Antiviral Research
Available online 3 April 2020, 104787
In Press, Journal Pre-proof

The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 *in vitro*

Leon Caly [1], Julian D. Druce [1], Mike G. Catton [1], David A. Jans [2], Kylie M. Wagstaff [2]

Show more

https://doi.org/10.1016/j.antiviral.2020.104787

Highlights

- Ivermectin is an inhibitor of the COVID-19 causative virus (SARS-CoV-2) *in vitro.*
- A single treatment able to effect ~5000-fold reduction in virus at 48h in cell culture.
- Ivermectin is FDA-approved for parasitic infections, and therefore has a potential for repurposing.
- Ivermectin is widely available, due to its inclusion on the WHO model list of essential medicines.

Abstract

Although several clinical trials are now underway to test possible therapies, the worldwide response to the COVID-19 outbreak has been largely limited to monitoring/containment. We report here that Ivermectin, an FDA-approved anti-parasitic previously shown to have broad-spectrum anti-viral activity *in vitro*, is an inhibitor of the causative virus (SARS-CoV-2), with a single addition to Vero-hSLAM cells 2 hours post infection with SARS-CoV-2 able to effect

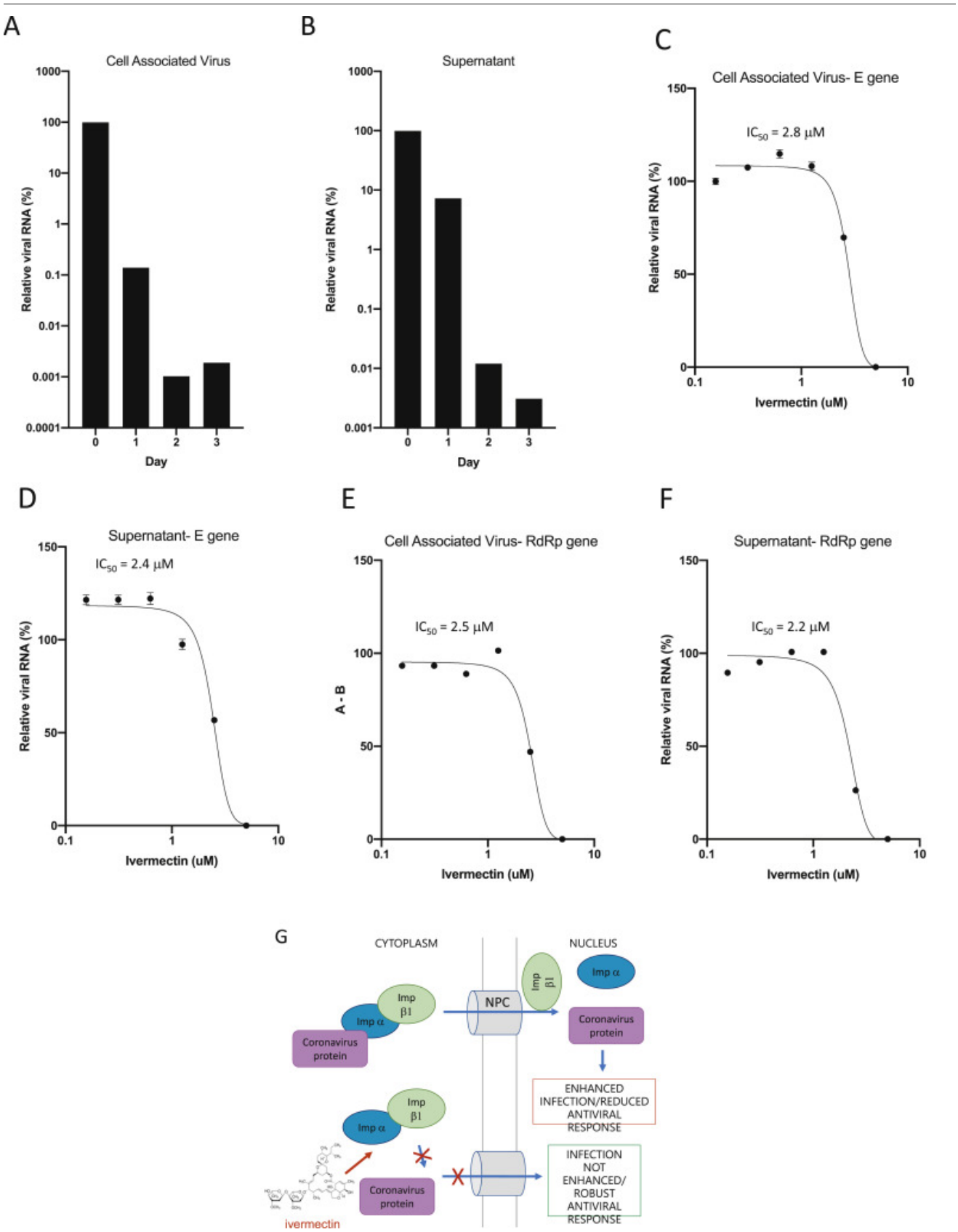
~5000-fold reduction in viral RNA at 48 h. Ivermectin therefore warrants further investigation for possible benefits in humans.

