

# From Hydroxychloroquine to Ivermectin: what are the anti-viral properties of anti-parasitic drugs to combat SARS-CoV-2?

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## Abstract

**Background:** Nearly a year into the COVID-19 pandemic we still lack effective anti SARS-CoV-2 drugs with substantial impact on mortality rates except for dexamethasone. As the search for effective antiviral agents continues, we aimed to review data on the potential of repurposing antiparasitic drugs against viruses in general, with an emphasis on coronaviruses.

**Methods:** We performed a review by screening *in vitro* and *in vivo* studies that assessed the antiviral activity of several antiparasitic agents: chloroquine, hydroxychloroquine, mefloquine, artesiminins, ivermectin, nitazoxanide, niclosamide, atovaquone, and albendazole.

**Results:** For hydroxychloroquine and chloroquine we found ample *in vitro* evidence of antiviral activity. Cohort studies that assessed the use of hydroxychloroquine for COVID-19 reported conflicting results, but randomized controlled trials (RCTs) demonstrated no effect on mortality rates and no substantial clinical benefits of HCQ (hydroxychloroquine) used either for prevention or treatment of COVID-19. We found two clinical studies of artesiminins and two studies of nitazoxanide for treatment of viruses other than COVID-19, all of which showed mixed results. Ivermectin was evaluated in one RCT and few observational studies, demonstrating conflicting results. As the level of evidence of these data is low, the efficacy of ivermectin against COVID-19 remains to be proven. For chloroquine, hydroxychloroquine, mefloquine, artesiminins, ivermectin, nitazoxanide and niclosamide we found *in vitro* studies showing some effects against a wide array of viruses. We found no relevant studies for atovaquone and albendazole.

Conclusions: As the search for an effective drug active against SARS-CoV-2 continues, we argue that pre-clinical research of possible antiviral effects of compounds that could have antiviral activity should be conducted. Clinical studies should be conducted when sufficient *in vitro* evidence exists, and drugs should be introduced into widespread clinical use only after being rigorously tested in RCTs. Such a search may prove beneficial in this pandemic, or in outbreaks yet to come.

## Introduction

The COVID-19 pandemic has already incurred a shocking price in terms of lives lost, worldwide economic recession, political tensions and psychological stress.<sup>1,2</sup> This pandemic's effects on high risk populations are still being studied.<sup>3</sup> Since time is critical, and no treatment except dexamethasone has been proven to decrease mortality,<sup>4</sup> there is now a worldwide rush to find an effective anti-viral agent.

Repurposing of existing antivirals is ongoing but results from most studies thus far have been poor, with many agents showing promise in small groups of relatively young patients, who are less likely to succumb to COVID-19. The few controlled trials that have been performed are somewhat disappointing; the protease inhibitor lopinavir / ritonavir has failed to demonstrate clear superiority over a placebo in one randomized controlled trial. A combination of lopinavir, ritonavir, ribavirin and interferon beta-1b was found to be effective in alleviating symptoms and shortening viral shedding, but such complex regimen is unlikely to become a mainstay of treatment.<sup>5</sup>

Another antiviral compound, remdesivir, was initially shown to be modestly effective in shortening illness duration in a randomized controlled trial.<sup>6</sup> However, recent clinical trials including the WHO solitary trail (that did not include a placebo group), did not show any mortality benefits<sup>7,8</sup>. The drug is administered only intravenously,

probably does not decrease mortality, and is unavailable to most clinicians worldwide.

Under these circumstances the use of older antiparasitic agents, based on *in vitro* antiviral activity, animal studies, or preliminary data on COVID-19 patients, is an interesting option. In this review we describe the available evidence on the activity of antiparasitic agents, including antimalarial drugs, on viruses and specifically on SARS-CoV-2 and reviewed interventional and observational studies that assessed the clinical effect of those therapies for COVID-19.

## Methods

### Type of studies

In this review we screened *in vivo* and *in vitro* studies that focus on antiviral activities of several antiparasitic agents, including specific activity against COVID-19 and other coronavirus-related infections (MERS, SARS and SARS-CoV-1). Antiparasitic agents include hydroxychloroquine (HCQ), chloroquine, mefloquine, artesimins, ivermectin, nitazoxanide (NTZ) and niclosamide, atovaquone, and albendazole. The structures and uses of these drugs are given in Table 2. We included studies published during the COVID-19 pandemic in English. We have included RCTs, non – randomized comparative cohorts, case series, and case reports containing data on the anti-viral activity of individual drugs. *In vitro* studies were included if detailing an experiment with a relevant drug and a specific virus.

