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Recent developments in anti-severe acute respiratory syndrome coronavirus chemotherapy

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Abstract

Severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in early 2003 to cause a very severe acute respiratory syndrome, which eventually resulted in a 10% case-fatality rate. Owing to excellent public health measures that isolated focus cases and their contacts, and the use of supportive therapies, the epidemic was suppressed to the point that further cases have not appeared since 2005. However, despite intensive research since then (over 3500 publications), it remains an untreatable disease. The potential for re-emergence of the SARS-CoV or a similar virus with unknown but potentially serious consequences remains high. This is due in part to the extreme genetic variability of RNA viruses such as the coronaviruses, the many animal reservoirs that seem to be able host the SARS-CoV in which reassortment or recombination events could occur and the ability coronaviruses have to transmit relatively rapidly from species to species in a short period of time. Thus, it seems prudent to continue to explore and develop antiviral chemotherapies to treat SARS-CoV infections. To this end, the various efficacious anti-SARS-CoV therapies recently published from 2007 to 2010 are reviewed in this article. In addition, compounds that have been tested in various animal models and were found to reduce virus lung titers and/or were protective against death in lethal models of disease, or otherwise have been shown to ameliorate the effects of viral infection, are also reported.

Keywords

animal models; antiviral; chemotherapy; coronavirus; *in vivo* inhibitors; SARS-CoV; SARS inhibitors; severe acute respiratory syndrome

Severe acute respiratory syndrome (SARS) is caused by a novel human coronavirus (SARS-CoV) that leads to pulmonary pathological features [1]. Some of the early cases of SARS were reported from a hospital in Hanoi, Vietnam, by Carlo Urbani, a WHO scientist who first identified this new disease and who, on 29 March 2003, died from the disease himself [1]. Owing to his unfortunate death, it was proposed that the first isolate of the virus be named the Urbani strain of SARS-associated coronavirus [2]. The ensuing outbreak in Asia, and its subsequent spread by air travel, illustrates the serious consequences of modern travel and how it enables the spread of an emerging disease with high virulence; by 31 July 2003,

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more than 8000 SARS cases and nearly 800 SARS-related deaths were reported around the world.

Studies on the molecular evolution of SARS-CoV have suggested that the virus emerged from nonhuman sources (for a review, see Cleri *et al.* [3]). Evidence has been presented that bats are the reservoir host of this virus, since sequences of closely related viruses were found in these animals [4,5]. It is likely that SARS-CoV was transmitted from bats to humans via an intermediate host such as palm civets [6–8]. In addition, at least seven other species have been found to harbor SARS-CoV, including raccoon dog, red fox, Chinese ferret, mink, pig, boar and rice field rat [9]. In one case, it was also shown that the pig may be capable of transmitting SARS-CoV to humans [10]. These facts suggest that SARS-CoV, or a virus like it, may be lurking in the environment awaiting a special set of circumstances to erupt in a human infection.

At the time of the first appearance of SARS, there were no approved therapies for treating human infections caused by coronaviruses. Anecdotal evidence from laboratory and other sources suggested that human coronaviruses might have been sensitive to ribavirin. Thus, a number of therapies were tried, including ribavirin and anti-inflammatory supportive therapies, but to no avail (reviewed in [11,12]). Since then, there have been numerous discovery efforts to find effective therapies to treat SARS infections caused by the SARS-CoV (see reviews [13–17]); however, no compound has yet been approved for use in humans, and very few have even been tested for efficacy or safety in animal models with the activity reported in a peer-reviewed journal.

At least five divergent species of coronaviruses are known to have had zoonotic transmission into the human population in the evolutionary recent past, and the zoonosis of SARS-CoV was just the consequence of one of these inter-species transmission events [18]. Importantly, there is nothing to suggest that such cross species events will not continue. Therefore, given the extreme genetic variability of RNA viruses, including coronaviruses [19], the many animal reservoirs for SARS-CoV, and the ability coronaviruses have to transmit relatively rapidly from species to species in a short period of time, the emergence of a new coronavirus, or re-emergence of a SARS-CoV-like virus, still poses a threat and represents a challenge for antiviral drug development and administration [20,21].

The specific challenge now is to find an appropriate target for intervention and to demonstrated proof-of-concept in a valid biological system that sustains virus replication. There are a number of targets for treating SARS-CoV infections (TABLE 1). TABLE 1 lists those targets against which antiviral agents have been evaluated in *in vitro* biological systems involving the SARS-CoV and the host system that supports SARS-CoV replication. The purpose of this article is to highlight those *in vitro* studies from the last several years that have demonstrated the efficacy of an anti-SARS-CoV therapy (nonvaccine) in a cell culture system that supports viral replication and to review the limited number of compounds that have shown efficacy in a suitable animal model.

Defined viral targets for drug therapy

Some of the strategies used in drug discovery included the targeting of host pathways or molecules essential for virus replication or modulating the host immune system to inhibit virus replication. Another strategy is to target the replication cycle of the virus (see reviews [16,22–27]). SARS-CoV is a positive-sense ssRNA virus [28]. The 30-kb-long genome is predicted to encode at least ten open reading frames [29,30]. Very few of the proteins derived from these open reading frames have been targeted for antiviral drug discovery.

The *Orf1a/1b* complex codes for 16 nonstructural proteins (Nsp) [31], one of which is Nsp3, a papain-like protease with deubiquitinating activity [32,33]. The SARS-CoV papain-like protease carries out N-terminal processing of the viral replicase polyprotein [34], catalyzes Lys48-linked polyubiquitin chain debranching [35] and appears to process fusion proteins of the ubiquitin-like modifier ISG15, encoded by an interferon-stimulated gene *in vitro* [33]. GRL0617 (5-amino-2-methyl-*N*-[(R)-1-(1-naphthyl)ethyl] benzamide) was found to be a potent competitive inhibitor of PLpro enzyme with a K_i value of 0.49 ± 0.08 μM and moderately inhibited virus replication in Vero E6 cell culture with a 50% effective concentration (EC_{50}) of 14.5 ± 0.8 μM [36]. There was no toxicity detected at concentrations as great as 50 μM, suggesting that, if the drug is not toxic at even higher concentrations, the drug could be moderately selective and worth pursuing. Another group of compounds that selectively and potently inhibited the SARS-CoV papain-like protease includes two isomeric benzodioxolane derivatives: 1-[(R)-1-(1-naphthyl)ethyl]-4-[3,4-(methylenedioxy) benzylamino]carbonylpiperidine and 1-[(S)-1-(1-naphthyl)ethyl]-4-[3,4-(methylenedioxy) benzylamino]carbonylpiperidine [37]. The compounds inhibited the PLpro inhibition enzyme with IC_{50} values equal to 0.56 and 0.32 μM, respectively. In an antiviral assay using Vero E6 cells as the host cell line, the compounds had IC_{50} values of 9.1 μM. Toxicity was not detected at concentrations as great as 25 μM. Based on these data, the compounds had a selective index (SI) of 3, suggesting that they were not active unless the real cytotoxicity value was greater than 90 μM. Although potent enzymatic inhibitors, it remains to be seen if these compounds will be selective enough to pursue as antiviral agents.

