

Journal Pre-proof

New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19?

Christian A. Devaux , Jean-Marc Rolain , Philippe Colson ,
Didier Raoult

PII: S0924-8579(20)30088-1
DOI: <https://doi.org/10.1016/j.ijantimicag.2020.105938>
Reference: ANTAGE 105938



To appear in: *International Journal of Antimicrobial Agents*

Please cite this article as: Christian A. Devaux , Jean-Marc Rolain , Philippe Colson ,
Didier Raoult , New insights on the antiviral effects of chloroquine against coronavirus:
what to expect for COVID-19?, *International Journal of Antimicrobial Agents* (2020), doi:
<https://doi.org/10.1016/j.ijantimicag.2020.105938>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2020 Published by Elsevier B.V.

Highlights

- This is an invited paper on the topic COVID-19 (VSI COVID-19).

Journal Pre-proof

New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19?

Christian A. Devaux^{a,b,c,*}, Jean-Marc Rolain^{a,c}, Philippe Colson^{a,c}, Didier Raoult^{a,c}

^a *Aix-Marseille Université, IRD, APHM, MEPHI, IHU–Méditerranée Infection, Marseille, France*

^b *CNRS, Marseille, France*

^c *IHU–Méditerranée Infection, 19–21 boulevard Jean Moulin, 13005 Marseille, France*

Keywords:

COVID-19

SARS-CoV-2

Coronavirus

Chloroquine

* Corresponding author. Present address: IHU–Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13385 Marseille, France. Tel.: +33 4 13 73 20 51; fax: +33 4 13 73 20 52.

E-mail address: christian.devault@mediterranee-infection.com (C.A. Devaux).

ABSTRACT

Recently, a novel coronavirus (2019-nCoV), officially known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in China. Despite drastic containment measures, the spread of this virus is ongoing. SARS-CoV-2 is the aetiological agent of coronavirus disease 2019 (COVID-19) characterised by pulmonary infection in humans. The efforts of international health authorities have since focused on rapid diagnosis and isolation of patients as well as the search for therapies able to counter the most severe effects of the disease. In the absence of a known efficient therapy and because of the situation of a public-health emergency, it made sense to investigate the possible effect of chloroquine/hydroxychloroquine against SARS-CoV-2 since this molecule was previously described as a potent inhibitor of most coronaviruses, including SARS-CoV-1. Preliminary trials of chloroquine repurposing in the treatment of COVID-19 in China have been encouraging, leading to several new trials. Here we discuss the possible mechanisms of chloroquine interference with the SARS-CoV-2 replication cycle.

1. Introduction

Chloroquine is an amine acidotropic form of quinine that was synthesised in Germany by Bayer in 1934 and emerged approximately 70 years ago as an effective substitute for natural quinine [1,2]. Quinine is a compound found in the bark of *Cinchona* trees native to Peru and was the previous drug of choice against malaria [3]. For decades, chloroquine was a front-line drug for the treatment and prophylaxis of malaria and is one of the most prescribed drugs worldwide [4]. Chloroquine and the 4-aminoquinoline drug hydroxychloroquine belong to the same molecular family. Hydroxychloroquine differs from chloroquine by the presence of a hydroxyl group at the end of the side chain: the *N*-ethyl substituent is β -hydroxylated. This molecule is available for oral administration in the form of hydroxychloroquine sulfate.

Hydroxychloroquine has pharmacokinetics similar to that of chloroquine, with rapid gastrointestinal absorption and renal elimination. However, the clinical indications and toxic doses of these drugs slightly differ. In malaria, the indication for chloroquine was a high dose for a short period of time (due to its toxicity at high doses) or a low dose for a long period of time. Hydroxychloroquine was reported to be as active as chloroquine against *Plasmodium falciparum* malaria and less toxic, but it is much less active than chloroquine against chloroquine-resistant *P.*

falciparum owing to its physicochemical properties. What is advantageous with hydroxychloroquine is that it can be used in high doses for long periods with very good tolerance. Unfortunately, the efficacy of chloroquine gradually declined due to the continuous emergence of chloroquine-resistant *P. falciparum* strains [5].

Chloroquine is also utilised in the treatment of autoimmune diseases [6]. Yet the activity of the molecule is not limited to malaria and the control of inflammatory processes, as illustrated by its broad-spectrum activity against a range of bacterial,

fungal and viral infections [7–10]. Indeed, in the mid-1990s, due to its tolerability, rare toxicity reports, inexpensive cost and immunomodulatory properties [11], chloroquine repurposing was explored against human immunodeficiency virus (HIV) and other viruses associated with inflammation and was found to be efficient in inhibiting their replication cycle [12].

Recently, a novel coronavirus emerged in the Chinese city of Wuhan in December 2019. After human coronavirus 229E (HCoV-229E) (classified in the genus *Alphacoronavirus*) and HCoV-OC43 (*Betacoronavirus* lineage 2a member) described in the 1960s, SARS-CoV-1 (*Betacoronavirus* lineage 2b member) that emerged in March 2003, HCoV-NL63 (*Alphacoronavirus* lineage 1b member) described in 2004, HCoV-HKU1 (*Betacoronavirus* lineage 2a member) discovered in 2005, and finally MERS-CoV that emerged in 2012 (classified in *Betacoronavirus* lineage 2c), the novel coronavirus is the seventh human coronavirus described to date as being responsible for respiratory infection. Evidence was rapidly reported that patients were suffering from an infection with a novel *Betacoronavirus* tentatively named 2019 novel coronavirus (2019-nCoV) [13,14]. Despite drastic containment measures, the spread of 2019-nCoV, now officially known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is ongoing. Phylogenetic analysis of this virus indicated that it is different (~80% nucleotide identity) but related to SARS-CoV-1 [15]. Because the world is threatened by the possibility of a SARS-CoV-2 pandemic, the broad-spectrum antiviral effects of chloroquine warranted particular attention for repurposing this drug in the therapy of the disease caused by SARS-CoV-2, named coronavirus disease 2019 (COVID-19).

