

Journal Pre-proof

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

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



PII: S0166-3542(20)30201-1

DOI: <https://doi.org/10.1016/j.antiviral.2020.104787>

Reference: AVR 104787

To appear in: *Antiviral Research*

Received Date: 18 March 2020

Revised Date: 27 March 2020

Accepted Date: 29 March 2020

Please cite this article as: Caly, L., Druce, J.D., Catton, M.G., Jans, D.A., Wagstaff, K.M., The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 *in vitro*, *Antiviral Research*, <https://doi.org/10.1016/j.antiviral.2020.104787>.

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 The Author(s). Published by Elsevier B.V.

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

2

3 Leon Caly¹, Julian D. Druce¹, Mike G. Catton¹, David A. Jans² and Kylie M. Wagstaff^{2*}

4 ¹ *Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital, at the*
5 *Peter Doherty Institute for Infection and Immunity, Victoria, 3000, Australia.*

6 ² *Biomedicine Discovery Institute, Monash University, Clayton, Vic, 3800 Australia*

7

8 *** Corresponding author:**

9 Dr. Kylie Wagstaff, Biomedicine Discovery Institute, Monash University, Clayton, Vic, 3800

10 Australia

11 Tel: +61 3 9902 9348

12 Email: kylie.wagstaff@monash.edu

13

14

15

16 **Summary**

17 Although several clinical trials are now underway to test possible therapies, the worldwide
18 response to the COVID-19 outbreak has been largely limited to monitoring/containment. We
19 report here that Ivermectin, an FDA-approved anti-parasitic previously shown to have broad-
20 spectrum anti-viral activity *in vitro*, is an inhibitor of the causative virus (SARS-CoV-2), with
21 a single addition to Vero-hSLAM cells 2 hours post infection with SARS-CoV-2 able to
22 effect ~5000-fold reduction in viral RNA at 48 h. Ivermectin therefore warrants further
23 investigation for possible benefits in humans.

24

25

26

27 Ivermectin is an FDA-approved broad spectrum anti-parasitic agent¹ that in recent years we,
28 along with other groups, have shown to have anti-viral activity against a broad range of
29 viruses²⁻⁵ *in vitro*. Originally identified as an inhibitor of interaction between the human
30 immunodeficiency virus-1 (HIV-1) integrase protein (IN) and the importin (IMP) α/β 1
31 heterodimer responsible for IN nuclear import⁶, Ivermectin has since been confirmed to
32 inhibit IN nuclear import and HIV-1 replication⁵. Other actions of ivermectin have been
33 reported⁷, but ivermectin has been shown to inhibit nuclear import of host (eg. ^{8, 9}) and viral
34 proteins, including simian virus SV40 large tumour antigen (T-ag) and dengue virus (DENV)
35 non-structural protein 5^{5, 6}. Importantly, it has been demonstrated to limit infection by RNA
36 viruses such as DENV 1-4⁴, West Nile Virus¹⁰, Venezuelan equine encephalitis virus
37 (VEEV)³ and influenza², with this broad spectrum activity believed to be due to the reliance
38 by many different RNA viruses on IMP α/β 1 during infection^{11, 12}. Ivermectin has similarly
39 been shown to be effective against the DNA virus pseudorabies virus (PRV) both *in vitro* and
40 *in vivo*, with ivermectin treatment shown to increase survival in PRV-infected mice¹³.
41 Efficacy was not observed for ivermectin against Zika virus (ZIKV) in mice, but the authors
42 acknowledged that study limitations justified re-evaluation of ivermectin's anti-ZIKV
43 activity¹⁴. Finally, ivermectin was the focus of a phase III clinical trial in Thailand in 2014-
44 2017, against DENV infection, in which a single daily oral dose was observed to be safe and
45 resulted in a significant reduction in serum levels of viral NS1 protein, but no change in
46 viremia or clinical benefit was observed (see below)¹⁵.