### Types of outcome measures

Clinically relevant outcomes such as mortality rates, hospital admission rates, lengths of hospital stay and disease severity were recorded. In addition, virologic parameters such as viral clearance time, viral shedding duration, and viral load when relevant were also described.

## Search methods for identification of studies

We conducted a systematic electronic literature screen using the Pubmed search engine by searching one or more of the following terms: "COVID", "MERS", "SARS" or "antiviral," combined with the names of several antiparasitic agents (chloroquine, hydroxychloroquine, mefloquine, artemisinins, nitazoxanide, niclosamide, ivermectin, albendazole and atovaquone). We used references of retrieved papers, including reviews or systematic reviews to identify further studies. Two reviewers independently screened all studies published before 15 November 2020. We excluded all retrieved articles that did not fulfill the inclusion criteria. In cases of disagreement, a third reviewer acted as arbitrator.

## **Results**

The numbers of all *in vitro* and *in vivo* articles that were included are shown in table 1. Our main findings are summarized in tables 2 and 3. As we found neither *in vitro* nor *in vivo* studies for albendazole or atovaquone, these two agents are not included in this review.

## **Chloroquine and hydroxychloroquine**

Chloroquine and HCQ are 4-aminoquinoline organic compounds that have long been used for the treatment and prevention of malaria, as anti-bacterial therapy for Q fever, and as immune modulators. These two drugs are very similar in structure, and their mechanism of action appears identical. HCQ is well tolerated and is generally regarded to have a better safety profile than chloroquine.

Most adverse reactions of HCQ are mild and include gastrointestinal adverse effects, skin rash and photosensitivity. Large cumulative dosages, commonly administered over a prolonged period of time, have been associated with irreversible toxic retinopathy. Cardiac toxicity is rare but may be life threatening.<sup>9</sup> According to current knowledge, significant QT prolongation occurs in 3-13% of patients, with

increasing incidence with age and among patients that are co-treated with azithromycin. Although torsades de pointes has not been recorded in small cohorts of COVID-19 patients, it is possible that fatal arrhythmias will be encountered if the drug will be used extensively.<sup>10-12</sup>

Chloroquine is active *in vitro* and in animal models against several viruses, including human immunodeficiency virus (HIV),<sup>13</sup> hepatitis C virus,<sup>14</sup> influenza type H1N1,<sup>15</sup> H5N1,<sup>16</sup> flaviviruses such as dengue virus and Zika,<sup>17</sup> Middle East Respiratory Syndrome (MERS) virus,<sup>18-20</sup> and SARS-CoV-1.<sup>21,2223-25</sup>

In early *in vitro* studies, chloroquine was found effective in blocking SARS-CoV-2 infection at low concentrations.<sup>23</sup> Recent *in vitro* and physiologically based pharmacokinetic (PBPK) modeling studies found higher potency for HCQ compared to chloroquine in reducing SARS-CoV-2.<sup>26,27</sup>

#### Clinical experience with HCQ and chloroquine

From the onset of the COVID-19 pandemic, HCQ was administered in most affected countries. A dose of 400mg twice daily followed by 200mg twice daily of HCQ was predicted to achieve an antiviral effect based on PK modeling, although dosage and treatment duration varied between guidelines.<sup>27</sup> In the current review we did not specify the exact doses that were used; we refer the reader to a previous systematic review for more specific details.<sup>28</sup>

In some non-randomized comparative studies or small cohorts, a shortening of clinical disease, a faster viral clearance, and a decrease in the likelihood to develop pneumonia were reported following the use of chloroquine or HCQ.<sup>29-32</sup> The combination of HCQ and azithromycin was also suggested to be beneficial in non-randomized small non-comparative studies.<sup>30,33</sup> Possible synergistic effects with ivermectin were also suggested.<sup>34</sup>

Other observational studies demonstrated no significant reduction in mortality rates following HCQ treatment.<sup>35-39</sup> In one study, all-cause mortality risk was significantly higher among HCQ-treated patients compared to patients that were not treated with HCQ (adjusted hazard ratio 1.83; 95% CI 1.16–2.89; P=0.009),<sup>40</sup>.