Ivermectin is an FDA-approved broad spectrum anti-parasitic agent[1] that in recent years we, along with other groups, have shown to have anti-viral activity against a broad range of viruses[2, 3, 4, 5] *in vitro*. Originally identified as an inhibitor of interaction between the human immunodeficiency virus-1 (HIV-1) integrase protein (IN) and the importin (IMP) α/β1 heterodimer responsible for IN nuclear import[6], Ivermectin has since been confirmed to inhibit IN nuclear import and HIV-1 replication[5]. Other actions of ivermectin have been reported[7], but ivermectin has been shown to inhibit nuclear import of host (eg.[8,9]) and viral proteins, including simian virus SV40 large tumour antigen (T-ag) and dengue virus (DENV) non-structural protein 5[5, 6]. Importantly, it has been demonstrated to limit infection by RNA viruses such as DENV 1-4[4], West Nile Virus[10], Venezuelan equine encephalitis virus (VEEV)[3] and influenza[2], with this broad spectrum activity believed to be due to the reliance by many different RNA viruses on IMPα/β1 during infection[11,12]. Ivermectin has similarly been shown to be effective against the DNA virus pseudorabies virus (PRV) both *in vitro* and *in vivo*, with ivermectin treatment shown to increase survival in PRV-infected mice[13]. Efficacy was not observed for ivermectin against Zika virus (ZIKV) in mice, but the authors acknowledged that study limitations justified re-evaluation of ivermectin's anti-ZIKV activity[14]. Finally, ivermectin was the focus of a phase III clinical trial in Thailand in 2014-2017, against DENV infection, in which a single daily oral dose was observed to be safe and resulted in a significant reduction in serum levels of viral NS1 protein, but no change in viremia or clinical benefit was observed (see below)[15].

The causative agent of the current COVID-19 pandemic, SARS-CoV-2, is a single stranded positive sense RNA virus that is closely related to severe acute respiratory syndrome coronavirus (SARS-CoV). Studies on SARS-CoV proteins have revealed a potential role for IMPα/β1 during infection in signal-dependent nucleocytoplasmic shutting of the SARS-CoV Nucleocapsid protein[16, 17, 18], that may impact host cell division[19,20]. In addition, the SARS-CoV accessory protein ORF6 has been shown to antagonize the antiviral activity of the STAT1 transcription factor by sequestering IMPα/β1 on the rough ER/Golgi membrane[21]. Taken together, these reports suggested that ivermectin's nuclear transport inhibitory activity may be effective against SARS-CoV-2.