2. Antiviral properties of chloroquine

In vitro, chloroquine appears as a versatile bioactive agent reported to possess antiviral activity against RNA viruses as diverse as rabies virus [16], poliovirus [17], HIV [12,18–20], hepatitis A virus [21,22], hepatitis C virus [23], influenza A and B viruses [24–27], influenza A H5N1 virus [28], Chikungunya virus [29–31], Dengue virus [32,33], Zika virus [34], Lassa virus [35], Hendra and Nipah viruses [36,37], Crimean–Congo hemorrhagic fever virus [38] and Ebola virus [39], as well as various DNA viruses such as hepatitis B virus [40] and herpes simplex virus [41]. The antiviral properties of chloroquine described in vitro have sometimes been confirmed during treatment of virus-infected patients but have not always been reproduced in clinical trials depending on the disease, the concentration of chloroquine used, the duration of treatment and the clinical team in charge of the trial.

Regarding coronaviruses, the potential therapeutic benefits of chloroquine were notably reported for SARS-CoV-1 [11,42]. Chloroquine was also reported to inhibit in vitro the replication of HCoV-229E in epithelial lung cell cultures [43,44]. In 2009, it was reported that lethal infections of newborn mice with the HCoV-O43 coronavirus could be averted by administering chloroquine through the mother's milk. In vitro experiments also showed a strong antiviral effect of chloroquine on a recombinant HCoV-O43 coronavirus [45]. Although chloroquine was reported to be active against Middle East respiratory syndrome coronavirus (MERS-CoV) in vitro [46], this observation remains controversial [47].

3. Potential antiviral effect of chloroquine against SARS-CoV-2

Because of its broad spectrum of action against viruses, including most coronaviruses and particularly its close relative SARS-CoV-1, and because coronavirus cell entry occurs through the endolysosomal pathway [48], it made sense in a situation of a public-health emergency and the absence of any known efficient therapy to investigate the possible effect of chloroquine against SARS-CoV-2. A recent paper reported that both chloroquine and the antiviral drug remdesivir inhibited SARS-CoV-2 in vitro and suggested these drugs be assessed in human patients suffering from COVID-19 [49].

Recently, the China National Center for Biotechnology Development indicated that chloroquine is one of the three drugs with a promising profile against the new SARS-CoV-2 coronavirus that causes COVID-19. Chloroquine repurposing was investigated in hospitals in Beijing, in central China's Hunan Province and South China's Guangdong Province. According to preliminary reports [50,51] from the Chinese authorities suggesting that approximately 100 infected patients treated with chloroquine experienced a more rapid decline in fever and improvement of lung computed tomography (CT) images and required a shorter time to recover compared with control groups, with no obvious serious adverse effects, the Chinese medical advisory board has suggested chloroquine inclusion in the SARS-CoV-2 treatment guidelines. As a result, chloroquine is probably the first molecule to be used in China and abroad on the front line for the treatment of severe SARS-CoV-2 infections. Although the long use of this drug in malaria therapy demonstrates the safety of acute chloroquine administration to humans, one cannot ignore the minor risk of macular retinopathy, which depends on the cumulative dose [52], and the existence

of some reports on cardiomyopathy as a severe adverse effect caused by chloroquine [53,54]. A survey of SARS-CoV-2-infected patients for adverse effects of chloroquine therapy remains to be performed. However, chloroquine is currently among the best available candidates to impact the severity of SARS-CoV-2 infections in humans. Currently, at least ten clinical trials are testing chloroquine as an anti-COVID-19 therapy [55].

4. Mode of action of chloroquine

Chloroquine has multiple mechanisms of action that may differ according to the pathogen studied.

Chloroquine can inhibit a pre-entry step of the viral cycle by interfering with viral particles binding to their cellular cell surface receptor. Chloroquine was shown to inhibit quinone reductase 2 [56], a structural neighbour of UDP-*N*-acetylglucosamine 2-epimerases [57] that are involved in the biosynthesis of sialic acids. The sialic acids are acidic monosaccharides found at the extremity of sugar chains present on cell transmembrane proteins and are critical components of ligand recognition. The possible interference of chloroquine with sialic acid biosynthesis could account for the broad antiviral spectrum of that drug since viruses such as the human coronavirus HCoV-O43 and the orthomyxoviruses use sialic acid moieties as receptors [58]. The potent anti-SARS-CoV-1 effects of chloroquine in vitro were considered attributable to a deficit in the glycosylation of a virus cell surface receptor, the angiotensin-converting enzyme 2 (ACE2) on Vero cells [59].

Chloroquine can also impair another early stage of virus replication by interfering with the pH-dependent endosome-mediated viral entry of enveloped viruses such as Dengue virus or Chikungunya virus [60,61]. Due to the alkalinisation of endosomes, chloroquine was an effective in vitro treatment against Chikungunya virus when added to Vero cells prior to virus exposure [30]. The mechanism of inhibition likely involved the prevention of endocytosis and/or rapid elevation of the endosomal pH and abrogation of virus–endosome fusion. A pH-dependant mechanism of entry of coronavirus into target cells was also reported for SARS-CoV-1 after binding of the DC-SIGN receptor [62]. The activation step that occurs in endosomes at acidic pH results in fusion of the viral and endosomal membranes leading to the release of the viral SARS-CoV-1 genome into the cytosol [63]. In the absence of antiviral drug, the virus is targeted to the lysosomal compartment where the low pH, along with the action of enzymes, disrupts the viral particle, thus liberating the infectious nucleic acid and, in several cases, enzymes necessary for its replication [64]. Chloroquine-mediated inhibition of hepatitis A virus was found to be associated with uncoating, thus blocking its entire replication cycle [22].