Recently, the results of three large scale RCTs were published. The "RECOVERY Collaborative group" trial compared 1561 patients treated with HCQ to 3155 patients that received standard of care. Death within 28 days occurred in 421 patients (27.0%) in the HCQ group compared to 790 (25.0%) in the usual-care group (rate ratio, 1.09; 95% confidence interval [CI], 0.97 to 1.23; P=0.15). Furthermore, patients in the HCQ group that were not undergoing mechanical ventilation at baseline had a higher frequency of invasive mechanical ventilation or death (30.7% vs. 26.9%; risk ratio, 1.14; 95% CI, 1.03 to 1.27).<sup>41</sup> The WHO solitary study compared 954 patients treated with HCQ to 909 patients receiving its control. Death occurred in 10.9% of patients receiving HCQ and in 9.2% receiving its control (rate ratio, 1.19; 95% CI, 0.89 to 1.59; P=0.23). The use of HCQ did not reduced initiation of ventilation or hospitalization duration.<sup>7</sup> The third study evaluated 479 hospitalized adults with respiratory symptoms from COVID-19. It demonstrated no significant differences in the distribution of the day 14 clinical status score (measured using a 7-category ordinal scale) for patients receiving HCQ compared with placebo (adjusted odds ratio, 1.02).<sup>42</sup>

Few smaller RCTs were also published. Two of them, demonstrated no significant differences in the virological cure rates between HCQ treatment and the standard of care groups.<sup>43,44</sup> In three others, no mortality and clinical status benefits were observed using HCQ (or HCQ and AZ) compared with standard of care treatment.<sup>45-48</sup>

One large RCT assessed the role of HCQ in post-exposure prevention of COVID-19. It demonstrated no significant difference in the new cases of COVID-19 between the HCQ treatment (800 mg once, followed by 600 mg in 6 to 8 hours, then 600 mg daily for 4 additional days) and the placebo groups.<sup>49</sup>

A specific alert cautioning against HCQ use without appropriate monitoring was issued by the FDA.<sup>50</sup> Similarly, the WHO recently discontinued the Solidarity Trial citing lack of any benefit of HCQ.<sup>51</sup>

## Artemisinins

Produced from the plant *Artemisia annua*, artemisinin and its derivatives have become the main weapon in the fight against malaria. The antimalarial effect of artemisinins is probably related to the formation of reactive oxygen species, although its full mechanism of action is unknown.<sup>52</sup> Modulation of heme oxygenase - 1 by artesunate has been also suggested as a treatment that lowers mortality and improves sepsis – related lung injury in an animal model.<sup>53</sup>

The effect of artemisinins when used as antiviral agents has been assessed for several viruses.<sup>54,55</sup> Artemisinin and artesunate inhibited the viral production of hepatitis B virus and EBV.<sup>56</sup> Artemisinins, either alone or synergistically with other antiviral agents, was shown to be active against CMV,<sup>57-60</sup> inhibited replication of JC virus.<sup>61</sup> and BK virus<sup>62</sup> and has been used anecdotally to treat HHV6 myocarditis.<sup>63</sup>

There has been one notable demonstration of widespread artesiminin use as an “accidental” antiviral. During the 2014 Ebola epidemic in Liberia, all patients were prescribed either artesunate-amodiaquine or artemether-lumefantrine regimens empirically for suspected co-infection with malaria. Those who were treated with artesunate-amodiaquine had a 31% lower risk of death. This effect was stronger among patients without malaria.<sup>64,65</sup> This was, however, a non randomized trial, and unknown confounding factors, such as cases of low-level Plasmodium parasitemia that might have been missed, may have biased the results.

