Highlights in the fight against COVID-19: does autophagy play a role in SARS-CoV-2 infection?

Elizabeth Delorne-Axford and Daniel J. Klionsky

Department of Biological Sciences, Oakland University, Rochester, MI, USA; Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA

ABSTRACT
In the preceding months, the novel SARS-CoV-2 pandemic has devastated global communities. The need for safe and effective prophylactic and therapeutic treatments to combat COVID-19 – the human disease resulting from SARS-CoV-2 infection – is clear. Here, we present recent developments in the effort to combat COVID-19 and consider whether SARS-CoV-2 may potentially interact with the host autophagy pathway.

Abbreviations: ACE2, angiotensin converting enzyme II; βCoV, betacoronavirus; COVID-19, Coronavirus Disease 2019; CQ, chloroquine; DMV, double-membrane vesicle; GI, gastrointestinal; HCQ, hydroxychloroquine; IL, interleukin; MAP1LC3/LC3, microtubule associated protein 1 light chain 3; MEFs, mouse embryonic fibroblasts; MERS-CoV, Middle East respiratory syndrome coronavirus; MHV, murine hepatitis virus; PE, phosphatidylethanolamine; SARS-CoV, severe acute respiratory syndrome coronavirus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2; TNF, tumor necrosis factor; WHO, World Health Organization

SARS-CoV-2 global pandemic

In December 2019, an outbreak of severe pneumonia in Wuhan, Hubei province, China, was later identified to be caused by a novel betacoronavirus (βCoV) – initially named 2019-nCoV, and, later, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2]. SARS-CoV-2 is a member of the Coronaviridae family (Nidovirales order); coronaviruses are enveloped, single-stranded positive-sense RNA viruses [3]. SARS-CoV-2 is related to severe acute respiratory syndrome coronavirus (SARS-CoV; 2002–2003 endemic) and Middle East respiratory syndrome coronavirus (MERS-CoV; first identified in 2012); SARS-CoV and MERS-CoV are also βCoVs although of different lineages [4]. No vaccines or specific-drug treatments have been approved for either SARS-CoV or MERS-CoV [4]. Zoonotic origin has been attributed as the source of these outbreaks [5,6] and is further reviewed elsewhere [4]. SARS-CoV-2 is likely derived from a bat reservoir [5], although other sources have been proposed, including pangolins as an intermediate host [7,8], and multiple recombination events between bats and pangolins [9,10]. However, more recent data suggest that there is insufficient evidence to conclude that pangolins played a direct role in the emergence of SARS-CoV-2 [11]. At this time, the precise details of the zoonotic spillover event contributing to the current global crisis have yet to be determined. The potential mechanisms contributing to the evolution of SARS-CoV-2 are further reviewed elsewhere [11].

The World Health Organization (WHO) declared Coronavirus Disease 2019 (COVID-19) a global pandemic on 11 March 2020. As of 12 October 2020, over 37 million confirmed cases and 1 million deaths world-wide have been reported to the WHO [12]. The United States leads the world with the highest number of cases thus far (over 7.5 million confirmed cases as of 12 October 2020) [12]. The resulting human illness due to infection with the novel coronavirus SARS-CoV-2 is most frequently characterized by fever, fatigue, cough, acute pneumonia, and, less frequently, diarrhea [2]. Additionally, individuals infected with SARS-CoV-2 have also reported anosmia, or loss of smell [13]. Neurological symptoms, including headache, nausea, and vomiting, have been noted; the virus has also been identified in the brainstems of infected individuals [14]. The development of a cytokine storm has been associated with patients suffering from severe COVID-19 [15]. Elevated cytokine levels (IL6, IL10, and TNF/TNF-α), lymphopenia (in CD4+ and CD8+ T cells), and decreased IFNG/IFN-γ expression in CD4+ T cells have been reported [15]. This cytokine storm contributes to the development of acute respiratory distress syndrome/ARDS and may lead to increased organ damage and mortality [16].