Chloroquine can also interfere with the post-translational modification of viral proteins. These post-translational modifications, which involve proteases and glycosyltransferases, occur within the endoplasmic reticulum or the trans-Golgi network vesicles and may require a low pH. For HIV, the antiretroviral effect of chloroquine is attributable to a post-transcriptional inhibition of glycosylation of the gp120 envelope glycoprotein, and the neosynthesised virus particles are non-infectious [19,65]. Chloroquine also inhibits the replication Dengue-2 virus by affecting the normal proteolytic processing of the flavivirus prM protein to M protein

[32]. As a result, viral infectivity is impaired. In the herpes simplex virus (HSV) model, chloroquine inhibited budding with accumulation of non-infectious HSV-1 particles in the trans-Golgi network [66]. Using non-human coronavirus, it was shown that the intracellular site of coronavirus budding is determined by the localisation of its membrane M proteins that accumulate in the Golgi complex beyond the site of virion budding [67], suggesting a possible action of chloroquine on SARS-CoV-2 at this step of the replication cycle. It was recently reported that the C-terminal domain of the MERS-CoV M protein contains a trans-Golgi network localisation signal [68].

Beside affecting the virus maturation process, pH modulation by chloroquine can impair the proper maturation of viral protein [32] and the recognition of viral antigen by dendritic cells, which occurs through a Toll-like receptor-dependent pathway that requires endosomal acidification [69]. On the contrary, other proposed effects of chloroquine on the immune system include increasing the export of soluble antigens into the cytosol of dendritic cells and the enhancement of human cytotoxic CD8⁺ T-cell responses against viral antigens [70]. In the influenza virus model, it was reported that chloroquine improve the cross-presentation of non-replicating virus antigen by dendritic cells to CD8⁺ T-cells recruited to lymph nodes draining the site of infection, eliciting a broadly protective immune response [71].

Chloroquine can also act on the immune system through cell signalling and regulation of pro-inflammatory cytokines. Chloroquine is known to inhibit phosphorylation (activation) of the p38 mitogen-activated protein kinase (MAPK) in THP-1 cells as well as caspase-1 [72]. Activation of cells via MAPK signalling is frequently required by viruses to achieve their replication cycle [73]. In the model of

HCoV-229 coronavirus, chloroquine-induced virus inhibition occurs through inhibition of p38 MAPK [44]. Chloroquine is a well-known immunomodulatory agent capable of mediating an anti-inflammatory response [11]. Therefore, there are clinical applications of this drug in inflammatory diseases such as rheumatoid arthritis [74–76], lupus erythematosus [6,77] and sarcoidosis [78]. Chloroquine inhibits interleukin-1 beta (IL-1 β) mRNA expression in THP-1 cells and reduces IL-1 β release [72]. Chloroquine-induced reduction of IL-1 and IL-6 cytokines was also found in monocytes/macrophages [79]. Chloroquine-induced inhibition of tumour necrosis factor-alpha (TNF α) production by immune cells was reported to occur either through disruption of cellular iron metabolism [80], blockade of the conversion of pro-TNF into soluble mature TNF α molecules [81] and/or inhibition of TNF α mRNA expression [72,82,83]. Inhibition of the TNF α receptor was also reported in U937 monocytic cells treated with chloroquine [84]. In the Dengue virus model, chloroquine was found to inhibit interferon-alpha (IFN α), IFN β , IFN γ , TNF α , IL-6 and IL-12 gene expression in U937 cells infected with Dengue-2 virus [33].

5. Conclusion

Chloroquine has been shown to be capable of inhibiting the in vitro replication of several coronaviruses. Recent publications support the hypothesis that chloroquine can improve the clinical outcome of patients infected by SARS-CoV-2. The multiple molecular mechanisms by which chloroquine can achieve such results remain to be further explored. Since SARS-CoV-2 was found a few days ago to utilise the same cell surface receptor ACE2 (expressed in lung, heart, kidney and intestine) as SARS-CoV-1 [85,86] (Table 1), it may be hypothesised that chloroquine also interferes with

ACE2 receptor glycosylation thus preventing SARS-CoV-2 binding to target cells. Wang and Cheng reported that SARS-CoV and MERS-CoV upregulate the expression of ACE2 in lung tissue, a process that could accelerate their replication and spread [85]. Although the binding of SARS-CoV to sialic acids has not been reported so far (it is expected that *Betacoronavirus* adaptation to humans involves progressive loss of hemagglutinin-esterase lectin activity), if SARS-CoV-2 like other coronaviruses targets sialic acids on some cell subtypes, this interaction will be affected by chloroquine treatment [87,88]. Today, preliminary data indicate that chloroquine interferes with SARS-CoV-2 attempts to acidify the lysosomes and presumably inhibits cathepsins, which require a low pH for optimal cleavage of SARS-CoV-2 spike protein [89], a prerequisite to the formation of the autophagosome [49]. Obviously, it can be hypothesised that SARS-CoV-2 molecular crosstalk with its target cell can be altered by chloroquine through inhibition of kinases such as MAPK. Chloroquine could also interfere with proteolytic processing of the M protein and alter virion assembly and budding (Fig. 1). Finally, in COVID-19 disease this drug could act indirectly through reducing the production of pro-inflammatory cytokines and/or by activating anti-SARS-CoV-2 CD8⁺ T-cells.

Already in 2007, some of us emphasised in this journal the possibility of using chloroquine to fight orphan viral infections [10]. The worldwide ongoing trials, including those involving the care of patients in our institute [90], will verify whether the hopes raised by chloroquine in the treatment of COVID-19 can be confirmed.

Acknowledgment: The figure was designed using the Servier Medical Art supply of images available under a Creative Commons CC BY 3.0 license.

Funding: This study was supported by IHU–Méditerranée Infection, University of Marseille and CNRS (Marseille, France). This work has benefited from French state support, managed by the Agence nationale de la recherche (ANR), including the ‘Programme d’investissement d’avenir’ under the reference Méditerranée Infection 10-1AHU-03.

Competing interests: None declared.

Ethical approval: Not required.