47 The causative agent of the current COVID-19 pandemic, SARS-CoV-2, is a single
48 stranded positive sense RNA virus that is closely related to severe acute respiratory syndrome
49 coronavirus (SARS-CoV). Studies on SARS-CoV proteins have revealed a potential role for
50 IMP α/β 1 during infection in signal-dependent nucleocytoplasmic shuttling of the SARS-CoV
51 Nucleocapsid protein¹⁶⁻¹⁸, that may impact host cell division^{19, 20}. In addition, the SARS-CoV

52 accessory protein ORF6 has been shown to antagonize the antiviral activity of the STAT1
53 transcription factor by sequestering $IMP\alpha/\beta 1$ on the rough ER/Golgi membrane²¹. Taken
54 together, these reports suggested that ivermectin's nuclear transport inhibitory activity may
55 be effective against SARS-CoV-2.

56 To test the antiviral activity of ivermectin towards SARS-CoV-2, we infected
57 Vero/hSLAM cells with SARS-CoV-2 isolate Australia/VIC01/2020 at an MOI of 0.1 for 2
58 h, followed by the addition of 5 μ M ivermectin. Supernatant and cell pellets were harvested
59 at days 0-3 and analysed by RT-PCR for the replication of SARS-CoV-2 RNA (**Fig. 1A/B**).
60 At 24 h, there was a 93% reduction in viral RNA present in the supernatant (indicative of
61 released virions) of samples treated with ivermectin compared to the vehicle DMSO.
62 Similarly a 99.8% reduction in cell-associated viral RNA (indicative of unreleased and
63 unpackaged virions) was observed with ivermectin treatment. By 48h this effect increased to
64 an ~5000-fold reduction of viral RNA in ivermectin-treated compared to control samples,
65 indicating that ivermectin treatment resulted in the effective loss of essentially all viral
66 material by 48 h. Consistent with this idea, no further reduction in viral RNA was observed at
67 72 h. As we have observed previously³⁻⁵, no toxicity of ivermectin was observed at any of the
68 timepoints tested, in either the sample wells or in parallel tested drug alone samples.

69 To further determine the effectiveness of ivermectin, cells infected with SARS-CoV-2 were
70 treated with serial dilutions of ivermectin 2 h post infection and supernatant and cell pellets
71 collected for real-time RT-PCR at 48 h (**Fig. 1C/D**). As above, a >5000 reduction in viral
72 RNA was observed in both supernatant and cell pellets from samples treated with 5 μ M
73 ivermectin at 48 h, equating to a 99.98% reduction in viral RNA in these samples. Again, no
74 toxicity was observed with ivermectin at any of the concentrations tested. The IC₅₀ of
75 ivermectin treatment was determined to be ~2 μ M under these conditions. Underlining the
76 fact that the assay indeed specifically detected SARS-CoV-2, RT-PCR experiments were

77 repeated using primers specific for the viral RdRp gene (**Fig. 1E/F**) rather than the E gene
78 (above), with nearly identical results observed for both released (supernatant) and cell-
79 associated virus.

80 Taken together these results demonstrate that ivermectin has antiviral action against
81 the SARS-CoV-2 clinical isolate *in vitro*, with a single dose able to control viral replication
82 within 24-48 h in our system. We hypothesise that this is likely through inhibiting IMP α / β 1-
83 mediated nuclear import of viral proteins (**Fig. 1G**), as shown for other RNA viruses^{4, 5, 10};
84 confirmation of this mechanism in the case of SARS-CoV-2, and identification of the specific
85 SARS-CoV-2 and/or host component(s) impacted (see¹⁰) is an important focus future work
86 in this laboratory. Ultimately, development of an effective anti-viral for SARS-CoV-2, if
87 given to patients early in infection, could help to limit the viral load, prevent severe disease
88 progression and limit person-person transmission. Benchmarking testing of ivermectin
89 against other potential antivirals for SARS-CoV-2 with alternative mechanisms of action²²⁻²⁶
90 would thus be important as soon as practicable. This Brief Report raises the possibility that
91 ivermectin could be a useful antiviral to limit SARS-CoV-2, in similar fashion to those
92 already reported²²⁻²⁶; until one of these is proven to be beneficial in a clinical setting, all
93 should be pursued as rapidly as possible.