*Artemisia annua* extract exhibited activity against SARS CoV-1 as measured by a cytopathic effect reduction (that does not prove an effect on the virus itself).<sup>66</sup> In another study, artesunate did not show an inhibitory activity against SARS CoV-1 in a cell line.<sup>67</sup> A recent in silico study showed a potential interaction between artemisinins and the SARS-CoV-2 Spike protein Lys353 and Lys31 binding hotspots, but did not directly assess antiviral activity.<sup>68</sup> These in vitro and in silico studies, and the fact that artesunate leads to a decrease in sepsis - related lung injury in an animal model, suggest that artemisinins may prove to be interesting candidates for further testing as anti-COVID-19 agents.<sup>53</sup>



## Mefloquine

Mefloquine is an antimalarial similar in structure to quinine. It is active through the destruction of the asexual forms of the *Plasmodium parasite*. In the past it has been used for patients with progressive multifocal encephalopathy due to *in vitro* anti-JC virus activity.<sup>69</sup> A randomized controlled trial failed to demonstrate any benefit, and its use as an antiviral has been largely abandoned.<sup>70</sup> *In vitro*, mefloquine demonstrates antiviral activity against coronaviruses, similar to earlier reports with JC virus. In a cell culture mefloquine inhibits the cytopathic effect of two coronaviruses closely related to SARS CoV-2: the pangolin coronavirus GX\_P2V/pangolin/2017/Guangxi and feline coronavirus.<sup>71,72</sup> To the best of our knowledge, no RCT is currently assessing the possible use of mefloquine for COVID-19.

## Ivermectin

Ivermectin is used to treat many parasitic infections such as filariasis, soil – transmitted helminths, scabies, and head lice, and it is relatively safe. The discoverers of avermectin, its precursor, received the 2015 Nobel Prize for their achievement.<sup>73</sup> Its antiparasitic effect involves an increase in parasite cell membrane permeability, a mechanism naturally irrelevant to viruses. It has been shown, however, to display some *in vitro* activity against dengue virus, chikungunya virus, and other flaviviruses.<sup>74-76</sup> One RCT that was conducted in Thailand and published only as an abstract demonstrated shorter duration of NS1 antigenemia in patients with dengue fever, however it failed to demonstrate any clinical benefit.<sup>77</sup>

*In vitro* studies suggest that ivermectin leads to a ~ 5000 fold reduction in SARS-CoV-2 RNA at 48 hours, likely through inhibiting IMP $\alpha$ / $\beta$ 1-mediated nuclear import of viral proteins<sup>78</sup>. It remains unclear, however, if relevant concentrations can be achieved with current human or veterinary drug formulations, and what the *in vivo*

effect would be. A synergistic effect of ivermectin and HCQ has also been suggested.<sup>34</sup>

Recently, few countries in Latin America used ivermectin as a routine, yet unproven, treatment of COVID-19. In northern Bolivia 350,000 doses were given to health care workers and in Peru around 20,000 bottles of animal-grade ivermectin were sold on the black market as a treatment for COVID-19.<sup>79</sup> A recent RCT evaluated the effect of ivermectin or ivermectin and doxycycline therapy on hospitalized COVID-19 patients. No significant difference in fever, cough or sore throat were observed between placebo and treatment groups after 7 days. Earlier virological clearance was observed in the ivermectin group compared to placebo, but not in the ivermectin and doxycycline group.<sup>80</sup>

A retrospective cohort study demonstrated lower mortality in the ivermectin group compared with the standard of care (15.0 % versus 25.2%, OR 0.52, 95% CI 0.29-0.96,  $P=.03$ ) and lower mortality of patients with severe pulmonary disease treated with ivermectin (38.8% vs 80.7%, OR 0.15, CI 0.05-0.47,  $P=.001$ ), but with no significant differences in the rates of successful weaning from mechanical ventilation (36.1% vs 15.4%, OR 3.11 (0.88-11.00),  $p=.07$ ).<sup>81</sup> Other small, non-randomized trials, that are potentially biased, have reported conflicting results regarding the benefit of ivermectin.<sup>82-84</sup>

As the level of evidence of these data is likely low, the efficacy of ivermectin against COVID-19 remains to be proven. However, based on current evidence, it is unlikely that Ivermectin will be a game changer in COVID-19 treatment.