Earlier in the pandemic, transmission was primarily attributed to respiratory droplets and fomites [17,18]. The risk of airborne transmission by aerosols has become more widely recognized [19,20], and the role of fomites as a transmission risk has been minimized [21]. Asymptomatic or mild infections have now been identified to be a source of underrecognized transmission [22,23], further exacerbating the spread.

SARS-CoV-2 entry mechanism

To gain entry into host cells, SARS-CoV-2 uses the SARS-CoV receptor ACE2 (angiotensin I converting enzyme 2)
and the transmembrane serine protease 2 (TMPRSS2) for spike (S) protein priming by host cell proteases [24]. Structural studies suggest that the receptor binding domain of SARS-CoV-2 interacts with ACE2, and that two trimeric S proteins bind to an ACE2 dimer [26]. The S protein of SARS-CoV-2 binds ACE2 with higher affinity than the S protein of SARS-CoV [27]. The SARS-CoV-2 S protein also mediates fusion of the virion with the host cell plasma membrane (reviewed in [27]). Recent work by Bruchez et al. also demonstrates that CD74, which is the major histocompatibility/MHC class II invariant chain for antigen presentation, inhibits SARS-CoV-2 entry in Vero cells [28].

Sungnak and colleagues applied single-cell RNA sequencing/scRNA-seq to identify the localization of SARS-CoV-2 entry factors – ACE2 and TMPRSS2 – to sites associated with transmission and infection, including nasal, respiratory, corneal, and gastrointestinal epithelial cells, supporting an underlying basis for SARS-CoV-2 transmissibility [29]. Lamers et al. also reported that ACE2 is highly expressed on differentiated human gut enterocytes [30]. Likewise, both SARS-CoV and SARS-CoV-2 productively infect human small intestinal organoids/hSIOs in culture [30]. These studies [29,30] provide a physiological basis for the gastrointestinal symptoms reported in some SARS-CoV-2 patients [2].

**Coronaviruses and autophagy**

Historically, the relationship between the host autophagy pathway and viruses has been somewhat provocative. Nevertheless, more recently, the dual roles of autophagy in the context of unique virus infections and cell types has become more widely recognized. Host autophagy may function in an antiviral capacity (also referred to as xenophagy or virophagy) to suppress virus infection. However, some viruses, may usurp and exploit the autophagy machinery to support replication (for further review on virus-host autophagy interactions, see [31,32]).

Earlier work examining a potential link between coronavirus-host autophagy interactions primarily focused on SARS-CoV or murine hepatitis virus (MHV) [33,34]. Briefly, from previous studies, coronaviruses form double-membrane vesicles (DMVs) during infection which may function as scaffolds for RNA replication [35–38], suggesting potential interactions between these viruses and the host autophagy pathway. These DMV scaffolds may function as a platform to improve the efficiency of viral RNA synthesis [3]. Similar to mammalian autophagosomes, DMVs are likely derived from the endoplasmic reticulum/ER and other cellular membranes [35,39,40]. Additional evidence from work by Prentice et al. in 2004 identified the colocalization of SARS-CoV replicase protein NSP8 with the autophagy marker MAP1LC3/LC3 [41], suggesting that SARS-CoV may interact with the autophagy machinery. LC3 is a mammalian ortholog of the highly conserved Atg8-family proteins and associates with both the emerging phagophore and complete autophagosome [42]. In contrast, another study showed that SARS-CoV replication was not significantly affected in autophagy-deficient atg5 null MEF cells exogenously expressing human ACE2 [33], even though ATG5 is required for canonical autophagy activity [43].

Additional studies investigating the relationship between coronaviruses and host autophagy have focused on the βCoV MHV [34,44]. Prentice and colleagues found that MHV replication complexes colocalize with autophagy proteins LC3 and ATG12 [34], further suggesting a role for autophagy in supporting MHV infection. ATG12 functions in the heterotrimetric ATG12–ATG5–ATG16L1 complex to facilitate LC3 conjugation to phosphatidylethanolamine (reviewed in [45]). MHV-dependent DMV formation and MHV replication are inhibited in embryonic stem cells lacking Atg5; the expression of exogenous ATG5 is sufficient to restore MHV replication [34]. However, another study by Zhao et al. determined that ATG5 is not required for MHV infection in bone marrow-derived macrophages or low-passage mouse embryonic fibroblasts (MEFs) [44].