Another target that has been heavily focused on for discovery of antiviral inhibitory agents is Nsp5 [38,39]. This protein is the SARS main protease (3C-like cysteine proteinase [$3CL^{pro}$]) and is primarily responsible for proteolytic processing of the polyproteins encoded by the SARS genome, along with SARS papain-like protease (Nsp3), making it an ideal selective target for drug discovery [40,41]. Numerous drug-discovery studies have been performed to develop inhibitors of this protease, but very few have been evaluated in antiviral cell culture assays or in relevant animal models. Of those that have been evaluated in antiviral assays, a 5-chloropyridinyl indole-carboxylate with the carboxylate at the 4-position has been reported to have potent activity against the SARS $3CL^{pro}$ (IC_{50} = 0.03 ± 0.01 μM), which translated into moderate inhibition of virus replication in Vero cells (EC_{50} = 6.9 ± 0.9 μM) [42]. Unfortunately, the selectivity of this inhibition could not be ascertained because no toxicity data were reported. However, a docking analysis from this same study suggests a possible mechanism of action: after covalently linking to residue CYS145 in the enzyme active site, the indolyl moiety of the compound shifts towards the S1 pocket of the active site and stacks with the shifted imidazole ring of HIS41, which locks the orientation of the binding pocket. With this information, more potent and biologically useful compounds may be synthesized. In another study, a Phe–Phe dipeptide inhibitor (ethyl 4-{2-[4-(dimethylamino) cinnaniyl]-amino-1-oxo-3-phenyl} propylamino-5-phenyl-2-pentenoate, JMF1521) designed on the basis of computer modeling of the SARS $3CL^{pro}$-inhibitor complex was found to be nontoxic up to 200 μM, to competitively inhibit the SARS $3CL^{pro}$ (K_i = 0.52 μM), and to inhibit SARS-CoV replication in cell culture with an EC_{50} of 0.18 μM [43].

One of the most obvious targets is the RNA-dependent RNA polymerase (Nsp12) that produces genome- and subgenome-sized RNAs of both polarities [44,45]. The zinc ionophore, pyrithione, when in combination with $ZnOAc_2$, inhibited SARS-CoV replication in Vero E6 cells, even at a high multiplicity of infection of 4, EC_{50} of 0.5 μM and with a SI of 164 [46]. The combination of the ionophore-promoting uptake of zinc into cells plus exogenously added zinc increased the intracellular Zn^{2+} concentrations to levels that presumably inhibited RNA synthesis. This postulate was supported by the fact that $ZnOAc_2$ was shown to inhibit RNA transcription in SARS-CoV transcription complexes and SARS-

CoV RNA polymerase activity in an RNA-dependent RNA polymerase assay [46]. In both cases, adding the Zn^{2+} chelator Mg-ethylenediaminetetraacetic acid abrogated the inhibition. This study provides an interesting mechanism to study the function of the Nsp12 protein, and perhaps it provides basic data that could enable the development of zinc ionophores as antiviral agents, although one might expect such an approach to be fraught with difficulties because of the global effects on host homeostasis [47].

Nonstructural protein 13 is a 5′–3′ helicase with associated NTPase and RNA 5′-triphosphatase activities [48–50]. Bismuth compounds have been shown to inhibit SARS-CoV replication [51,52]. Ranitidine bismuth citrate was found to be a good inhibitor of the ATPase activity of the SARS-CoV helicase protein, with an IC_{50} value of 0.3 μM. FRET-based assays demonstrated inhibition of the DNA duplex unwinding activity with an IC_{50} value of 0.6 μM. These data were fitted to the logistic equation to give an EC_{50} value of 5.9 μM for viable SARS-CoV. Expressing this by using a logarithmic scale for cell viability, the compound was relatively nontoxic with a CC_{50} of 5 mM. Thus, the SI of the compound was 847. In addition, a 90% effective concentration (EC_{90}) was determined and was equal to 50 μM. These data suggest that bismuth-based drugs should be evaluated *in vivo* for the treatment of SARS infections.

The E protein (Orf4), or envelope protein, has ion-channel-like properties [53,54] and is thought to be involved in viral budding/release [55]. It has been shown that siRNA could reduce viral FRhK cell culture titers up to approximately 25-fold with a half-life of approximately 24 h in FRhK-4 cells [56]. An interesting observation is that in another experiment, the siRNA alone reduced viral titers by 2 $\log_{10}$, but in combination with IFN-α, the viral titers were reduced by 5 $\log_{10}$.

M protein (Orf6), or membrane protein, is a surface protein responsible for viral assembly [55] and budding, which has been shown to suppress NF-κB [57]; it may also induce apoptosis [58,59]. It has no enzymatic activity or ligand interactions with cells, so it is a more difficult protein to target with an antiviral agent. Thus, most efforts have concentrated on using siRNA technology to inhibit the transcription of M protein RNA. It has been shown that siRNA could reduce viral titers in FRhK cells by up to approximately 23-fold with a half-life of approximately 24 h in FRhK-4 cells [56]. An interesting observation is that in another experiment, the siRNA alone reduced viral titers by 2 $\log_{10}$, but in combination with IFN-α, the viral lung titers were reduced by 5 $\log_{10}$.

N protein (Orf9), or nucleocapsid protein [60], facilitates binding and packaging of viral RNA in assembly of the virion [61]. Similar to the other structural proteins, the siRNA approach has been used to inhibit production of the N protein with similar results to those previously described for the E and M proteins [56]. A rather unique approach for inhibiting the N protein is the development of ‘intrabodies’. These are antibodies used inside living cells, which provide an alternative to standard methods of manipulating gene expression inside cells, and allow proteins to be targeted in a domain-, conformation- and modification-specific fashion [62]. Using mRNA display selection and directed evolution, a number of antibody-like proteins were designed that targeted SARS-CoV N protein with high affinity and selectivity [62]. They were placed on a fibronectin type III domain of human fibronectin scaffold. Two of the intrabodies bound to the N-terminal domain of the N protein and six recognized the C-terminus; one had a K_d equal to 1.7 nM. Of the intrabodies evaluated, seven of them reduced virus replication from 11- to 5900-fold when expressed in cells prior to infection. They also reduced N protein gene expression.

The remaining open reading frames code for proteins that are designated as accessory proteins. For example, *Orf7a* codes for a protein that is a type I transmembrane protein [63].