### **Nitazoxanide**

Nitazoxanide (NTZ) is an antiparasitic drug used for treatment of *Giardia* and *Cryptosporidium* infections, and the research of its possible repurposing for treatment of several viruses is ongoing. These viruses include, among others, parainfluenza, respiratory syncytial virus (RSV), canine coronavirus, rhinovirus,

flaviviruses, including hepatitis C virus (HCV), hepatitis B virus, HIV, and influenza.<sup>85-90</sup>

NTZ has an *in vitro* synergistic effect against influenza A virus when combined with oseltamivir and zanamivir.<sup>91</sup> NTZ acts against influenza viruses by blocking the maturation of the viral hemagglutinin at the post-translational level.<sup>92</sup> Tizoxanide, the active circulating metabolite of NTZ, is capable of inhibiting the replication of several strains of influenza A and B - 16 strains of influenza A/H1N1, H3N2, H3N2v, H3N8, H5N9, H7N1, and one strain of influenza B.<sup>89</sup> In an RCT that included 624 patients with influenza virus infection, NTZ was associated with a reduction in the duration of symptoms compared to a placebo.<sup>90</sup> In another RCT conducted among patients with acute respiratory illness that required hospital admission in Mexico (6.6% of whom were infected with various coronaviruses), NTZ was not associated with any benefits compared to placebo.<sup>93</sup> Patients in this study, however, had multiple causative agents, both bacterial and viral, so the measure of effect of NTZ on specific pathogens was impossible to assess.

NTZ exhibits antiviral activity against MERS-CoV and other coronaviruses by inhibiting the expression of the viral N protein,<sup>94,95</sup> and inhibits the SARS-CoV-2 at a low-micromolar concentration.<sup>23</sup> One small prospective non controlled study that assessed NTZ treatment of in and outpatients with COVID-19 was performed. Among 20 women (pregnant or in immediate puerperium) that were treated with NTZ, two women died. Five hospitalized patients showed a positive outcome with two patients weaned from mechanical ventilation. 16 outpatients treated with NTZ were cured.<sup>96</sup> The low number of patients and the relatively high mortality rate do not support the use of this agent against COVID-19. However larger studies are needed in order to provide more solid data.

### Niclosamide

Niclosamide (2',5-dichloro-4'-nitrosalicylanilide) is another FDA approved drug with anticestodal activity that was discovered in 1958. It has an *in vitro* antiviral effect against a broad range of viruses such as SARS-CoV-1,<sup>97,98</sup> MERS-CoV,<sup>99</sup> Zika virus, Japanese encephalitis virus, Human immunodeficiency virus, Hepatitis C virus and human adenovirus.<sup>100,101</sup> The broad antiviral activity of niclosamide is attributed to

its ability to neutralize endo-lysosomal pH and interfere with pH-dependent membrane fusion, which is an essential step in viral entry mechanisms. The antiviral effect of niclosamide should be further assessed *in vitro* before any consideration of clinical utility could be made.

## Discussion

The current COVID-19 pandemic has strained national and international resources. The research community has produced a vast amount of new research. However, efforts were at times fragmented and the methodology of many clinical trials that were performed was of low quality. Several months into the outbreak, the number of large, well-conducted RCTs that have been published is still small, despite the millions of infected people worldwide. The only anti-viral drug with proven benefit, remdesivir, has been shown to shorten symptom duration in a large RCT that included more than 1000 patients. A non-significant trend towards lower mortality rates has been observed in this trial although absolute risk reduction was small in the standard of infectious diseases. Remdesivir is therefore only modestly effective, and the search for more active anti SARS-CoV-2 continues.<sup>6</sup>

We included in this review several antiparasitic agents that have been shown to have some *in vitro* or *in vivo* antiviral activities. We elaborated on drug activity against different viruses, including the new SARS-CoV-2 and its suggested mechanisms of action (Tables 2,3).

Most drugs in Table 2 were shown to reduce the viral replication stage by affecting cellular organelles. However, intensive *in vitro* / *in vivo* studies are required to establish the detailed mechanism of action of these agents against viruses, particularly against SARS-CoV-2. Combination therapies of drugs with effects on the various stages of the viral life cycle should be also considered.

The limitations of this review are those of the studies themselves. *In vitro* studies have used very different methodologies, and at least some were authored by researchers with potential conflict of interests. Some clinical studies were limited to small cohorts that were prone to selection bias, i.e. inclusion of patients with relatively good prognosis. The rate of publication of well-conducted RCTs, as mentioned before, has been disappointingly slow, and recent studies have been retracted from major journals due to methodological or ethical issues.