A later study by Reggiori et al. demonstrated an autophagy-independent role for nonlipidated LC3-I in MHV infection [39]. In the same study, the authors concluded that MHV replication does not require a functional autophagy pathway based on studies in atg7 null MEFs [39]. These results suggest that with MHV, infection may occur independent of the canonical autophagy machinery, and/or the role of autophagy in MHV infection could be dependent on the cell type under study (for a more extensive review on the relationship between coronaviruses and autophagy, see [46] and the review by Miller et al. in this issue [47]). At the time this editorial went to press, there are currently no published studies on SARS-CoV-2 and autophagy. Thus, there remains a need for validated experiments on SARS-CoV-2 in the relevant cell types to provide conclusive evidence (or lack thereof) for the role of autophagy in its infectious life cycle.

**Potential treatments for COVID-19**

**Chloroquine and hydroxychloroquine**

There has been an ongoing search to find a safe and effective therapy to prevent or cure SARS-CoV-2 infection. The application of chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) as a treatment for COVID-19 has been met with controversy. CQ and HCQ are routinely used in the clinic for the treatment of malaria and various autoimmune diseases (reviewed in [48]). CQ and HCQ inhibit autophagy by preventing the fusion of the autophagosome with the lysosome and deacidifying the lysosome (reviewed in [49,50]). HCQ exhibits antiviral activity toward SARS-CoV-2 in cell culture studies [51]. However, the Federal Drug Administration has cautioned against the use of CQ or HCQ for COVID-19 treatment outside of clinical trials or hospital settings due to cardiac concerns and other serious side-effects [52]. In addition, a study examining postexposure prophylaxis with HCQ did not find any significant difference in new infection in those treated with HCQ compared to placebo within 4 days after exposure [53]. Furthermore, a clinical study published in the *New England Journal of Medicine* did not find any benefit to the administration of HCQ in >4,700 patients [54]. In fact, the study demonstrated that
patients who received HCQ suffered from an elevated risk of receiving mechanical ventilation or death within 28 days [54].
COVID-19, autophagy, and autophagy-modifying drugs are further discussed in recent Autophagy commentaries by Bonam and colleagues [55], Brest et al. [56], and Tang et al. [57].

Remdesivir and other potential treatments

According to its manufacturer Gilead Sciences, remdesivir (GS-5734) is “an investigational nucleotide analog with broad-spectrum antiviral activity” [58]. This drug inhibits the viral RNA-dependent RNA polymerase and was previously demonstrated to have antiviral efficacy in vitro against SARS-CoV, MERS-CoV, and, recently, SARS-CoV-2 [51,59,60]. Remdesivir was used to treat the United States’ first confirmed case of COVID-19 in the clinic with no obvious side-effects reported, but the report also called for ongoing trials to assess the safety and efficacy of the drug [61]. The Federal Drug Administration authorized remdesivir for emergency use as a COVID-19 treatment [62]. Preliminary results of the clinical trial administered by the National Institutes of Allergy and Infectious Disease/NIAID cited a quicker recovery time in patients receiving remdesivir compared to placebo [63]. A study published in the Lancet around the same time, found no statistically significant differences in the improvement of critically ill COVID-19 patients when treated with remdesivir; however, the authors did note that illness duration decreased in the remdesivir group compared to control [64], which corroborates the results stated by the NIAID clinical trial [63]. A recently published report in the New England Journal of Medicine demonstrates that patients who received intravenous remdesivir benefited from a shortened recovery time and decreased mortality [59]. When patients were given remdesivir earlier in the illness, a greater benefit was observed [59].