It is involved in viral assembly as it interacts with M and N structural proteins [61]. It appears to be essential for induction of cell cycle arrest [64] and interacts with human Ap4A-hydrolase [65], but it is not essential for replication *in vitro* or *in vivo*. Nevertheless, it has been targeted using siRNAs [66]. A protein encoded from the *Orf7b* region that overlaps with *Orf7a* but is expressed in a different reading frame has an unknown function, but is incorporated into virions [67]; it is also not essential for replication *in vitro* or *in vivo*. The targeting of either protein by siRNA has been shown to produce an antiviral effect in transfected Vero E6 cells and in stable cell lines subsequently infected with SARS-CoV [66]. The reduction in virus titer was greater than 1 $\log_{10}$ in stable cell lines and greater than 1.5 $\log_{10}$ in transfected Vero cells. The siRNAs were also shown to specifically target the intended *Orf7a/7b* RNA and not off-target RNAs.

Orf3a forms a potassium-sensitive ion channel [68], it is virion associated [69] and it may promote virus budding and release [68,70]. It promotes membrane rearrangement and cell death [71]. It also induces apoptosis [68,70], but is not essential for replication *in vitro* or *in vivo*. In the aforementioned study, a 21-nucleotide siRNA was synthesized to inhibit the *Orf3a* subgenomic RNA [66]. The sequence corresponding to the consensus sequence for the *Orf3a* RNA was flanked by five to seven nucleotides before and six to ten nucleotides after the consensus sequence, and was chosen to give the best differentiation between the various synthesized subgenomic RNAs, which would allow the knockdown of the *Orf3a*-specific subgenomic RNA. The reduction in virus titer was similar to that observed for the subgenomic 7a/7b siRNA – almost 1 $\log_{10}$ in stable cell lines and greater than 1.5 $\log_{10}$ in transfected Vero cells. The *Orf3a* siRNA also specifically targeted the intended *Orf3a* RNA.

Host targets

Other potential targets for inhibition of SARS-CoV include host factors necessary for infection or those that promote efficiency of infection. One such target is TNF-α-converting enzyme (TACE). TACE (ADAM-17) was shown to be a requisite for viral entry [72]. TACE, a metalloprotease, is required for the membrane-associated cytokine pro-TNF-α to be processed to the soluble form found in serum [73]. It has also been shown to be involved in the cleavage and release of the ACE2 ectodomain [74]. In addition, downregulation of ACE2 caused by the SARS-CoV spike protein has been postulated to be correlated with the severe respiratory failure [75]. TAPI-2, a hydroxamate TACE inhibitor, reduced the production of viral RNA by 50% at 200 nM [76]. However, the toxicity of the TAPI-2 was not evaluated in this experiment. Inhibitors of cathepsin L, an enzyme that processes antigen for binding to MHC class II molecules and has been shown to facilitate virus entry, inhibited SARS-CoV replication in cell culture at approximately 0.1 μM at 24 h post-virus exposure [77]. These data suggest that cathepsin might be a good target for inhibiting SARS-CoV infections if very selective inhibitors can be developed.

Therapeutic antibodies

Another chemotherapy strategy that has been developed is affinity-selected neutralizing antibodies as potential drugs [78]. For many virus infections, the development of neutralizing antibody is responsible for combating a virus infection, and this appears to be no different for infections caused by SARS-CoV [79]. Most neutralizing antibodies seem to be directed against the S protein [80], which is responsible not only for attachment to cellular receptors, but is also involved in the fusion of the virus to the cellular membrane [81]. However, it has been found that the target epitope for neutralizing antibody seems to localize to the fusion region of S protein [82]. Thus, targeting this region may be critical for the development of clinically relevant therapeutic antibodies.

Using a combination of human framework reassembly technology and DNA display, a number of humanized mouse antibodies were selected that were potent neutralizers of viral infectivity. A more potent antibody was then developed using a complete site-saturation mutagenesis methodology (GSSM™; Diversa Corp., CA, USA) that focused on the complementarity-determining regions of the most potent antibodies with the highest affinities. Using these methodologies, an antibody (2978/10) that neutralized virus infectivity in Vero cell culture and that had had high affinity for the recognized virus epitope was selected. In plaque reduction assays at 1.56 μg/ml, 80% of the virus was neutralized.

Miscellaneous anti-SARS-CoV agents with less-well-defined targets of inhibition

A number of anti-SARS-CoV agents have been studied whose targets of inhibition have yet to be elucidated or are not well characterized (TABLE 2). Plant lectins have also been shown to be potent inhibitors of coronavirus replication in cell culture [83–85]. Potent plant lectin inhibitors included the mannose-specific HHA with an EC_{50} of 3.2 μg/ml ± 2.8 and a SI of >31.3, the GlcNAc-specific agglutinin Nictaba with an EC_{50} of 1.7 ± 0.3 and a SI of >58.8, and the (GlcNAc)*n*-specific agglutinin *Urtica dioica* agglutinin (UDA) with an EC_{50} of1.3 ± 0.1 and a SI of >76.9 [83]. UDA inhibited the SARS-CoV Urbani strain in Vero cells with an EC_{50} of 0.86 ± 0.39 μg/ml and a mouse-adapted Urbani strain with an EC_{50} of 0.76 ± 0.22 μg/ml [85]. The EC_{90} values obtained from the inhibition of virus yields from infected cells demonstrated that UDA was a potent inhibitor of SARS-CoV replication; the EC_{90} was 1.1 μg/ml for inhibition of the Urbani strain and the EC_{90} = 0.95 μg/ml for the mouse-adapted Urbani strain. For all *in vitro* studies cited, the lectins had no detectable cytotoxicity at the concentrations tested. The major question to be answered is whether or not these plant lectins will be toxic in animals, since the carbohydrate moieties to which they specifically bind are found on all sorts of molecules within an animal. Amiodarone, an antiarrhythmic agent used to treat supraventricular and ventricular arrhythmias, has been shown to alter compartments of the endocytic pathway used late in endosomal processing of the virus [86]. When SARS-CoV-infected Vero cells were treated with various concentrations of amiodarone, virus titers were reduced by 50% at approximately 30 μM [86]. However, this activity was not due to disruption of the virion and yet the compound was not cytotoxic even at 50 μM, so the degree to which reported inhibition was selective for virus replication remains to be determined. For further development as a useful lead drug, the compound would have to be nontoxic (>50% cell viability) at concentrations greater than 300 μM.

Arbidol, an anti-influenza drug, and arbidol mesylate were shown to inhibit SARS-CoV infection of GMK-AH-1 cells at 25, 50 and 100 μg/ml [87]. The antiviral effect seemed to occur after adsorption. Arbidol mesylate was nearly five times as effective as arbidol in reducing virus replication. The data are rather difficult to interpret since an English translation of the entire manuscript is not available. It is likely that that arbidol might inhibit the fusion process [88].