Journal Pre-proof

References

- [1] Winzeler EA. Malaria research in the post-genomic era. *Nature* 2008;455:751–6.
- [2] Parhizgar AR, Tahghighi A. Introducing new antimalarial analogues of chloroquine and amodiaquine: a narrative review. *Iran J Med Sci* 2017;42:115–28.
- [3] Bruce-Chwatt LJ, editor. *Chemotherapy of malaria*. 2nd ed. Geneva, Switzerland: WHO; 1981. WHO Monograph Series 27.
- [4] White NJ, Pukrittayakamee S, Hien TT, Faiz MA, Mokuolu OA, Dondorp AM. Malaria. *Lancet* 2014;383:723–35. doi: 10.1016/S0140-6736(13)60024-0.
- [5] Wellems TE, Plowe CV. Chloroquine-resistant malaria. *J Infect Dis* 2001;184:770–6.
- [6] Lee SJ, Silverman E, Bargman JM. The role of antimalarial agents in the treatment of SLE and lupus nephritis. *Nat Rev Nephrol* 2011;7:718–29. doi: 10.1038/nrneph.2011.150.
- [7] Raoult D, Drancourt M, Vestris G. Bactericidal effect of doxycycline associated with lysosomotropic agents on *Coxiella burnetii* in P388D1 cells. *Antimicrob Agents Chemother* 1990;34:1512–4. doi: 10.1128/aac.34.8.1512.
- [8] Raoult D, Houpiqian P, Tissot DH, Riss JM, Arditi-Djjane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. *Arch Intern Med* 1999;159:167–73. doi: 10.1001/archinte.159.2.167.
- [9] Boulos A, Rolain JM, Raoult D. Antibiotic susceptibility of *Tropheryma whipplei* in MRC5 cells. *Antimicrob Agents Chemother* 2004;48:747–52.

- [10] Rolain JM, Colson P, Raoult D. Recycling of chloroquine and its hydroxyl analogue to face bacterial, fungal and viral infection in the 21st century. *Int J Antimicrob Agents* 2007;30:297–308.
- [11] Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases? *Lancet Infect Dis* 2003;3:722–7.
- [12] Boelaert JR, Piette J, Sperber K. The potential place of chloroquine in the treatment of HIV-1-infected patients. *J Clin Virol* 2001;20:137–40.
- [13] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497–506. doi: 10.1016/S0140-6736(20)30183-5.
- [14] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
- [15] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *bioRxiv* 2020 Jan 23. doi: 10.1101/2020.01.22.914952.
- [16] Tsiang H, Superti F. Ammonium chloride and chloroquine inhibit rabies virus infection in neuroblastoma cells. *Arch Virol* 1984;81:377–82.
- [17] Kronenberger P, Vrijisen R, Boeyé A. Chloroquine induces empty capsid formation during poliovirus eclipse. *J Virol* 1991;65:7008–11.
- [18] Tsai WP, Nara PL, Kung HF, Oroszlan S. Inhibition of human immunodeficiency virus infectivity by chloroquine. *AIDS Res Hum Retroviruses* 1990;6:481–9. doi: 10.1089/aid.1990.6.481.

- [19] Savarino A, Gennero L, Sperber K, Boelaert JR. The anti-HIV-1 activity of chloroquine. *J Clin Virol* 2001;20:131–5.
- [20] Romanelli F, Smith KM, Hoven AD. Chloroquine and hydroxychloroquine as inhibitors of human immunodeficiency virus (HIV-1) activity. *Curr Pharm Des* 2004;10:2643–8.
- [21] Superti F, Seganti L, Orsi W, Divizia M, Gabrieli R, Pana A. The effect of lipophilic amines on the growth of hepatitis A virus in Frp/3 cells. *Arch Virol* 1987;96:289–96. doi: 10.1007/bf01320970.
- [22] Bishop NE. Practical guidelines in antiviral therapy. *Intervirology* 1998;41:261–71.
- [23] Mizui T, Yamashina S, Tanida I, Takei Y, Ueno T, Sakamoto N, et al. Inhibition of hepatitis C virus replication by chloroquine targeting virus-associated autophagy. *J Gastroenterol* 2010;45:195–203.
- [24] Miller DK, Lenard J. Antihistaminics, local anesthetics, and other amines as antiviral agents. *Proc Natl Acad Sci U S A* 1981;78:3605–9. doi: 10.1073/pnas.78.6.3605.
- [25] Shibata M, Aoki H, Tsurumi T, Sugiura Y, Nishiyama Y, Suzuki S, et al. Mechanism of uncoating of influenza B virus in MDCK cells: action of chloroquine. *J Gen Virol* 1983;64:1149–56. doi: 10.1099/0022-1317-64-5-1149.
- [26] Ooi EE, Chew JS, Loh JP, Chua RC. In vitro inhibition of human influenza A virus replication by chloroquine. *Viol J* 2006;3:39.
- [27] Paton NI, Lee L, Xu Y, Ooi EE, Cheung YB, Archuleta S, et al. Chloroquine for influenza prevention: a randomised, double-blind, placebo controlled trial. *Lancet Infect Dis* 2011;11:677–83.

- [28] Yan Y, Zou Z, Sun Y, Li X, Xu KF, Wei Y, et al. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. *Cell Res* 2013;23:300–2. doi: 10.1038/cr.2012.165.
- [29] De Lamballerie X, Boisson V, Reynier JC, Enault S, Charrel RN, Flahault A, et al. On Chikungunya acute infection and chloroquine treatment. *Vector Borne Zoonotic Dis* 2008;8:837–40. doi: 10.1089/vbz.2008.0049.
- [30] Khan M, Santhosh SR, Tiwari M, Lakshmana Rao PV, Parida M. Assessment of in vitro prophylactic and therapeutic efficacy of chloroquine against Chikungunya virus in Vero cells. *J Med Virol* 2010;82:817–24.
- [31] Delogu I, de Lamballerie X. Chikungunya disease and chloroquine treatment. *J Med Virol* 2011;83:1058–9.
- [32] Randolph VB, Winkler G, Stollar V. Acidotropic amines inhibit proteolytic processing of flavivirus prM protein. *Virology* 1990;174:450–8. doi: 10.1016/0042-6822(90)90099-d.
- [33] Farias KJ, Machado PR, de Almeida Junior RF, de Aquino AA, da Fonseca BA. Chloroquine interferes with dengue-2 virus replication in U937 cells. *Microbiol Immunol* 2014;58:318–26.
- [34] Delvecchio R, Higa LM, Pezzuto P, Valadao AL, Garcez PP, Monteiro FL, et al. Chloroquine, an endocytosis blocking agent, inhibits Zika virus infection in different cell models. *Viruses* 2016;8:E322. doi: 10.3390/v8120322.
- [35] Glushakova SE, Lukashevich IS. Early events in arenavirus replication are sensitive to lysosomotropic compounds. *Arch Virol* 1989;104:157–61.