Dexamethasone is a glucocorticoid that may alleviate inflammation underlying lung injury and respiratory failure in COVID-19 patients [65]. Patients receiving respiratory support who were administered intravenous dexamethasone were found to have decreased mortality in a preliminary report [65]. Other potential therapies under consideration include tocilizumab, a humanized recombinant anti-human IL6 receptor monoclonal antibody, which could suppress severe inflammatory responses and alleviate the cytokine storm exhibited by patients with severe COVID-19 [66]. Additional potential drug candidates are in development targeting various aspects of the virus life cycle, including the main SARS-CoV-2 protease Mpro that mediates viral replication and transcription [67]. The ribonucleoside analog β-D-N4-hydroxycytidine (NHC; EIDD-1931) has demonstrated in vitro efficacy against various coronaviruses including SARS-CoV-2, MERS-CoV, and SARS-CoV [68]. Furthermore, prophylactic and therapeutic administration of EIDD-2801, an orally bioactive NHC prodrug (β-D-N4-hydroxycytidine-5’-isopropyl ester), in mice reduces MERS-CoV and SARS-CoV titers [68]. Similar to other antiviral therapies, such as Tamiflu®, the timing of the administration for drugs targeting SARS-CoV-2 during patient infection is likely critical, and, moving forward, will require optimization once a promising candidate is identified. In addition, a number of vaccines are currently under investigation [69].

Conclusions

At the time this article went to press, there is a lack of direct experimental evidence to conclude whether autophagy is involved (either in an antiviral or proviral manner, or in any biologically significant capacity) in SARS-CoV-2 infection either in vitro in cell culture or in vivo – in animal models or clinical findings. Direct inquiry into the factors involved in the physiologically relevant cell types is necessary to determine the full range of the host response to SARS-CoV-2. In addition, investigation into how unique gene variants (such as host factors important for viral infection) may influence COVID-19 pathophysiology – for example, asymptomatic infection and, on the other end of the spectrum, severe disease pathology – will be key to not only furthering our understanding of SARS-CoV-2, but also that of the host response to emerging viruses.

Acknowledgments

The authors apologize to those whose work was not included here due to space limitations.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the National Institute of General Medical Sciences [GM131919].

ORCID

Elizabeth Delorme-Axford http://orcid.org/0000-0002-7455-7616
Daniel J. Klionsky http://orcid.org/0000-0002-7828-8118

References

[1] Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–733.
[2] Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506.
[3] Maier HJ, Britton P. Involvement of autophagy in coronavirus replication. Viruses. 2012;4(12):3400–51.
[4] Fung TS, Liu DX. Human coronavirus: host-pathogen interaction. Annu Rev Microbiol. 2019;73:529–557.
[5] Zhou P, Yang X-L, Wang X-G, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–273.
[6] Zhang YZ, Holmes EC. A genomic perspective on the origin and emergence of SARS-CoV-2. Cell. 2020;181(2):223–227.
[7] Lam TT, Jia N, Zhang Y-W, et al. Identifying SARS-CoV-2 related coronaviruses in Malayang pangolins. Nature. 2020;583:282–285.
[8] Zhang T, Wu Q, Zhang Z. Probable pangolin origin of SARS-CoV-2 associated with the COVID-19 outbreak. Curr Biol. 2020;30(7):1346–1351.e2
[9] Li X, Giorgi EE, Marichannegowda MH, et al. Emergence of SARS-CoV-2 through recombination and strong purifying selection. Sci Adv. 2020;6(27):eaaw9153.

[10] Xiao K, Zhai J, Feng Y, et al. Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins. Nature. 2020;583(7815):286–289.

[11] Hu B, Guo H, Zhou P, et al. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2020; in press. DOI: 10.1038/s41579-020-00459-7.

[12] WHO. WHO coronavirus disease (COVID-19) dashboard Oct 12, 2020; Available from: https://covid19.who.int.

[13] Yan CH, Faraji F, Prajapati AD, et al. Association of chemosensory dysfunction and Covid-19 in patients presenting with influenza-like symptoms. Int Forum Allergy Rhinol. 2020;10(7):806–813.