Extracts/natural products

Diterpenoids, sesquiterpenoids, triterpenoids, lignoids and curcumin were shown to inhibit SARS-CoV in the range of 3–10 μM [89]. The most selective of these compounds were a diterpenoid, 8b-hydroxyabieta-9(11),13-dien-12-one, with a SI of >557 and a lignin, savinin, with a SI >667. Betulinic acid with a SI of 180 and savinin were competitive inhibitors of SARS-CoV 3CL protease with K_i values of 8.2 ± 0.7 and 9.1 ± 2.4 μM, respectively. The study is somewhat problematic to interpret because the authors pretreated cells with compound prior to addition of the virus to cells. It is not clear whether the compound was

removed from the plate wells prior to the addition of the virus. Thus, one cannot rule out the possibility that the inhibitory activity was due to a virucidal effect or the removal of virus receptor from cells. However, the authors do present some evidence suggesting that the observed effect was due to binding of these compounds to the spike protein of SARS. In addition, some test phytochemicals mentioned in the paper inhibited SARS 3CL protease activity in enzyme assays.

Traditional herbs from many geographical areas and environments have been considered as potential new drugs for treatment of viral infections, including those caused by SARS-CoV. An extract from the leaves of *Toona sinensis Roem* inhibited SARS-CoV replication with EC_{50} values ranging from 30 to 40 μg/ml and SI values ranging from 12 to 17 [90]. The extract was not toxic up to 500 μg/ml. It is, therefore, unknown as to how much of the antiviral activity could be attributed to physical inactivation of the virus. In addition, a variety of compounds have been extracted from the tender leaf of the *Toona sinensis Roem* plant, and it is unknown which one compound or combination of compounds was responsible for the activity reported above, or what the target of inhibition may have been. In another study, ginsenoside-Rb1 purified from Panax ginseng showed antiviral activity at 100 μM, although the toxicity of the compound is unknown [91].

SARS-CoV antiviral drug discovery in animal models

There have been some compounds evaluated for efficacy in SARS-CoV animal models, including mice, hamsters, ferrets and nonhuman primates (summarized in TABLE 3). However, the majority of these types of studies have been performed in mice.

Animal models

Several inbred mouse species (BALB/c, C57BL/6 [B6] and 129S) have been shown to support SARS-CoV replication (reviewed in [92]). BALB/c mice have been shown to be permissive to virus replication in the lungs without showing pathology in the lungs [92], 129S mice present with pneumonitis [93] and aged BALB/c mice (senescent mouse model, mice >1 year old) show clinical signs of SARS disease, including weight loss, viral lung titers, lung pathology characterized by diffuse alveolar damage including edema, hyaline membrane formation and pneumonitis [94]. In addition, Day *et al.* have adapted an Urbani strain of SARS-CoV (v2163) to infect and cause mortality of mice. The virus was serially passaged 25 times in the lungs of mice using virus homogenates from each successive passage. The virus causes a lethal infection. In contrast to other SARS virus infections of mice in which mice must be euthanized upon losing 20–25% of their initial bodyweight even though they would survive to the end of the experiment, mortality in mouse-adapted virus mouse models is entirely due to mice succumbing to virus infection [85]. In this model, the lungs support robust replication of virus peaking at days 3–4, and abundant proinflammatory cytokines, especially IL-6, can be detected in the lung. The lung pathology at day 6 post-virus exposure is characterized by acute-to-subacute alveolitis with some perivascular edema, and moderate neutrophil and macrophage infiltration. Another lethal mouse model was developed using a similar strategy of serially passaging mouse lung homogenate through mice to develop a virus that was lethal for mice (strain MA15) [95]. However, for the MA15 virus, it required 15 passages to adapt to mice, and mice were sacrificed when weight loss was 20% to achieve 70–100% mortality. Similar pathogenesis and cytokine profiles are seen with this model and virus strain, although MA15 appears to be slightly less virulent than the v2163 strain [85].

The golden Syrian hamster (strain LVG) has been shown to be very permissive to SARS-CoV replication in the lung [96]. In addition, the viral replication is accompanied by pathological changes in the lungs, including pneumonitis and consolidation. Peak viral

replication in the lungs occurs at day 3 post-virus exposure, and virus is cleared from the lungs by day 7 of the infection. Virus can also be recovered from the spleen and liver. As with the mouse models described above, clinical symptoms and signs associated with human disease have been difficult to identify.

Ferrets (*Mustela furo*) also support the replication of SARS-CoV, with peak viral lung titers being detected at day 4 post-virus exposure and reaching levels as high as 10^6 median tissue culture infective dose/ml [97,98]. The lung pathology is characterized by multifocal pulmonary lesions only involving a small percentage of the surface area of the lung [98]. In another study, intranasally infected ferrets had clinical signs similar to that of humans – increased body temperature from days 2–6 post-virus exposure (sneezing was also detected during this time period) – but the lung pathology was considered mild and the infection was self-limiting [99].

A number of nonhuman primates have been demonstrated to support SARS-CoV replication with an accompanying pneumonitis [92]. However, most drug efficacy studies have been performed in rhesus macaques. In addition to finding that SARS-CoV replicates to high titers in the lungs, viral shedding can be demonstrated in respiratory secretions, and some histopathological changes can be seen that seem to correlate with human infection of type I and II pneumocytes and diffuse alveolar damage can also be shown [92].

Animal efficacy studies

Mice—A number of compounds have been evaluated in both mouse lung replication models and in lethal models of the BALB/c mouse using two similar mouse-adapted SARS coronaviruses. Several studies have evaluated the therapeutic efficacy of antibody therapy [78,100]. In an aerosol infection model using aged mice, one group of mice was treated intraperitoneally with 15 mg/kg of a humanized mouse antibody (2978/10) selected by human framework reassembly technology and optimized for neutralization and specificity [78]. Immediately following the treatment, mice were challenged by aerosol exposure to SARS-CoV. Day 3 viral lung titers were evaluated and the antibody treatment of mice resulted in an approximately 15,000-fold reduction in virus titers, with several animals showing no evidence of SARS virus in the lung at the detectable limits of the virus assay. In a similar-aged model, equine anti-SARS-CoV F(ab')(2) was used to treat a SARS-CoV infection in BALB/c mice [100]. When the equine anti-SARS-CoV F(ab')(2) antibody was intraperitoneally administered 24 h after infection, the 40-mg/kg treatment significantly decreased viral lung titers by more than 1000-fold compared with the lung virus titers in the placebo control group, and reduced the detectable copies of mRNA for the N protein in the lung homogenates by a similar amount ($p < 0.01$).Thus, immunotherapy with antibody may be a useful way of treating SARS infections.