To test the antiviral activity of ivermectin towards SARS-CoV-2, we infected Vero/hSLAM cells with SARS-CoV-2 isolate Australia/VIC01/2020 at an MOI of 0.1 for 2 h, followed by the addition of 5 μM ivermectin. Supernatant and cell pellets were harvested at days 0-3 and analysed by RT-PCR for the replication of SARS-CoV-2 RNA (Fig. 1A/B). At 24 h, there was a 93% reduction in viral RNA present in the supernatant (indicative of released virions) of samples treated with ivermectin compared to the vehicle DMSO. Similarly a 99.8% reduction in cell-associated viral RNA (indicative of unreleased and unpackaged virions) was observed with ivermectin treatment. By 48h this effect increased to an ~5000-fold reduction of viral RNA in ivermectin-treated compared to control samples, indicating that ivermectin treatment resulted in the effective loss of essentially all viral material by 48 h. Consistent with this idea,

no further reduction in viral RNA was observed at 72 h. As we have observed previously[3, 4, 5], no toxicity of ivermectin was observed at any of the timepoints tested, in either the sample wells or in parallel tested drug alone samples.



Download : Download high-res image (712KB) Download : Download full-size image

Figure 1. **Ivermectin is a potent inhibitor of the SARS-CoV-2 clinical isolate Australia/VIC01/2020.** Vero/hSLAM cells were in infected with SARS-CoV-2 clinical isolate Australia/VIC01/2020 (MOI = 0.1) for 2 h prior to addition of vehicle (DMSO) or Ivermectin at the indicated concentrations. Samples were taken at 0-3 days post infection for quantitation of viral load using real-time PCR of cell associated virus (**A**) or supernatant (**B**). IC_{50} values were determined in subsequent experiments at 48 h post infection using the indicated concentrations of Ivermectin (treated at 2 h post infection as per **A/B**). Triplicate real-time PCR analysis was performed on cell associated virus (**C/E**) or supernatant (**D/F**) using probes against either the SARS-CoV-2 E (**C/D**) or RdRp (**E/F**) genes. Results represent mean ± SD (n=3). 3 parameter dose response curves were fitted using GraphPad prism to determine IC_{50} values (indicated). **G.** Schematic of ivermectin's proposed antiviral action on coronavirus. IMPα/β1 binds to the coronavirus cargo protein in the cytoplasm (top) and translocates it through the nuclear pore complex (NPC) into the nucleus where the complex falls apart and the viral cargo can reduce the host cell's antiviral response, leading to enhanced infection. Ivermectin binds to and destabilises the Impα/β1 heterodimer thereby preventing Impα/β1 from binding to the viral protein (bottom) and preventing it from entering the nucleus. This likely results in reduced inhibition of the antiviral responses, leading to a normal, more efficient antiviral response.

To further determine the effectiveness of ivemectin, cells infected with SARS-CoV-2 were treated with serial dilutions of ivermectin 2 h post infection and supernatant and cell pellets collected for real-time RT-PCR at 48 h (Fig. 1C/D). As above, a >5000 reduction in viral RNA was observed in both supernatant and cell pellets from samples treated with 5 μM ivermectin at 48 h, equating to a 99.98% reduction in viral RNA in these samples. Again, no toxicity was observed with ivermectin at any of the concentrations tested. The IC50 of ivermectin treatment was determined to be ~2μM under these conditions. Underlining the fact that the assay indeed specifically detected SARS-CoV-2, RT-PCR experiments were repeated using primers specific for the viral RdRp gene (Fig. 1E/F) rather than the E gene (above), with nearly identical results observed for both released (supernatant) and cell-associated virus.

Taken together these results demonstrate that ivermectin has antiviral action against the SARS-CoV-2 clinical isolate *in vitro*, with a single dose able to control viral replication within 24-48 h in our system. We hypothesise that this is likely through inhibiting IMPα/β1-mediated nuclear import of viral proteins (Fig. 1G), as shown for other RNA viruses[4,5,10]; confirmation of this mechanism in the case of SARS-CoV-2, and identification of the specific SARS-CoV-2 and/or host component(s) impacted (see[10]) is an important focus future work in this laboratory. Ultimately, development of an effective anti-viral for SARS-CoV-2, if given to patients early in infection, could help to limit the viral load, prevent severe disease progression and limit person-person transmission. Benchmarking testing of ivermectin against other potential antivirals for SARS-CoV-2 with alternative mechanisms of action22, 23, 24, 25, 26 would thus be important as soon as practicable. This Brief Report raises the possibility that ivermectin could be a useful antiviral to limit SARS-CoV-2, in similar fashion

to those already reported[22, 23, 24, 25, 26]; until one of these is proven to be beneficial in a clinical setting, all should be pursued as rapidly as possible.