- [36] Porotto M, Orefice G, Yokoyama CC, Mungall BA, Realubit R, Sganga ML, et al. Simulating *Henipavirus* multicycle replication in a screening assay leads to identification of a promising candidate for therapy. *J Virol* 2009;83:5148–55.
- [37] Freiberg AN, Worthy MN, Lee B, Holbrook MR. Combined chloroquine and ribavirin treatment does not prevent death in a hamster model of Nipah and Hendra virus infection. *J Gen Virol* 2010;91:765–72. doi: 10.1099/vir.0.017269-0.
- [38] Ferraris O, Moroso M, Pernet O, Emonet S, Ferrier Rembert A, Paranhos-Baccala G, et al. Evaluation of Crimean–Congo hemorrhagic fever virus in vitro inhibition by chloroquine and chlorpromazine, two FDA approved molecules. *Antiviral Res* 2015;118:75–81. doi: 10.1016/j.antiviral.2015.03.005.
- [39] Dowall SD, Bosworth A, Watson R, Bewley K, Taylor I, Rayner E, et al. Chloroquine inhibited Ebola virus replication in vitro but failed to protect against infection and disease in the in vivo guinea pig model. *J Gen Virol* 2015;96:3484–92.
- [40] Kouroumalis EA, Koskinas J. Treatment of chronic active hepatitis B (CAH B) with chloroquine: a preliminary report. *Ann Acad Med Singapore* 1986;15:149–52.
- [41] Koyama AH, Uchida T. Inhibition of multiplication of herpes simplex virus type 1 by ammonium chloride and chloroquine. *Virology* 1984;138:332–5.
- [42] Keyaerts E, Li S, Vijgen L, Rysman E, Verbeeck J, Van Ranst M, et al. Antiviral activity of chloroquine against human coronavirus OC43 infection in newborn mice. *Antimicrob Agents Chemother* 2009;53:3416–21.

- [43] Blau D, Holmes K. Human coronavirus HCoV-229E enters susceptible cells via the endocytic pathway. In: Lavi E, Weiss SR, Hingley ST, editors. *The nidoviruses (coronaviruses and arteriviruses)*. New York, NY: Kluwer; 2001. p. 193–7.
- [44] Kono M, Tatsumi K, Imai AM, Saito K, Kuriyama T, Shirasawa H. Inhibition of human coronavirus 229E infection in human epithelial lung cells (L132) by chloroquine: involvement of p38 MAPK and ERK. *Antiviral Res* 2008;77:150–2. doi: 10.1016/j.antiviral.2007.10.011.
- [45] Shen L, Yang Y, Ye F, Liu G, Desforges M, Talbot PJ, et al. Safe and sensitive antiviral screening platform based on recombinant human coronavirus OC43 expressing the luciferase reporter gene. *Antimicrob Agents Chemother* 2016;60:5492–503. doi: 10.1128/AAC.00814-16.
- [46] de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, et al. Screening of an FDA-approved compound library identifies four small-molecule inhibitors of Middle East respiratory syndrome coronavirus replication in cell culture. *Antimicrob Agents Chemother* 2014;58:4875–84. doi: 10.1128/AAC.03011-14.
- [47] Mo Y, Fisher D. A review of treatment modalities for Middle East respiratory syndrome. *J Antimicrob Chemother* 2016;71:3340–50.
- [48] Burkard C, Verheije MH, Wicht O, van Kasteren SI, van Kuppeveld FJ, Haagmans BL, et al. Coronavirus cell entry occurs through the endo-/lysosomal pathway in a proteolysis-dependent manner. *PLoS Pathog* 2014;10:e1004502.

- [49] Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020;30:269–71. doi: 10.1038/s41422-020-0282-0.
- [50] Gao J, Tian Z, Yang X. Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* 2020 Feb [Epub ahead of print]. doi: 10.5582/bst.2020.01047.
- [51] Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for chloroquine in the treatment of novel coronavirus pneumonia. Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia [in Chinese]. *Zhonghua Jie He He Hu Xi Za Zhi* 2020;43:E019. doi: 10.3760/cma.j.issn.1001-0939.2020.0019.
- [52] Bernstein HN. Ocular safety of hydroxychloroquine. *Ann Ophthalmol* 1991;23:292–6.
- [53] Ratliff NB, Estes ML, Myles JL, Shirey EK, McMahon JT. Diagnosis of chloroquine cardiomyopathy by endomyocardial biopsy. *N Engl J Med* 1987;316:191–3.
- [54] Cubero GJ, Rodriguez Reguero JJ, Rojo Ortega JM. Restrictive cardiomyopathy caused by chloroquine. *Br Heart J* 1993;69:451–2.
- [55] Harrison C. Coronavirus puts drug repurposing on the fast track. *Nature Biotechnology* 2020 Feb 27. doi: 10.1038/d41587-020-00003-1.
- [56] Kwiek JJ, Haystead TA, Rudolph J. Kinetic mechanism of quinone oxidoreductase 2 and its inhibition by the antimalarial quinolines. *Biochemistry* 2004;43:4538–47.