Another immunological approach for treating infections is to use substances found as part of the innate immune system of vertebrates. For example, rhesus θ-defensin 1 (RTD-1) is an 18-residue, macrocyclic, tri-disulfide antibiotic peptide first found in the granules of neutrophils and monocytes of rhesus macaque leukocytes [101]. All BALB/c mice treated intranasally with RTD-1 at 5 mg/kg 15 min prior to exposure to a mouse-adapted strain of SARS-CoV (MA15) survived the infection with minimal weight loss. SARS-CoV-infected mice had altered lung tissue cytokine patterns at days 2 and 4 post-virus exposure compared with those of untreated animals, including a robust IL-6 response in RTD-1-treated mice, which was significantly greater than the IL-6 response in infected, untreated mice at day 2 post-virus exposure ($p < 0.05$). RTD-1 did not significantly inhibit virus replication in the lungs, nor did the treatment seem to affect the observable lung pathology in a profound way. Thus, RTD-1 probably has immune modulatory properties, or its antiviral activity may be to

delay or limit viral replication to a level or rate below a critical threshold beyond which the host is unable to adequately control the virus infection.

Another example of an antimicrobial substance associated with the innate immune system is the inducible bronchus-associated lymphoid tissue, which functions as an inducible secondary lymphoid tissue for respiratory immune responses to specific infections [102]. The approach used to harness this activity to become a useful therapy for treating SARS infections in mice was to develop a protein cage nanoparticle (PCN) to significantly accelerate clearance of SARS-CoV after primary infection and to elicit a host immune response in the inducible bronchus-associated lymphoid tissue tissue that would result in less lung damage [103]. The PCN molecules used in the study were derived from the small heat-shock protein 16.5 of the hyperthermophilic archaeon *Methanococcus jannaschii*. When BALB/c mice were pretreated with the PCN (100 μg/mouse) 24 h prior to virus exposure and then challenged with an extremely lethal dose of mouse-adapted SARS-CoV, all treated mice survived the infection and did not lose any weight throughout the infection. Gross lung pathology also seemed to be reduced. Therapeutic dosing regimens did not protect mice against death nearly as well. The PCN was also not toxic in uninfected mice [Kumaki Y, Unpublished Data]. Protection against death and weight loss due to the SARS-CoV infection was already apparent by 3 days after infection, which is well before an adaptive primary immune response expands to the point of effectiveness. It is likely that innate immune mechanisms were modulated by this PCN treatment. Thus, the data show that the ability to nonspecifically enhance immune protection against a respiratory virus, most notably in the absence of significant pulmonary inflammation, is a feasible way of preventing SARS infections.

A more direct approach to modulating the immune system to prevent or inhibit a SARS infection might be to treat with interferons or perhaps with compounds that induce interferon. In the virus lung replication model of SARS-CoV in BALB/c mice, compounds previously approved for use for other diseases and *in vitro* inhibitors of SARS-CoV were evaluated for inhibition in the mouse SARS-CoV replication model. A hybrid interferon, IFN-α B/D, and a mismatched dsRNA interferon inducer, Ampligen® (poly I:poly $C_{12}U$), were the only compounds that potently inhibited virus titers in the lungs of infected mice as assessed by CPE titration assays [104]. Mice were dosed intraperitoneally with IFN-α B/D once-daily for 3 days beginning 4 h after virus exposure, and lung titers reduced by 1 $\log_{10}$ at 10,000 and 32,000 international units compared with lung virus titers in infected, untreated mice. Ampligen used intraperitoneally at 10 mg/kg 4 h prior to virus exposure reduced virus lung titers to below detectable limits. Alferon (human leukocyte IFN-α-n3) did not significantly reduce lung virus titers in mice as would be expected when using human interferon protein to induce mouse antiviral proteins responsive to mouse interferon. The inhibitory activity of Ampligen was later confirmed in a lethal mouse model of SARS-CoV using a mouse-adapted Urbani strain [85]. In that study, all mice survived who were given Ampligen at 10 mg/kg 4 h prior to virus exposure. The lung gross pathology scores at day 6 were significantly lower compared with those for mice treated with placebo ($p < 0.05$), and the weight loss associated with infection was dramatically reduced ($p < 0.001$). Interestingly, viral lung titers were not significantly reduced at day 3 or 6 post-virus exposure compared with viral lung titers in the placebo-treated mice. However, by day 6 of the infection, Ampligen treatment led to a more rapid decline of virus detected in the lungs compared with virus detected in the lungs of untreated animals.

Another interferon inducer, polyinosinic-polycytidylic (poly-ICLC), was also found to be effective in the SARS-CoV lethal mouse model. It is a double-stranded poly-ICLC RNA stabilized with poly-lysine and carboxymethylcellulose and acts as a TLR-3 ligand. TLR-3 ligands serve as natural inducers of proinflammatory cytokines capable of promoting type-1

adaptive immunity [105]. Treatment with poly-ICLC (5 mg/kg) by the intranasal route beginning at 24 h and continued twice a day for 5 days protected all treated mice against the lethal infection with mouse-adapted SARS-CoV and reduced day-3 viral lung titers by over 1 $\log_{10}$ half-maximal cell culture infectious dose ($CCID_{50}$) [106]. Gross lung pathology at day 6 was also greatly reduced in mice administered poly-ICLC up to 16 h post-virus exposure. The severe weight loss attributable to virus infection was also greatly reduced for mice receiving the treatments initiated up to 8 h post-virus exposure. However, therapeutic intervention initiated 8 h after virus exposure was not effective in preventing death.

One issue that that makes it more difficult to treat infections with recombinant interferons is the short serum half-life. Thus, a number of things have been done to interferons to stabilize them, including PEGylating the interferon. To overcome the fast *in vivo* decay of IFN-α, an adenovirus 5 (Ad5) gene-delivery platform was made to deliver the mouse *IFN*-α gene (subtype 5) that constitutively drives IFN-α production *in situ* (mDEF201), although an interferon gene from any species may be inserted into the Ad5 platform and it will be expressed [107]. To subvert the immunological response to the Ad5 platform, the material was delivered intranasally. In a SARS-CoV lethal mouse model, single-dose intranasal administration with mDEF201 (measured as PFU of platform virus, 10^6 or 10^5 PFU) protected from the lethality of the mouse-adapted SARS-CoV Urbani strain [108]. When mice were given a single dose of mDEF201 administered intranasally 1, 3, 5, 7 or 14 days prior to lethal SARS-CoV challenge ($p < 0.001$), all treated mice survived. Bodyweight loss due to viral challenge was abrogated at the higher dose of mDEF201. In addition, even low doses of mDEF201 reduced the gross pathology caused by the virus infection. Therapeutic intranasal treatment with mDEF201 ranging from 10^6 to 10^8 PFU significantly protected mice against a lethal SARS-CoV infection in a dose-dependent manner up to 12 h postinfection ($p < 0.001$). There was no reduction of viral lung titers in treated mice, a phenomenon that has been reported by others when using interferon to treat SARS infections in animals [109].