94 Ivermectin has an established safety profile for human use^{1, 12, 27}, and is FDA-
95 approved for a number of parasitic infections^{1, 27}. Importantly, recent reviews and meta-
96 analysis indicate that high dose ivermectin has comparable safety as the standard low-dose
97 treatment, although there is not enough evidence to make conclusions about the safety profile
98 in pregnancy^{28, 29}. The critical next step in further evaluation for possible benefit in COVID-
99 19 patients will be to examine a multiple addition dosing regimen that mimics the current
100 approved usage of ivermectin in humans. As noted, ivermectin was the focus of a recent
101 phase III clinical trial in dengue patients in Thailand, in which a single daily dose was found

102 to be safe but did not produce any clinical benefit. However, the investigators noted that an
103 improved dosing regimen might be developed, based on pharmacokinetic data¹⁵. Although
104 DENV is clearly very different to SARS-CoV-2, this trial design should inform future work
105 going forward. Altogether the current report, combined with a known-safety profile,
106 demonstrates that ivermectin is worthy of further consideration as a possible SARS-CoV-2
107 antiviral.

108

109 **Methods**

110 *Cell culture, viral infection and drug treatment*

111 Vero/hSLAM cells³⁰ were maintained in Earle's Minimum Essential Medium (EMEM)
112 containing 7% Fetal Bovine Serum (FBS) (Bovogen Biologicals, Keilor East, AUS) 2 mM L-
113 Glutamine, 1 mM Sodium pyruvate, 1500 mg/L sodium bicarbonate, 15 mM HEPES and 0.4
114 mg/ml geneticin at 37°C, 5% CO₂. Cells were seeded into 12-well tissue culture plates 24 h
115 prior to infection with SARS-CoV-2 (Australia/VIC01/2020 isolate) at an MOI of 0.1 in
116 infection media (as per maintenance media but containing only 2% FBS) for 2 h. Media
117 containing inoculum was removed and replaced with 1 mL fresh media (2% FBS) containing
118 Ivermectin at the indicated concentrations or DMSO alone and incubated as indicated for 0-3
119 days. At the appropriate timepoint, cell supernatant was collected and spun for 10 min at
120 6,000g to remove debris and the supernatant transferred to fresh collection tubes. The cell
121 monolayers were collected by scraping and resuspension into 1 mL fresh media (2% FBS).
122 Toxicity controls were set up in parallel in every experiment on uninfected cells.

123

124 *Generation of SARS-CoV-2 cDNA*

125 RNA was extracted from 200 µL aliquots of sample supernatant or cell suspension using the
126 QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany) and eluted in 60 µl. Reverse

127 transcription was performed using the BioLine SensiFAST cDNA kit (Bioline, London,
128 United Kingdom), total reaction mixture (20 μ l), containing 10 μ L of RNA extract, 4 μ l of 5x
129 TransAmp buffer, 1 μ l of Reverse Transcriptase and 5 μ l of Nuclease free water. The
130 reactions were incubated at 25°C for 10 min, 42°C for 15 min and 85°C for 5 min.

131

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

133 TaqMan RT-PCR assay were performed using 2.5 μ l cDNA, 10 μ l Primer Design
134 PrecisionPLUS qPCR Master Mix 1 μ M Forward (5'- AAA TTC TAT GGT GGT TGG CAC
135 AAC ATG TT-3'), 1 μ M Reverse (5'- TAG GCA TAG CTC TRT CAC AYT T-3') primers
136 and 0.2 μ M probe (5'-FAM- TGG GTT GGG ATT ATC-MGBNFQ-3') targeting the
137 BetaCoV RdRp (RNA-dependent RNA polymerase) gene or Forward (5'-ACA GGT ACG
138 TTA ATA GTT AAT AGC GT -3'), 1 μ M Reverse (5'-ATA TTG CAG CAG TAC GCA
139 CAC A-3') primers and 0.2 μ M probe (5'-FAM-ACA CTA GCC ATC CTT ACT GCG CTT
140 CG-

141 286 NFQ-3') targeting the BetaCoV E-gene³¹. Real-time RT-PCR assays were performed on
142 an Applied Biosystems ABI 7500 Fast real-time PCR machine (Applied Biosystems, Foster
143 City, CA, USA) using cycling conditions of 95°C for 2 min, 95°C for 5 s, 60°C for 24 s.
144 SARS-CoV-2 cDNA (Ct~28) was used as a positive control. Calculated Ct values were
145 converted to fold-reduction of treated samples compared to control using the Δ Ct method
146 (fold changed in viral RNA = $2^{-\Delta$ Ct) and expressed as % of DMSO alone sample. IC50
147 values were fitted using 3 parameter dose response curves in GraphPad prism.