Ivermectin has an established safety profile for human use[1,12,27], and is FDA-approved for a number of parasitic infections[1,27]. Importantly, recent reviews and meta-analysis indicate that high dose ivermectin has comparable safety as the standard low-dose treatment, although there is not enough evidence to make conclusions about the safety profile in pregnancy[28,29]. The critical next step in further evaluation for possible benefit in COVID-19 patients will be to examine a multiple addition dosing regimen that mimics the current approved usage of ivermectin in humans. As noted, ivermectin was the focus of a recent phase III clinical trial in dengue patients in Thailand, in which a single daily dose was found to be safe but did not produce any clinical benefit. However, the investigators noted that an improved dosing regimen might be developed, based on pharmacokinetic data[15]. Although DENV is clearly very different to SARS-CoV-2, this trial design should inform future work going forward. Altogether the current report, combined with a known-safety profile, demonstrates that ivermectin is worthy of further consideration as a possible SARS-CoV-2 antiviral.

Methods

Cell culture, viral infection and drug treatment

Vero/hSLAM cells[30] were maintained in Earle's Minimum Essential Medium (EMEM) containing 7% Fetal Bovine Serum (FBS) (Bovogen Biologicals, Keilor East, AUS) 2 mM L-Glutamine, 1 mM Sodium pyruvate, 1500 mg/L sodium bicarbonate, 15 mM HEPES and 0.4 mg/ml geneticin at 37°C, 5% CO_2. Cells were seeded into 12-well tissue culture plates 24 h prior to infection with SARS-CoV-2 (Australia/VIC01/2020 isolate) at an MOI of 0.1 in infection media (as per maintenance media but containing only 2% FBS) for 2 h. Media containing inoculum was removed and replaced with 1 mL fresh media (2% FBS) containing Ivermectin at the indicated concentrations or DMSO alone and incubated as indicated for 0-3 days. At the appropriate timepoint, cell supernatant was collected and spun for 10 min at 6,000g to remove debris and the supernatant transferred to fresh collection tubes. The cell monolayers were collected by scraping and resuspension into 1 mL fresh media (2% FBS). Toxicity controls were set up in parallel in every experiment on uninfected cells.

Generation of SARS-CoV-2 cDNA

RNA was extracted from 200 µL aliquots of sample supernatant or cell suspension using the QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany) and eluted in 60 µl. Reverse transcription was performed using the BioLine SensiFAST cDNA kit (Bioline, London, United Kingdom), total reaction mixture (20 µl), containing 10 µL of RNA extract, 4 µl of 5x TransAmp buffer, 1µl of Reverse Transcriptase and 5 µl of Nuclease free water. The reactions were incubated at 25°C for 10 min, 42°C for 15 min and 85°C for 5 min.

Detection of SARS-CoV-2 using a TaqMan Real-time RT-PCR assay.

TaqMan RT-PCR assay were performed using 2.5 µl cDNA, 10 µl Primer Design PrecisonPLUS qPCR Master Mix 1 µM Forward (5'- AAA TTC TAT GGT GGT TGG CAC AAC ATG TT-3'), 1 µM Reverse (5'- TAG GCA TAG CTC TRT CAC AYT T-3') primers and 0.2 µM probe (5'-FAM-TGG GTT GGG ATT ATC-MGBNFQ-3') targeting the BetaCoV RdRp (RNA-dependent RNA polymerase) gene or Forward (5'-ACA GGT ACG TTA ATA GTT AAT AGC GT -3'), 1 µM Reverse (5'-ATA TTG CAG CAG TAC GCA CAC A-3') primers and 0.2 µM probe (5'-FAM-ACA CTA GCC ATC CTT ACT GCG CTT CG-286 NFQ-3') targeting the BetaCoV E-gene[31]. Real-time RT-PCR assays were performed on an Applied Biosystems ABI 7500 Fast real-time PCR machine (Applied Biosystems, Foster City, CA, USA) using cycling conditions of 95°C for 2 min, 95°C for 5 s, 60°C for 24 s. SARS-CoV-2 cDNA (Ct~28) was used as a positive control. Calculated Ct values were converted to fold-reduction of treated samples compared to control using the ΔCt method (fold changed in viral RNA = 2^ΔCt) and expressed as % of DMSO alone sample. IC50 values were fitted using 3 parameter dose response curves in GraphPad prism.