A different way of inhibiting virus infections may be to disrupt the attachment of virus by blocking virus interactions with the host cell. For example, TACE antagonists (i.e., TAPI-2) that block ACE2 shedding caused by the spike protein of SARS-CoV have been shown to inhibit SARS-CoV replication *in vitro* [76]. An efficacy study was done in 7-week-old BALB/c mice to validate the *in vitro* activity [76]. For this study, mice were either pretreated intranasally with TAPI-2 20 μM (in 20 μl) at 1 h before virus exposure and then 3 and 27 h after infection, or they were treated with the compound at 3 and 27 h after virus exposure. Virus titers from lung lavage fluids taken at day 3 post-virus exposure were at least 1.5 $\log_{10}$ $CCID_{50}$ lower in the mice receiving the combination prophylactic–therapeutic treatment. The viral lung titers in mice receiving the therapeutic dosing regimen were no different than viral lung titers in the untreated, infected control mice. The data suggested a positive effect on reducing viral lung replication, but the sample sizes ($n = 4$) used in the experiment did not provide enough statistical power to show any significant differences.

Inhibiting virus attachment by binding to the virus may be another way of preventing a SARS infection *in vivo*. Stinging nettle lectin, UDA, is an N-acetyl glucosamine-specific lectin that was reported to be a potent and selective inhibitor of the SARS-CoV strain Frankfurt-1 [84]. Plant lectins like UDA probably target viral attachment and fusion, and exocytosis or egress of the virus from the cell [110]. UDA treatment (mg/kg/day) of BALB/c mice infected with a lethal mouse-adapted strain of Urbani (v2163) resulted in 50% protection from death up to 10 days after infection but no reduction in lung virus titer [85]. In addition, reduction of IL-6 in lungs of mice treated at day 3 post-virus exposure was detected, in contrast with infected mice in which IL-6 levels were extremely high at day 3

post-virus infection [85]. A replicate 21-day study showed that when mice were treated with 15 mg/kg/day, 40% protection from death was achieved with no measurable toxicity.

Another virus attachment inhibitor is griffithsin. The antiviral protein griffithsin was originally isolated from the red alga *Griffithsia* species based upon its activity against HIV [111]. This unique 12.7-kDa protein was shown to bind specifically to oligosaccharides on the surface of viruses. It has been shown to specifically bind to the SARS-CoV spike glycoprotein and inhibit viral entry [112]. In a lethal mouse model of SARS-CoV (MA15 strain), animals that received griffithsin (2 mg/kg/dose) 4 h before inoculation with virus followed by twice-daily griffithsin treatment for 4 days did not lose weight, and 100% of the griffithsin-treated mice survived [112]. Griffithsin treatment significantly reduced day-2 viral lung titers by approximately 2 $\log_{10}$ $CCID_{50}$ compared with placebo-treated mice. Mice receiving griffithsin also had reduced levels of pulmonary edema at both 2 and 4 days postinfection and reduced severity of necrotizing bronchiolitis at 2 days postinfection compared with placebo-treated mice. Interestingly, there might be some ancillary toxicity associated with griffithsin treatment of mice; uninfected mice administered griffithsin demonstrated some perivascular infiltrate in the lung that was largely resolved 6 days after the last dose of compound.

Hamsters—The concept of passively transferring antibodies specific for SARS-CoV as a therapeutic treatment for SARS infections has been tested in hamster models for SARS infection [113–115]. In one study, golden Syrian hamsters were intranasally inoculated with SARS-CoV and treated with various doses of mAb 201 24 h after inoculation [115]. Antibody treatment reduced viral lung titers by 2.4–3.9 $\log_{10}$ $CCID_{50}$/g of lung tissue. Interstitial pneumonitis and consolidation were also dramatically reduced.

In a Chinese hamster SARS-CoV infection model, the same antibody was also shown to prophylactically protect animals from SARS-CoV infection [114]. More importantly, its usefulness as a therapeutic was demonstrated. Antibody treatment resulted in almost a 4 $\log_{10}$ drop in viral lung titers and amelioration of lung pathology compared with infected, untreated hamsters. Thus, this study suggests that equine anti-SARS-CoV F(ab')(2) might be useful in treating SARS infections in humans. It remains to be determined if the administration of antibody from another species to humans has any short- or long-term negative consequences.

Ferrets—A human IgG1 monoclonal antibody, CR3014, reactive with whole inactivated SARS-CoV, was developed using antibody phage display technology by screening a large naive antibody library [116]. Since it neutralized SARS-CoV in *in vitro* assays, it was tested *in vivo* as an immunoprophylactic in a ferret model for SARS-CoV infection. Prophylactic administration of CR3014 given 24 h prior to virus exposure at 10 mg/kg significantly reduced replication of SARS coronavirus in the lungs of infected ferrets by 3.3 $\log_{10}$ ($p < 0.001$), significantly inhibited the development of SARS coronavirus-induced macroscopic lung pathology ($p = 0.013$) and abolished shedding of virus in pharyngeal secretions. Perhaps the more important finding was that shedding of SARS-CoV from the throat of treated ferrets was completely abolished in three of the four treated animals.

Macaques—Several studies have been performed to determine the feasibility of treating SARS infections in rhesus macaques with siRNA [117,118]. In one study, siRNA duplexes were made that targeted the SARS-CoV S protein-coding region and the *ORF1b* (*Nsp12*) regions of the viral genome [117]. The siRNAs (30 μg/macaque), solubilized in 5% dextrose in water solution, were administered intranasally to the animals either 4 h prior to virus exposure, at 0, 24, 72 or 120 h or at 4, 24 or 72 h after virus exposure. Macaques receiving all dosing regimens of siRNA had reduced body temperatures, decreased virus replication in

the lungs and less acute diffuse alveoli damage. However, the significance of the histological scores reported is inconclusive as the authors incorrectly analyzed the data by using a Student's t-test versus one control to analyze nonparametric data in an experiment with multiple treatment groups. The siRNA was not toxic at 10–40-mg/kg accumulated dosages of siRNA.

Modulation of the immune system has also been explored as a potential method for treating SARS infections. The therapeutic effects of recombinant IFN-α2b administered as a nasal spray to treat SARS-CoV infection in rhesus macaques was studied [119] based on previous *in vitro* and *in vivo* mouse studies showing that SARS-CoV replication was very sensitive to inhibition by interferon [104,120]. In this rhesus macaque infection model, the level of SARS-CoV-specific IgG and neutralizing antibody induced by SARS-CoV was lower in the interferon treatment group than in the control group [119]. Pathological examination of the lung tissues from animals receiving the interferon treatment revealed that the pathology in the lungs appeared to be similar to that detected in normal macaques. The lungs of untreated, infected macaques had the typical pathological signs of interstitial pneumonia, with thickened septum and infiltration of mononuclear cells being the more predominant findings. This study was too small to achieve statistical significance, but it does support the findings that have been demonstrated in a mouse model: interferon treatment can ameliorate the detrimental effects of a SARS-CoV infection [104]. In another study in which interferon was administered to aged rhesus macaques, therapeutic treatment of SARS-CoV-infected aged macaques with type I interferon reduced pathology and decreased proinflammatory gene expression, including IL-8 levels, a potent chemotactic factor essential in acute inflammation [109]. However, viral lung titers in treated macaques were equivalent to the viral lung titers in untreated, infected macaques. Thus, the acute lung injury induced by SARS-CoV infection that exacerbated the innate host response was abrogated by treatment with type I interferon. The findings of this study support the findings from the SARS mouse models in which interferons were used therapeutically, and suggest that interferon treatment or induction of interferon would likely be successful in treating SARS infections in humans.