The only antiparasitic agent that has been included in major RCTs is HCQ. Although initial observational trials suggested benefit of HCQ, larger high-quality trials provide ample evidence that HCQ should not be used neither for treatment nor for prevention of COVID-19. Its inclusion in clinical trials using differing protocols is ethical as no excess mortality was shown in the Solidarity trials.<sup>10</sup> We call for the continuation of pre-clinical research of existing compounds with potential antiviral effect against SARS-COV-2. However, as *in vitro* results and virologic tests often correlate poorly with actual efficacy, we argue that drugs should be introduced into widespread clinical use only after being rigorously tested in RCTs. Such a search may prove beneficial in this pandemic, or in future outbreaks yet to come.

Table 1: Articles screened, reviewed and included in the review

Drug *	Total abstract screened	In vitro and animal model studies included	In vivo studies included
Chloroquine and HCQ	1191	10	21
Artesiminins	116	14	2
Mefloquine	78	3	0
Ivermectin	93	7	5
Nitazoxanide	88	14	3
Niclosamide	72	17	0

\*No articles were found for albendazole and atovaquone.

Table 2: Mechanism of action of antiparasitic drugs when used as antiviral agents

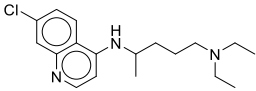
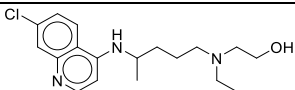
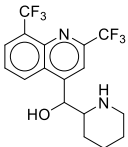
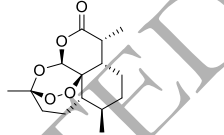
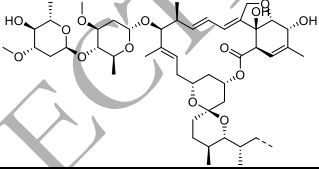
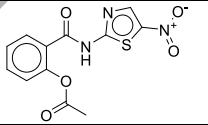
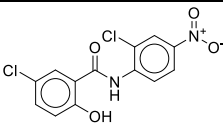
Drug name	Chemical structure	Pathogen for which the drug is indicated (reference)	Proposed Antiviral Mechanism of action
Chloroquine		<i>Plasmodium spp.</i>	Prevents viral entry, transport and post-entry events. It is a weak base that increases the pH of low-pH organelles, such as endosomes, Golgi vesicles, and lysosomes; suppressing the production and release of TNF $\alpha$ and interleukin 6, which mediate the inflammatory complications of several viral diseases. <sup>13,24,102</sup>
HCQ		<i>Plasmodium spp., Coxiella burnetii</i>	Same as chloroquine
Mefloquine		<i>Plasmodium spp.</i>	Inhibits viral DNA replication in cells after viral entry <sup>69</sup>
Artesiminins		<i>Plasmodium spp., Schistosoma spp.</i>	Reduce virus DNA binding activity of NF- $\kappa$ B and Sp1; <sup>55</sup> affect viral replication <sup>25</sup>
Ivermectin		Helminths and ecto parasites	Inhibits nuclear import of host and viral proteins thereby inhibiting viral replication <sup>78</sup>
Nitazoxanide		Helminthic and protozoal infections	Blocks the maturation of the viral hemagglutinin at the post-translational stage <sup>92</sup>
Niclosamide		Protozoal infections	Neutralize endolysosomal pH and interfere with pH-dependent membrane fusion <sup>100</sup>

Table 3: Activity of selected antiparasitic agents against different coronaviruses

Drug	In-vitro study	Animal Model	Clinical trial	comments
HCQ <sup>26,27,30-41,43-49</sup>	SARS-CoV-2	SARS-CoV-2	Conflicting results,	Widely used worldwide, conflicting results in observational studies, no benefit in RCTs.
Chloroquine <sup>21-25,29</sup>	SARS-CoV-1 SARS-CoV2 MERS-CoV	None	None	None
Artesiminin <sup>s 66-68</sup>	SARS-CoV-1	None	None	In silico study suggests possible activity against SARS-CoV-2
Mefloquine <sup>71,72</sup>	Pangolin & Feline coronavirus es	None	None	-
Ivermectin <sup>3 4,78,81</sup>	SARS-CoV-2	None	None	Conflicting results. A synergistic effect with HCQ has been suggested.
Nitazoxanide <sup>23,94-96</sup>	MERS-CoV-1 Canine coronavirus SARS-CoV-2	None	one clinical trial	Benefit unclear
Niclosamide <sup>97-99</sup>	SARS-CoV-1, MERS-CoV	None	None	-



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