Funding

This work was supported by a National Breast Cancer Foundation Fellowship (ECF-17-007) for KMW and an NHMRC SPRF (APP1103050) for DAJ.

Recommended articles Citing articles (0)

References

1 A. Gonzalez Canga, *et al.*
The pharmacokinetics and interactions of ivermectin in humans--a mini-review
AAPS J, 10 (1) (2008), pp. 42-46
CrossRef View Record in Scopus Google Scholar

2 V. Gotz, *et al.*
Influenza A viruses escape from MxA restriction at the expense of efficient nuclear vRNP import
Sci Rep, 6 (2016), p. 23138
Google Scholar

3 L. Lundberg, *et al.*
Nuclear import and export inhibitors alter capsid protein distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus replication
Antiviral Res, 100 (3) (2013), pp. 662-672
Article Download PDF View Record in Scopus Google Scholar

4 M.Y. Tay, *et al.*
Nuclear localization of dengue virus (DENV) 1-4 non-structural protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin
Antiviral Res, 99 (3) (2013), pp. 301-306

Article Download PDF View Record in Scopus Google Scholar

5 K.M. Wagstaff, *et al.*
Ivermectin is a specific inhibitor of importin alpha/beta-mediated nuclear import able to inhibit replication of HIV-1 and dengue virus
The Biochemical journal, 443 (3) (2012), pp. 851-856
View Record in Scopus Google Scholar

6 K.M. Wagstaff, *et al.*
An AlphaScreen(R)-based assay for high-throughput screening for specific inhibitors of nuclear import
Journal of biomolecular screening, 16 (2) (2011), pp. 192-200
CrossRef View Record in Scopus Google Scholar

7 E. Mastrangelo, *et al.*
Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug
The Journal of antimicrobial chemotherapy (2012)
Google Scholar

8 F.K. Kosyna, *et al.*
The importin alpha/beta-specific inhibitor Ivermectin affects HIF-dependent hypoxia response pathways
Biol Chem, 396 (12) (2015), pp. 1357-1367
CrossRef View Record in Scopus Google Scholar

9 P.J. van der Watt, *et al.*
Targeting the Nuclear Import Receptor Kpnbeta1 as an Anticancer Therapeutic
Mol Cancer Ther, 15 (4) (2016), pp. 560-573
CrossRef View Record in Scopus Google Scholar

10 S.N.Y. Yang, *et al.*
The broad spectrum antiviral ivermectin targets the host nuclear transport importin alpha/beta1 heterodimer
Antiviral Res (2020), p. 104760
Article Download PDF Google Scholar

11 L. Caly, K.M. Wagstaff, D.A. Jans
Nuclear trafficking of proteins from RNA viruses: Potential target for anti-virals?
Antiviral research, 95 (2012), pp. 202-206
Article Download PDF View Record in Scopus Google Scholar

12 D.A. Jans, A.J. Martin, K.M. Wagstaff
Inhibitors of nuclear transport
Curr Opin Cell Biol, 58 (2019), pp. 50-60
Article Download PDF View Record in Scopus Google Scholar

13 C. Lv, *et al.*
Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the nucleus and proliferation of the virus in vitro and vivo
Antiviral Res, 159 (2018), pp. 55-62
Article Download PDF View Record in Scopus Google Scholar