Finally, some of the animal studies provide evidence that suggests certain agents previously reported to inhibit SARS-CoV replication *in vitro* should not be used to treat SARS-CoV infections, including ribavirin [104,121,122], promazine [123] and IMP dehydrogenase inhibitors [122] because they are either ineffective, they exacerbate the infection and/or they promote the death of the animal.

Executive summary

Targets for anti-severe acute respiratory syndrome chemotherapy

- A number of targets in the severe acute respiratory syndrome coronavirus (SARS-CoV) replication cycle have so far been exploited.
- They include various structural and nonstructural viral proteins.
- They also include host processes or proteins found necessary for viral replication.

Compounds found active *in vitro*

- The following compounds are active *in vitro*:
  - Papain-like protease inhibitors;
  - SARS-CoV main protease inhibitors;
  - RNA-dependent RNA polymerase inhibitors;

- Helicase inhibitors;
- siRNAs inhibiting SARS-CoV structural proteins E, M and N;
- siRNAs inhibiting virion-associated accessory proteins from *Orf7*;
- siRNAs inhibiting virion-associated accessory proteins from *Orf3a*;
- Compounds inhibiting virus entry and fusion;
- Compounds inhibiting host functions.

Animal models most frequently used for efficacy testing of SARS–CoV inhibitors

- The most frequently tested animal models are mice (aged mice, 5–7-week-old mice, lung replication model mice and the lethal model using mouse-adapted SARS-CoV), hamsters, ferrets and macaques (aged and mature cynomolgus macaques and rhesus macaques).

Compounds found active *in vitro*

- The following compounds are active *in vitro*:
  - TNF-α-converting enzyme inhibitor (TAPI-2);
  - IFN-α (B/D, mDEF201 by adenovirus 5 vector, CR3014 humanized monoclonal antibody, recombinant IFN-α2b and type I IFN-β);
  - Interferon inducers (Ampligen and polyinosinic–polycytidylic);
  - Therapeutic antibodies (2978/10, equine anti-SARS-CoV F[ab'] [2] and monoclonal antibody 201);
  - Attachment inhibitors (*Urtica Dioica* lectin and griffithsin);
  - Host immune system;
  - iBALT inducer (nanoparticle heat-shock protein cage);
  - Rhesus θ-defensin 1;
  - Nonstructural protein 12 siRNA.

Future perspective

In 2003, SARS-CoV emerged as a virus of grave concern to the world community due to its ability to cause severe, life-threatening disease with an appalling mortality rate. Since then it has 'disappeared' from the public health scene due, in part, to vigilant public health measures. Owing to the potential of SARS-CoV to reemerge due to a variety of factors or the possibility of a SARS-like virus to arise to cause serious disease, it is still prudent to develop and get approved antiviral therapies that could be used to treat the disease caused by this as yet untreatable virus.

Three approaches should be actively pursued: vaccines, postexposure prophylaxis to help isolate focus cases and contacts to prevent spread, and therapeutic efficacious drugs targeting either virus-encoded functions, host targets necessary for virus replication or host functions modulated by virus infection that exacerbate disease. Any of these remedies should be developed to be able to contend with rapid virus evolution and host safety, and to be able to cope with the rapidity with which this disease can become pandemic.

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Table 1

Anti-severe acute respiratory syndrome coronavirus agents that inhibit defined targets (2007–2011).

Target	Targeted function/activity	Antiviral agent	Significant antiviral activity (EC_{50}/IC_{50})†	Ref.
Nonstructural proteins				
Nsp3	Papain-like proteinases; putative catalytic triad (Cys273-His112-Asp287) [32,33]	GRL0617 (5-amino-2-methyl-N-[(R)-1-(1-naphthyl)ethyl]benzamide)	14.5 μM	[36]
	Papain-like proteinases; putative catalytic triad (Cys273-His112-Asp287) [32,33]	Benzodioxolane derivatives: 1-[(R)-1-(1-naphthyl)ethyl]-4-[3,4-(methylenedioxy)benzylamino] carbonylpiperidine, 1-[(S)-1-(1-naphthyl) ethyl]-4-[3,4-(methylenedioxy)benzylamino] carbonylpiperidine	9.1 μM	[37]
Nsp5	3C-like main protease cysteine proteinase cleaving polyproteins to form key functional enzymes such as replicase and helicase [38,39,41,124]; may induce apoptosis [124]	5-chloropyridinyl indolecarboxylate	6.9 μM	[42]
Nsp12	RNA-dependent RNA polymerase that produces genome- and subgenome-sized RNAs of both polarities [44,45]	Zinc + ionophore (pyrithione + $ZnOAc_2$)	0.5 μM	[46]
Nsp13	5′–3′ helicase (NTPase and RNA 5’-tri-phosphatase activities) [48–50]	Ranitidine bismuth citrate	5.9 μM	[51,52]
Structural proteins				
(*Orf4*) E protein	The envelope protein appears to act as a ion-channel protein [54]; putatively involved in viral budding and release [53,55]	siRNA	~4 μM	[56]
(*Orf5*) M protein	Membrane protein [55]; surface protein responsible for viral assembly and budding; suppresses NF-κB [57]; may also induce apoptosis [58,59]	siRNA	~4 μM	[56]
(*Orf9a*) N protein	Nucleocapsid protein [60]; binding and packaging of viral RNA in assembly of the virion [61]	siRNA	~4 μM	[56]
		10Fn3 intrabodies	EC_{90} = 6.7–60 ng transfected plasmid	[62]
Virion-associated accessory proteins				
Orf7a	Type I transmembrane protein and possible structural protein [63]; involved in viral assembly by interacting with M and N proteins [61]; essential for induction of cell cycle arrest [64]; SARS coronavirus protein 7a interacts with human Ap4A-hydrolase [65]; not	siRNA	1–1.5 $\log_{10}$ reduction in virus titer	[66]

Target	Targeted function/activity	Antiviral agent	Significant antiviral activity (EC_{50}/IC_{50})†	Ref.
	essential for replication *in vitro* or *in vivo*			
Orf7b	Not known; incorporated into virions [67]; not essential for replication *in vitro* or *in vivo*	siRNA	1–1.5 $\log_{10}$ reduction in virus titer	[66]
Viral-encoded cellular accessory proteins				
Orf3a	Forms potassium-sensitive ion channels [68]; virion associated [69]; may promote virus budding and release [68,70]; also induces apoptosis [68,70]; not essential for replication *in vitro* or *in vivo*; promotes membrane rearrangement and cell death [71]	siRNA	1–1.5 $\log_{10}$ reduction in virus titer	[66]

† Selective index = antiviral efficacy value (EC_{50} or IC_{50})/toxicity value (IC_{50} or CC_{50}).