148

149

150

151

152 ***Funding***

153 This work was supported by a National Breast Cancer Foundation Fellowship (ECF-17-007)

154 for KMW and an NHMRC SPRF (APP1103050) for DAJ.

155

156

Journal Pre-proof

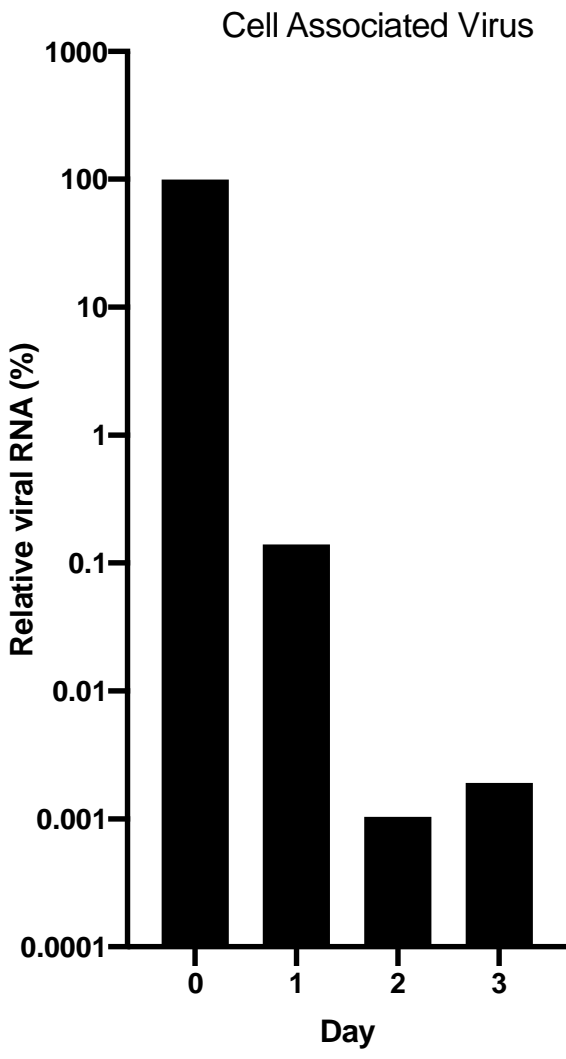
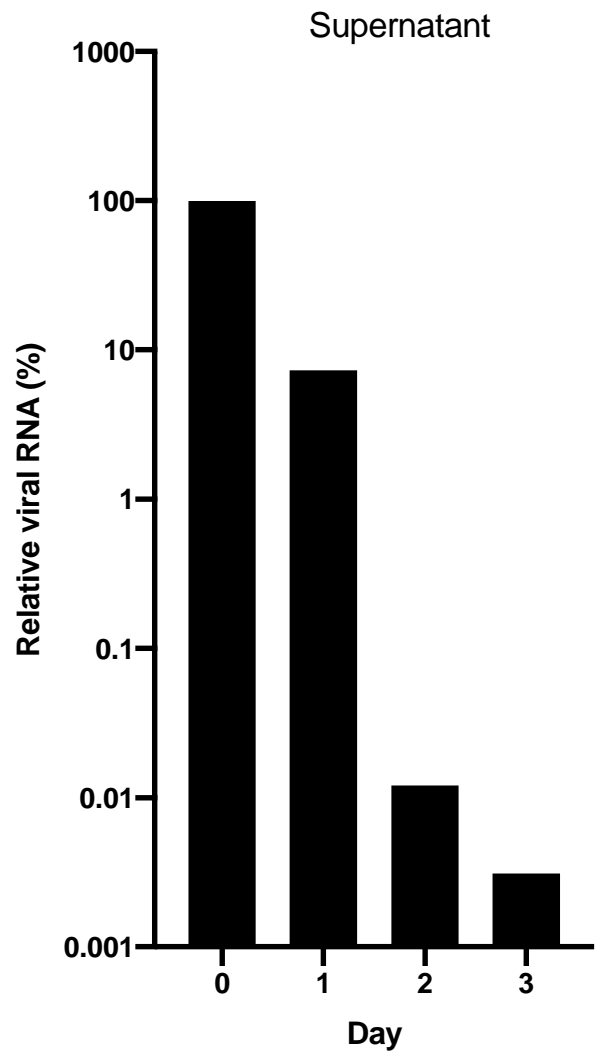
157 **References**