[14] Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV-2 may play a role in the respiratory failure of COVID-19 patients. J Med Virol. 2020; in press. DOI: 10.1002/jmv.25728.

[15] Pedersen SF, Ho YC, SARS-CoV-2: a storm is raging. J Clin Invest. 2020;130(5):2202–2205.

[16] Barnes BJ, Adrover JM, Baxter-Stoltzfus A, et al. Targeting potential drivers of COVID-19: neutrophil extracellular traps. J Exp Med. 2020;217(6):e20200652.

[17] Chan JF, Yuan S, Kok K-H, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395(10223):514–523.

[18] van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med. 2020;382(16):1564–1567.

[19] Morawska L, Milton DK. It is time to address airborne transmission of COVID-19. Clin Infect Dis. 2020; in press. DOI: 10.1093/cid/ciaa939.

[20] Tang S, Mao Y, Jones RM, et al. Aerosol transmission of SARS-CoV-2. Evidence, prevention and control. Environ Int. 2020;144(14):106039.

[21] Goldman E. Exaggerated risk of transmission of COVID-19 by fomites. Lancet Infect Dis. 2020;20(8):892–893.

[22] Li R, Pei S, Chen B, et al. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science. 2020;368(6490):489–493.

[23] Ing A, Cocks C, Green J. COVID-19: in the footsteps of Ernest Shackleton. Thorax. 2020;75(3):693–694.

[24] Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271–280. e8.

[25] Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450–4.

[26] Yan R, Zhang Y, Li Y, et al. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020;367(6485):1444–1448.

[27] Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020;367(6483):1260–1263.

[28] Bruchez A, Sha K, Johnson J, et al. MHC class II transactivator CIITA induces cell resistance to Ebola virus and SARS-like coronaviruses. Science. 2020;370(6513):241–247.

[29] Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med. 2020;26(5):681–687.

[30] Lamers MM, Beumer J, van der Vaart J, et al. SARS-CoV-2 productively infects human gut enteroocytes. Science. 2020;369(6499):50–54.

[31] Jackson WT. Viruses and the autophagy pathway. Virology. 2015;475:480–450.456.

[32] Viete C, Rozieres A, Faure M. Autophagy during early virus-host cell interactions. J Mol Biol. 2018;430(12):1696–1713.

[33] Schneider M, Ackermann K, Stuart M, et al. Severe acute respiratory syndrome coronavirus replication is severely impaired by MG132 due to proteasome-independent inhibition of M-calpain. J Virol. 2012;86(18):10112–22.

[34] Prentice E, Jerome WQ, Yoshimori T, et al. Coronavirus replication complex formation utilizes components of cellular autophagy. J Biol Chem. 2004;279(11):10136–41.

[35] Knoops K, Kikkert M, Worm SHEV, et al. SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol. 2008;6(9):e226.

[36] Usali M, Verheije MH, de Haan CAM, et al. Qualitative and quantitative ultrastructural analysis of the membrane rearrangements induced by coronavirus. Cell Microbiol. 2010;12(6):844–61.

[37] van den Worm SHE, Knoops K, Zevenhoven-Dobbe JC, et al. Development and RNA-synthesizing activity of coronavirus replication structures in the absence of protein synthesis. J Virol. 2011;85(11):5669–73.

[38] Gosert R, Kanjanahaluthai A, Egger D, et al. RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J Virol. 2002;76(8):3697–708.

[39] Reggiori F, Monastyrski I, Verheije MH, et al. Coronavirus Hijack the LC3-1-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. Cell Host Microbe. 2010;7(6):500–8.

[40] Axe EL, Walker SA, Manifava M, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositols 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol. 2008;182(4):685–701.

[41] Prentice E, McAlulife J, Lu X, et al. Identification and characterization of severe acute respiratory syndrome coronavirus replicase proteins. J Virol. 2004;78(18):9977–86.

[42] Kabeya Y, Mizushima N, Ueno T, et al. LC3, amamamal homologue of yeast Apg8p, is localized in autophagosome membranes after processing. Embo J. 2000;19(21):5720–8.