- [57] Varki A. Sialic acids as ligands in recognition phenomena. *FASEB J* 1997;11:248–55.
- [58] Olofsson S, Kumlin U, Dimock K, Arnberg N. Avian influenza and sialic acid receptors: more than meets the eye? *Lancet Infect Dis* 2005;5:184–8.
- [59] Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology* 2005;2:69. doi: 10.1186/1743-422X-2-69.
- [60] Tricou V, Minh NN, Van TP, Lee SJ, Farrar J, Wills B, et al. A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLoS Negl Trop Dis* 2010;4:e785. doi: 10.1371/journal.pntd.0000785.
- [61] Gay B, Bernard E, Solignat M, Chazal N, Devaux C, Briant L. pH-dependent entry of Chikungunya virus into *Aedes albopictus* cells. *Infect Genet Evol* 2012;12:1275–81. doi: 10.1016/j.meegid.2012.02.003.
- [62] Yang ZY, Huang Y, Ganesh L, Leung K, Kong WP, Schwartz O, et al. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. *J Virol* 2004;78:5642–50. doi: 10.1128/JVI.78.11.5642-5650.2004.
- [63] Wang H, Yang P, Liu K, Guo F, Zhang Y, Zhang G, et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. *Cell Res* 2008;18:290–301. doi: 10.1038/cr.2008.15.
- [64] Cassell S, Edwards J, Brown DT. Effects of lysosomotropic weak bases on infection of BHK-21 cells by Sindbis virus. *J Virol* 1984;52:857–64.

- [65] Savarino A, Lucia MB, Rastrelli E, Rutella S, Golotta C, Morra E, et al. Anti-HIV effects of chloroquine: inhibition of viral particle glycosylation and synergism with protease inhibitors. *J Acquir Immune Defic Syndr* 1996;35:223–32.
- [66] Harley CA, Dasgupta A, Wilson DW. Characterization of herpes simplex virus-containing organelles by subcellular fractionation: role for organelle acidification in assembly of infectious particles. *J Virol* 2001;75:1236–51.
- [67] Klumperman J, Locker JK, Meijer A, Horzinek MC, Geuze HJ, Rottier PJ. Coronavirus M proteins accumulate in the Golgi complex beyond the site of virion budding. *J Virol* 1994;68:6523–34.
- [68] Perrier A, Bonnin A, Desmarests L, Danneels A, Goffard A, Rouillé Y, et al. The C-terminal domain of the MERS coronavirus M protein contains a trans-Golgi network localization signal. *J Biol Chem* 2019;294:14406–21.
- [69] Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. *Science* 2004;303:1529–31.
- [70] Accapezzato D, Visco V, Francavilla V, Molette C, Donato T, Paroli M, et al. Chloroquine enhances human CD8⁺ T cell responses against soluble antigens in vivo. *J Exp Med* 2005;202:817–28.
- [71] Garulli B, Di Mario G, Sciaraffia E, Accapezzato D, Barnaba V, Castrucci MR. Enhancement of T cell-mediated immune responses to whole inactivated influenza virus by chloroquine treatment in vivo. *Vaccine* 2013;31:1717–24. doi: 10.1016/j.vaccine.2013.01.037.

- [72] Steiz M, Valbracht J, Quach J, Lotz M. Gold sodium thiomalate and chloroquine inhibit cytokine production in monocytic THP-1 cells through distinct transcriptional and posttranslational mechanisms. *J Clin Immunol* 2003;23:477–84. doi: 10.1023/B:JOCl.0000010424.41475.17.
- [73] Briant L, Robert-Hebmann V, Acquaviva C, Pelchen-Matthews A, Marsh M, Devaux C. The protein tyrosine kinase p56^{lck} is required for triggering NF- κ B activation upon interaction of human immunodeficiency virus type 1 envelope glycoprotein gp120 with cell surface CD4. *J Virol* 1998;72:6207–14.
- [74] Fuld H, Horwich L. Treatment of rheumatoid arthritis with chloroquine. *Br Med J* 1958;15:1199–201. doi: 10.1136/bmj.2.5106.1199.
- [75] Mackenzie AH. Antimalarial drugs for rheumatoid arthritis. *Am J Med* 1983;75:48–58.
- [76] Sharma TS, Do EJ, Wasko MCM. Anti-malarials: are there benefits beyond mild disease? *Curr Treat Options Rheumatol* 2016;2:1–12. doi: 10.1007/s40674-016-0036-9.
- [77] Wozniacka A, Lesiak A, Narbutt J, McCauliffe DP, Sysa-Jedrzejowska A. Chloroquine treatment influences proinflammatory cytokine levels in systemic lupus erythematosus patients. *Lupus* 2006;15:268–75.
- [78] Sharma OP. Effectiveness of chloroquine and hydroxychloroquine in treating selected patients with sarcoidosis with neurological involvement. *Arch Neurol* 1998;55:1248–54.
- [79] Jang CH, Choi JH, Byun MS, Jue DM. Chloroquine inhibits production of TNF- α , IL-1 β and IL-6 from lipopolysaccharide-stimulated human monocytes/macrophages by different modes. *Rheumatology* 2006;45:703–10.

