CoV, SARS-CoV, and novel influenza A strains (H5N1) has given rise to the hypothesis that

227   the pro-inflammatory response might be involved in the disease pathogenesis. Consequently,

228   immunosuppressants (e.g., corticosteroids) might be used as an adjunct for treating severe

229   forms of the disease. However, the therapeutic use of immunosuppressants is not free of

230   controversy. To date, no conclusive results have been found for the effects of

231   immunosuppressants in severe influenza virus infections (12). Furthermore, the use of

232   corticosteroids to treat influenza virus has been associated with an increased risk of

233   superinfection, prolonged viral replication, and an increased risk of death (41). In contrast,

234   corticosteroid treatment for MERS-CoV infections was not significantly associated with

235   mortality, although a delay in MERS-CoV RNA clearance was observed (42). Further studies

236   should be performed to clarify the potential clinical benefit of prescribing immunosuppressants

237   for coronavirus infections.

238

239   To end this minireview, we will discuss an interesting potential antiviral strategy. The spike

240   protein of SARS-CoV mediates viral entry into target cells. Intriguingly, the cleavage and

241   activation of the SARS-CoV spike protein by a host cell protease is essential for infectious viral

242   entry (43). This host protease could be a type II transmembrane serine protease, TMPRSS2,

243   which was shown to cleave and activate SARS-CoV spike protein in cell cultures. Therefore,

244   TMPRSS2 is a potential a target for antiviral interventions. For example, the serine protease

245   inhibitor, camostat mesylate, inhibits the enzymatic activity of TMPRSS2 (44). Recently, K11777,

246   a cysteine protease inhibitor, was shown in tissue cultures to inhibit SARS-CoV and MERS-CoV

247   replication in the sub-nanomolar range (45). Future tissue culture and animal model studies

248   should be conducted to clarify the potential antiviral activity of targeting TMPRSS2.

249

250   By the end of February 2020, two months after the first cases of SARS-CoV-2 were reported in

251   China, several hundreds of new infection cases had been registered, mainly in other Asian

252   regions and Europe. This news has strongly suggested that we are in the thick of a SARS-CoV-2

253   pandemic. Social alarm and health authorities have called for the development of therapeutic

254   alternatives for fighting the current, and possibly new, coronavirus epidemics. Animal models

255 and clinical studies are urgently needed for evaluating the effectiveness and safety of promising

256 antiviral compounds that target the virus and/or the immunopathology involved in the host

257 responses. The identification and characterization of novel compounds and therapeutic

258 alternatives will be required to better control this probable pandemic outbreak.

259

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