14 H. Ketkar, *et al.*
Lack of efficacy of ivermectin for prevention of a lethal Zika virus infection in a murine system
Diagn Microbiol Infect Dis, 95 (1) (2019), pp. 38-40
Article Download PDF View Record in Scopus Google Scholar

15 Yamasmith, E., et al., Efficacy and Safety of Ivermectin against Dengue Infection: A Phase III, Randomized, Double-blind, Placebo-controlled Trial, in he 34th Annual Meeting The Royal College of Physicians of Thailand- 'Internal Medicine and One Health'. 2018: Chonburi, Thailand.
Google Scholar

16 R.R. Rowland, *et al.*
Intracellular localization of the severe acute respiratory syndrome coronavirus nucleocapsid protein: absence of nucleolar accumulation during infection and after expression as a recombinant protein in vero cells
J Virol, 79 (17) (2005), pp. 11507-11512
CrossRef View Record in Scopus Google Scholar

17 K.A. Timani, *et al.*
Nuclear/nucleolar localization properties of C-terminal nucleocapsid protein of SARS coronavirus
Virus Res, 114 (1-2) (2005), pp. 23-34
Article Download PDF View Record in Scopus Google Scholar

18 W.N. Wulan, *et al.*
Nucleocytoplasmic transport of nucleocapsid proteins of enveloped RNA viruses
Front Microbiol, 6 (2015), p. 553
Google Scholar

19 J.A. Hiscox, *et al.*
The coronavirus infectious bronchitis virus nucleoprotein localizes to the nucleolus
J Virol, 75 (1) (2001), pp. 506-512
View Record in Scopus Google Scholar

20 T. Wurm, *et al.*
Localization to the nucleolus is a common feature of coronavirus nucleoproteins, and the protein may disrupt host cell division
J Virol, 75 (19) (2001), pp. 9345-9356

View Record in Scopus Google Scholar

21 M. Frieman, *et al.*
Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane
J Virol, 81 (18) (2007), pp. 9812-9824
CrossRef View Record in Scopus Google Scholar

22 L. Dong, S. Hu, J. Gao
Discovering drugs to treat coronavirus disease 2019 (COVID-19)
Drug Discov Ther, 14 (1) (2020), pp. 58-60
CrossRef View Record in Scopus Google Scholar

23 A.A. Elfiky
Anti-HCV, nucleotide inhibitors, repurposing against COVID-19
Life Sci, 248 (2020), p. 117477
Article Download PDF Google Scholar

24 C.J. Gordon, *et al.*
The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus
J Biol Chem (2020)
Google Scholar

25 G. Li, E. De Clercq
Therapeutic options for the 2019 novel coronavirus (2019-nCoV)
Nat Rev Drug Discov, 19 (3) (2020), pp. 149-150
CrossRef View Record in Scopus Google Scholar

26 M. Wang, *et al.*
Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro
Cell Res, 30 (3) (2020), pp. 269-271
CrossRef View Record in Scopus Google Scholar

27 D. Buonfrate, *et al.*
Multiple-dose versus single-dose ivermectin for Strongyloides stercoralis infection (Strong Treat 1 to 4): a multicentre, open-label, phase 3, randomised controlled superiority trial
Lancet Infect Dis, 19 (11) (2019), pp. 1181-1190
Article Download PDF View Record in Scopus Google Scholar

28 M. Navarro, *et al.*
Safety of high-dose ivermectin: a systematic review and meta-analysis
J Antimicrob Chemother, 75 (4) (2020), pp. 827-834

CrossRef View Record in Scopus Google Scholar

29 P. Nicolas, *et al.*
Safety of oral ivermectin during pregnancy: a systematic review and meta-analysis
Lancet Glob Health, 8 (1) (2020), pp. e92-e100
Article Download PDF View Record in Scopus Google Scholar

30 N. Ono, *et al.*
Measles viruses on throat swabs from measles patients use signaling lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor
J Virol, 75 (9) (2001), pp. 4399-4401
View Record in Scopus Google Scholar

31 V.M. Corman, *et al.*
Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR
Euro Surveill, 25 (3) (2020)
Google Scholar

View Abstract