- [80] Picot S, Peyron F, Donadille A, Vuillez J-P, Barbe G, Ambroise-Thomas P. Chloroquine-induced inhibition of the production of TNF, but not of IL-6, is affected by disruption of iron metabolism. *Immunology* 1993;80:127–33.
- [81] Jeong JY, Jue DM. Chloroquine inhibits processing of tumor necrosis factor in lipopolysaccharide-stimulated RAW 264.7 macrophages. *J Immunol* 1997;158:4901–7.
- [82] Zhu X, Ertel W, Ayala A, Morrison MH, Perrin MM, Chaudry IH. Chloroquine inhibits macrophage tumour necrosis factor- α mRNA transcription. *Immunology* 1993;80:122–6.
- [83] Weber SM, Levitz SM. Chloroquine interferes with lipopolysaccharide-induced TNF- α gene expression by a nonlysosomotropic mechanism. *J Immunol* 2000;165:1534–40. doi: 10.4049/jimmunol.165.3.1534.
- [84] Jeong JY, Choi JW, Jeon KI, Jue DM. Chloroquine decreases cell-surface expression of tumour necrosis factor receptors in human histiocytic U-937 cells. *Immunology* 2002;105:83–91. doi: 10.1046/j.0019-2805.2001.01339.x.
- [85] Wang PH, Cheng Y. Increasing host cellular receptor—angiotensin-converting enzyme 2 (ACE2) expression by coronavirus may facilitate 2019-nCoV infection. *bioRxiv* 2020 Feb 27. doi: 10.1101/2020.02.24.963348.
- [86] Li R, Qiao S, Zhang G. Analysis of angiotensin-converting enzyme 2 (ACE2) from different species sheds some light on cross-species receptor usage of a novel coronavirus 2019-nCoV. *J Infect* 2020 Feb 21 [Epub ahead of print]. doi: 10.1016/j.jinf.2020.02.013.

- [87] Zeng Q, Langereis MA, van Vliet ALW, Huizinga EG, de Groot RJ. Structure of coronavirus hemagglutinin-esterase offers insight into corona and influenza virus evolution. *Proc Natl Acad Sci U S A* 2008;105:9065–9.
- [88] Bakkers MJG, Lang Y, Feistsma LJ, Hulswit RJG, de Poot SAH, van Vliet ALW, et al. *Betacoronavirus* adaptation to humans involved progressive loss of hemagglutinin-esterase lectin activity. *Cell Host Microbe* 2017;21:356–66. doi: 10.1016/j.chom.2017.02.008.
- [89] Simmons G, Bertram S, Glowacka I, Steffen I, Chaipan C, Agudelo J, et al. Different host cell proteases activate the SARS-coronavirus spike-protein for cell–cell and virus–cell fusion. *Virology* 2011;413:265–74. doi: 10.1016/j.virol.2011.02.020.
- [90] Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. *Int J Antimicrob Agents* 2020 Mar 4:105932. doi: 10.1016/j.ijantimicag.2020.105932.
- [91] Graham RL, Donaldson EF, Baric RS. A decade after SARS: strategies to control emerging coronaviruses. *Nat Rev Microbiol* 2013;11:836–48.
- [92] Milewska A, Zarebski M, Nowak P, Stozek K, Potempa J, Pyrc K. Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells. *J Virol* 2014;88:13221–30.
- [93] Collins AR. HLA class I antigen serves as a receptor for human coronavirus OC43. *Immunol Invest* 1993;22:95–103.
- [94] Zhao X, Guo F, Liu F, Cuconati A, Chang J, Block TM, et al. Interferon induction of IFITM proteins promotes infection by human coronavirus OC43. *Proc Natl Acad Sci U S A* 2014;111:6756–61.

- [95] Vlasak R, Luytjes W, Spaan W, Palese P. Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. *Proc Natl Acad Sci U S A* 1988;85:4526–9.
- [96] Huang X, Dong W, Milewska A, Golda A, Qi Y, Zhu QK, et al. Human coronavirus HKU1 spike protein uses *O*-acetylated sialic acid as an attachment receptor determinant and employs hemagglutinin-esterase protein as a receptor-destroying enzyme. *J Virol* 2015;89:7202–13.
- [97] Chan CM, Lau SKP, Woo PCY, Tse H, Zheng BJ, Chen L, et al. Identification of major histocompatibility complex class I C molecule as an attachment factor that facilitates coronavirus HKU1 spike-mediated infection. *J Virol* 2009;83:1026–35.
- [98] Millet JK, Whittaker GR. Host cell entry of Middle East respiratory syndrome coronavirus after two-step, furin-mediated activation of the spike protein. *Proc Natl Acad Sci U S A* 2014;111:15214–9.
- [99] Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan 2019-nCov. *bioRxiv* 2020 Jan 26. doi: 10.1101/2020.01.26.919985
- [100] Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfefferle S, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. *J Virol* 2011;85:4122–34.
- [101] Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol Biol* 2015;1282:1–23. doi: 10.1007/978-1-4939-2438-7_1.