CC_{50}: Half-maximal cytotoxic concentration; CoV: Coronavirus; EC_{50}: Half-maximal effective concentration; IC_{50}: Half-maximal inhibitory concentration; SARS: Severe acute respiratory syndrome.

Table 2

Miscellaneous anti-severe acute respiratory syndrome coronavirus agents with less-well-defined targets of inhibition (2007–2011)

Target	Targeted function/molecule type	Antiviral agent
Viral entry	S protein-activated cellular factor TNF-α-converting enzyme, promotes viral entry [72]	N-(R)-(2-[hydroxyaminocarbonyl]methyl)-4-methylpentanoyl-L-t-butyl-glycine-L-alanine 2-aminoethyl ami
Virus-neutralizing epitopes	Virus infectivity	2978/10 humanized antibody
Viral entry	Glycoproteins	Plant lectins: HHA, Nictaba, UDA
Virus spreading	Inhibits endosomal processing of SARS-CoV	Amiodarone
Fusion	Presumably the S protein	Arbidol
Extracts/natural products		
Viral replication	SARS-CoV 3CL protease?	Diterpenoids, sesquiterpenoids, triterpenoids, lignoids, curcumin
Viral replication	Post-virus adsorption event	TSL-1

†Selective index = antiviral efficacy value (EC_{50} or IC_{50})/toxicity value (IC_{50} or CC_{50}).

CC_{50}: Half-maximal cytotoxic concentration; CoV: Coronavirus; EC_{50}: Half-maximal effective concentration; HHA: Hippeastrum hybrid lectin (amaryllis bulbs); IC_{50}: Half-maximal inhibitory concentration; SARS: Severe acute respiratory syndrome; TSL: Toona sinensis leaf; UDA: Urtica dioica agglutinin.

Table 3

Efficacy of various drugs-tested animal models of severe acute respiratory syndrome infections.

Animal species	Strain/type	Model	Antiviral agent	Efficacy	Ref.
Mouse	BALB/c	Virus lung replication model (7-week-old mice)	TACE inhibitor (TAPI-2), N-(R)-(2-(hydroxyaminocarbonyl) methyl)-4-methylpentanoyl-L-t-butyl-glycine-L-alanine 2-aminoethyl amide	1.5 $\log_{10}$ drop in viral lung titer	[76]
Mouse	BALB/c	Virus lung replication model (5–6-week-old mice)	IFN-α B/D; Ampligen® (poly I:poly $C_{12}U$)-mismatched dsRNA interferon inducer	Day-3 viral lung titers reduced by 1 $\log_{10}$ at 10,000 and 32,000 IU Day-3 viral lung titers reduced by 5 $\log_{10}$ at 10 mg/kg/mouse	[104]
Mouse	BALB/c	Senescent mouse model	Humanized mouse antibody 2978/10	~15,000-fold reduction in day-3 viral lung titers	[78]
Mouse	BALB/c	Senescent mouse model	Equine anti-SARS-CoV F(ab')(2)	Reduced viral lung titers by ~1000-fold and ~1000-fold fewer copies of N protein mRNA were detected	[100]
Mouse	BALB/c	Lethal mouse-adapted virus model (v2163 virus)	Stinging nettle lectin, UDA	50% survival at 5 mg/kg/day, 40% survival at 15 mg/kg/day, reduction of IL-6 levels	[85]
Mouse	BALB/c	Lethal mouse-adapted virus model (MA15 virus)	Griffithsin	100% survival, no weight loss, 2 $\log_{10}$ drop in day-2 viral lung titers, reduction in lung pathology	[112]
Mouse	BALB/c	Lethal mouse-adapted virus model (v2163 virus)	PCN induction iBALT tissue	100% survival, no weight loss	[103]
Mouse	BALB/c	Lethal mouse-adapted virus model (MA15 virus)	Rhesus θ-defensin 1	100% survival, minimal weight loss, robust IL-6 response, no effect on viral lung titers	[101]
Mouse	BALB/c	Lethal mouse-adapted virus model (V2163 virus)	Ampligen (poly I:poly $C_{12}U$)-mismatched dsRNA interferon inducer	100% survival, day-6 lung scores significantly lower, weight loss significantly reduced	[85]
Mouse	BALB/c	Lethal mouse-adapted virus model (v2163 virus)	Poly IC:LC (Hiltonol®)	100% survival, protected against severe weight loss, 1.2 $\log_{10}$ drop in viral lung titers, decreased gross lung pathology	[125]
Mouse	BALB/c	Lethal mouse-adapted virus model (v2163 virus)	mDEF201	Prophylaxis: 100% survival, no weight loss, viral lung titers unaffected; Therapeutic: 100% survival to 12-h post-virus exposure	[108]
Hamster	Golden Syrian (LVG)	Virus lung replication model, golden Syrian hamster	mAb 201	2.4–3.9 $\log_{10}$ drop in viral lung titers, reduced interstitial pneumonitis and lung consolidation	[115]
Hamster	Chinese	Virus lung replication model	Equine neutralizing antibody F(ab')(2)	4-$\log_{10}$ drop in viral lung titers, amelioration of lung pathology	[114]
Ferret	Unknown	Virus lung replication model	CR3014 (human IgG1 neutralizing mAb)	3.3 $\log_{10}$ drop in viral lung titers, reduced pathology	[116]
Macaque	Rhesus	Infection model	siRNA siSC2	Reduced body temperatures, viral lung titers and acute diffuse alveoli damage	[117]

Animal species	Strain/type	Model	Antiviral agent	Efficacy	Ref.
Macaque	Rhesus	Infection model	Recombinant IFN-α2b	Amelioration of lung pathology	[119]
Macaque	Cynomolgus	Old-age infection model	Type 1 IFN-β	Abrogation of acute lung injury, decreased levels of IL-8	[109]

CoV: Coronavirus; iBALT: Inducible bronchus-associated lymphoid tissue; IU: International unit; mAb: Monoclonal antibody; PCN: Protein cage nanoparticle; SARS: Severe acute respiratory syndrome; SiSC2: siRNA duplexes targeting severe acute respiratory syndrome coronavirus RNA; TACE: TNF-α-converting enzyme; UDA: Urtica dioica agglutinin.