[43] Kametaka S, Matsuura A, Wada Y, et al. Structural and functional analyses of AGPS, a gene involved in autophagy in yeast. Gene. 1996;197(1–2):139–43.

[44] Zhao Z, Thackray LB, Miller BC, et al. Coronavirus replication does not require the autophagy gene ATG5. Autophagy. 2007;3(6):581–5.

[45] Delorme-Axford E, Klionsky DJ. Transcriptional and post-transcriptional regulation of autophagy in the yeast Saccharomyces cerevisiae. J Biol Chem. 2018;293(15):5396–5403.

[46] Carmona-Gutierrez D, Bauer MA, Zimmermann A, et al. Digesting the crisis: autophagy and coronaviruses. Microb Cell. 2020;7(5):119–128.

[47] Miller K, McGrath ME, Hu Z, et al. Coronavirus interactions with the cellular autophagy machinery. Autophagy. 2020; in press. DOI: 10.1080/15548627.2020.18117280.

[48] Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov. 2020;6:16.

[49] Yang Y-p, Hu L-f, Zheng H-f, et al. Application and interpretation of current autophagy inhibitors and activators. Acta Pharmacol Sin. 2013;34(5):625–35.

[50] Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nat Rev Cancer. 2017;17(9):528–542.

[51] Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269–271.

[52] FDA. FDA cautions against use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting or a clinical trial due to risk of heart rhythm problems. April 30, 2020; Access data May 12, 2020. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting.

[53] Boulware D, Pullen MF, Bangdiwala AS, et al. A randomized trial of hydroxychloroquine as postexposure prophylaxis for Covid-19. N Engl J Med. 2020;383(6):517–525.
[54] Horby P, Mafham M, Linsell L, et al. Effect of hydroxychloroquine in hospitalized patients with Covid-19. N Engl J Med. 2020; in press.DOI: 10.1056/NEJMoa2022926.

[55] Bonam S, Muller S, Bayry J, et al. Autophagy as an emerging target for COVID-19: lessons from an old friend. Autophagy. 2020; in press. DOI: 10.1080/15548627.2020.1779467.

[56] Bret P, Benzaquen J, Klionsky DJ, et al. Open questions for harnessing autophagy-modulating drugs in the SARS-CoV-2 war: hope or hype? Autophagy. 2020;1–4. DOI: 10.1080/15548627.2020.1779531.

[57] Tang D, Li J, Zhang R, et al. Chloroquine in fighting COVID-19: good, bad, or both? Autophagy. 2020; in press.DOI: 10.1080/15548627.2020.1796014.

[58] Gilead. About Remdesivir. May 13, 2020; Available from: https://www.gilead.com/purpose/advancing-global-health/covid-19/about-remdesivir

[59] Beigel J, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19 - final report. N Engl J Med. 2020. DOI:10.1056/NEJMoa2007764.

[60] Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017;9(396):eaal3653.

[61] Hohue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. N Engl J Med. 2020;382(10):929–936.

[62] FDA. Coronavirus (COVID-19) update: FDA Issues emergency use authorization for potential COVID-19 treatment. Access date May 13, 2020. Available from: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment

[63] Beigel J, Tomashek KM, Dodd LE, et al. Remdesivir for the treatment of Covid-19 - final report. N Engl J Med. 2020; in press.DOI: 10.1056/NEJMoa2007764.

[64] Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 2020;395(10236):1569-1578.

[65] Horby P, Lim WS, Emberson JR, et al. Dexamethasone in hospitalized patients with Covid-19 - preliminary report. N Engl J Med. 2020; in press.DOI: 10.1056/NEJMoa2021436.

[66] Xu X, Han M, Li T, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Nat Acad Sci. 2020;117(20):10970–10975.

[67] Dai W, Zhang B, Jiang X-M, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science. 2020;368(6497):1331–1335.

[68] Sheahan TP, Sims AC, Zhou S, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med. 2020;12(541):eabb5883.

[69] Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020;586(7830):516–527.