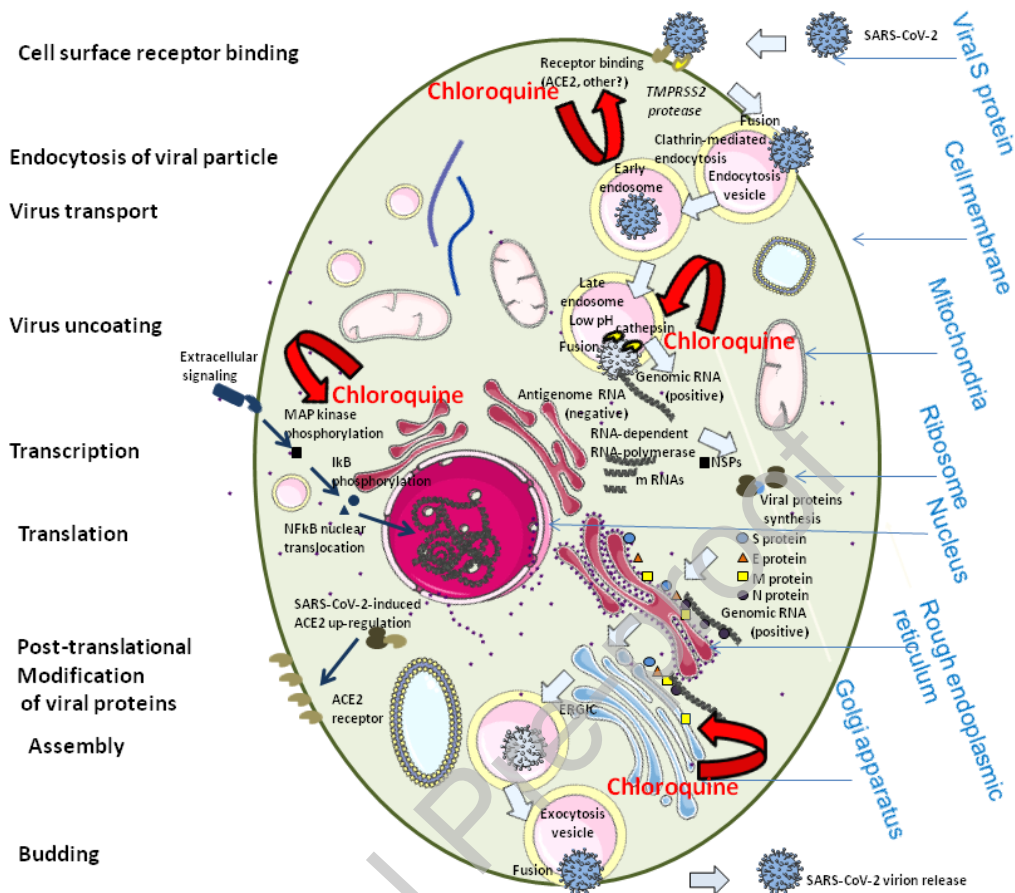


Fig. 1. Schematic representation of the possible effects of chloroquine on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication cycle. SARS-CoV2, like other human coronaviruses, harbours three envelope proteins, the spike (S) protein (180–220 kDa), the membrane (M) protein (25–35 kDa) and the envelope (E) protein (10–12 kDa), which are required for entry of infectious virions into target cells. The virion also contains the nucleocapsid (N), capable of binding to viral genomic RNA, and nsp3, a key component of the replicase complex. A subset of betacoronaviruses use a hemagglutinin-esterase (65 kDa) that binds sialic acids at the surface of glycoproteins. The S glycoprotein determines the host tropism. There is indication that SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) expressed on pneumocytes [85,99]. Binding to ACE2 is expected to trigger

conformational changes in the S glycoprotein allowing cleavage by the transmembrane protease TMPRSS2 of the S protein and the release of S fragments into the cellular supernatant that inhibit virus neutralisation by antibodies [100]. The virus is then transported into the cell through the early and late endosomes where the host protease cathepsin L further cleaves the S protein at low pH, leading to fusion of the viral envelope and phospholipidic membrane of the endosomes resulting in release of the viral genome into the cell cytoplasm. Replication then starts and the positive-strand viral genomic RNA is transcribed into a negative RNA strand that is used as a template for the synthesis of viral mRNA. Synthesis of the negative RNA strand peaks earlier and falls faster than synthesis of the positive strand. Infected cells contain between 10 and 100 times more positive strands than negative strands. The ribosome machinery of the infected cells is diverted in favour of the virus, which then synthesises its non-structural proteins (NSPs) that assemble into the replicase-transcriptase complex to favour viral subgenomic mRNA synthesis (see the review by Fehr and Perlman for details [101]). Following replication, the envelope proteins are translated and inserted into the endoplasmic reticulum and then move to the Golgi compartment. Viral genomic RNA is packaged into the nucleocapsid and then envelope proteins are incorporated during the budding step to form mature virions. The M protein, which localises to the trans-Golgi network, plays an essential role during viral assembly by interacting with the other proteins of the virus. Following assembly, the newly formed viral particles are transported to the cell surface in vesicles and are released by exocytosis. It is possible that chloroquine interferes with ACE2 receptor glycosylation, thus preventing SARS-CoV-2 binding to target cells. Chloroquine could also possibly limit the biosynthesis of sialic acids that may be required for cell surface binding of SARS-CoV-2. If binding of some viral

particles is achieved, chloroquine may modulate the acidification of endosomes thereby inhibiting formation of the autophagosome. Through reduction of cellular mitogen-activated protein (MAP) kinase activation, chloroquine may also inhibit virus replication. Moreover, chloroquine could alter M protein maturation and interfere with virion assembly and budding. With respect to the effect of chloroquine on the immune system, see the elegant review by Savarino et al. [11]. ERGIC, ER-Golgi intermediate compartment.

Journal Pre-proof

Table 1. Human coronavirus (HCoV) receptors/co-receptors as possible targets for chloroquine-induced inhibition of the virus replication cycle

Coronavirus	Receptor ^a	May also bind	Replication cycle inhibited by chloroquine ^b
<i>Alphacoronavirus</i>			
HCoV-229E	Aminopeptidase N (APN)/CD13		Yes
HCoV-NL63	Angiotensin-converting enzyme 2 (ACE2)		?
	Heparan sulfate proteoglycans ^c		
<i>Betacoronavirus</i>			
HCoV-OC43	HLA class I ^d , IFN-inducible transmembrane (IFITM) proteins in endocytic vesicles ^e	Sialic acid (O-acetylated sialic acid) ^f	Yes
SARS-CoV-1	Angiotensin-converting enzyme 2 (ACE2)	DC-SIGN/CD209, DC-SIGNr, DC-SIGN-related lectin LSECtin ^g	Yes
HCoV-HKU1	HLA class I ^h	Sialic acid (O-acetylated sialic acid)	?
MERS-CoV ⁱ	Dipeptidyl peptidase 4 (DPP4)/CD26		Yes
SARS-CoV-2	ACE2 ^l	Sialic acid?	Yes

HLA, human leukocyte antigen.

^a Adapted from Graham et al. [91].

^b Chloroquine could interfere with receptor (ACE2) glycosylation and/or sialic acid biosynthesis.

^c According to Milewska et al. [92].

^d According to Collins [93].

^e According to Zhao et al. [94].

^f According to Vlasak et al. [95].

^g According to Huang et al. [96].

^h According to Chan et al. [97].

ⁱ It is worth noting that different host cell proteases are required to activate the spike (S) protein for coronaviruses, such as SARS-CoV-1 S protein that requires activation by cathepsin L [89], or MERS-CoV that requires furin-mediated activation of the S protein [98].