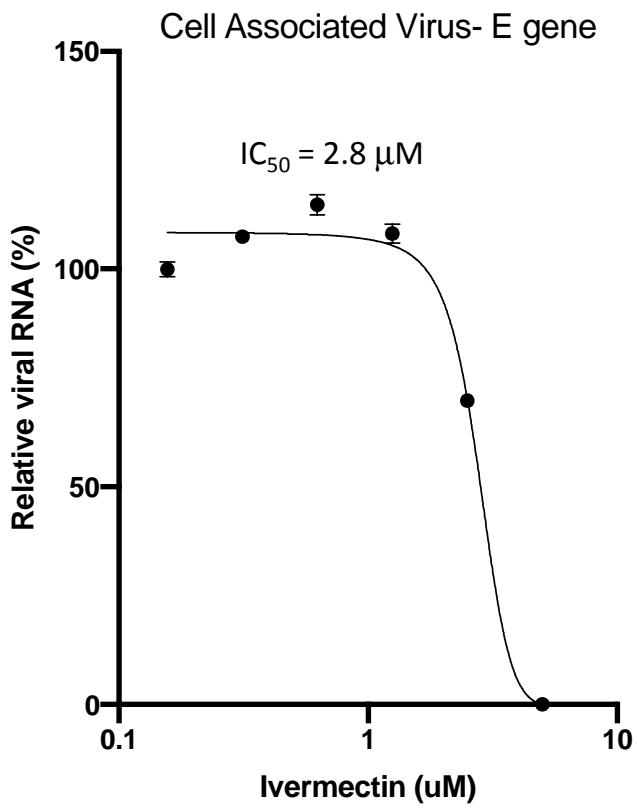
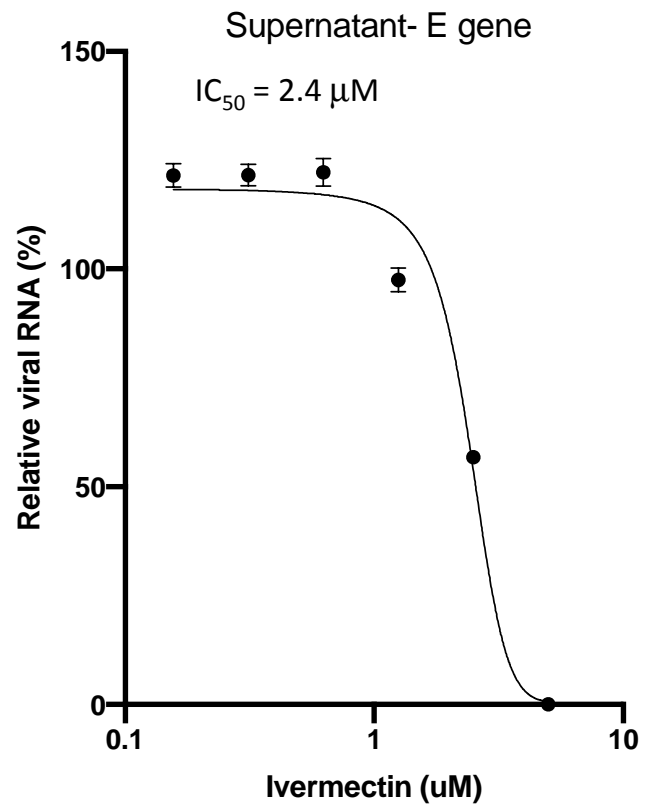
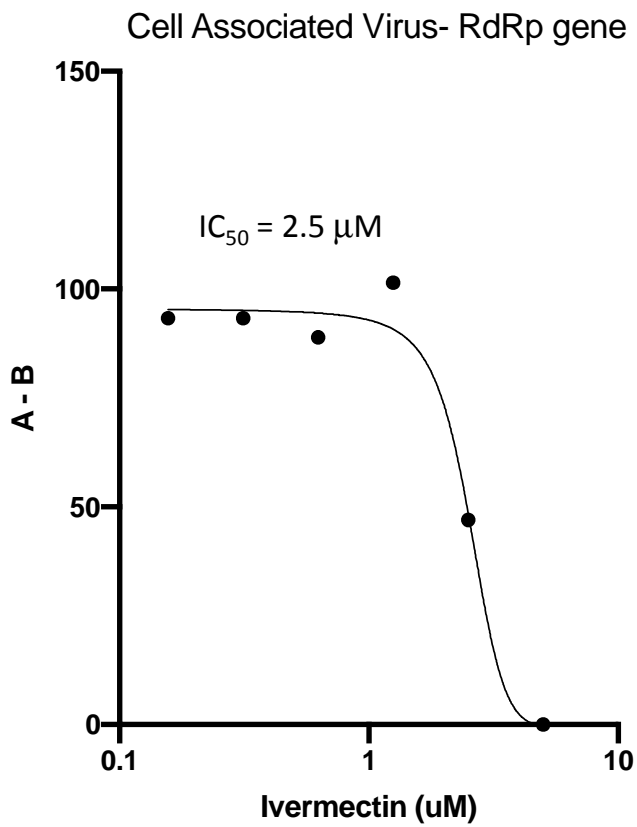
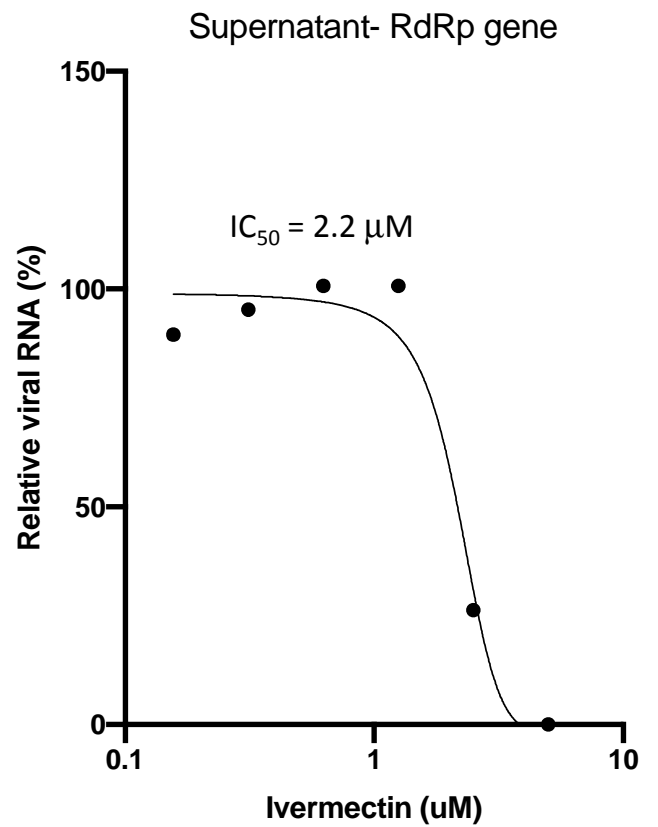
- 158 1. Gonzalez Canga, A., et al., *The pharmacokinetics and interactions of ivermectin in*
159 *humans--a mini-review*. AAPS J, 2008. **10**(1): p. 42-6.
- 160 2. Gotz, V., et al., *Influenza A viruses escape from MxA restriction at the expense of*
161 *efficient nuclear vRNP import*. Sci Rep, 2016. **6**: p. 23138.
- 162 3. Lundberg, L., et al., *Nuclear import and export inhibitors alter capsid protein*
163 *distribution in mammalian cells and reduce Venezuelan Equine Encephalitis Virus*
164 *replication*. Antiviral Res, 2013. **100**(3): p. 662-72.
- 165 4. Tay, M.Y., et al., *Nuclear localization of dengue virus (DENV) 1-4 non-structural*
166 *protein 5; protection against all 4 DENV serotypes by the inhibitor Ivermectin*.
167 Antiviral Res, 2013. **99**(3): p. 301-6.
- 168 5. Wagstaff, K.M., et al., *Ivermectin is a specific inhibitor of importin alpha/beta-*
169 *mediated nuclear import able to inhibit replication of HIV-1 and dengue virus*. The
170 Biochemical journal, 2012. **443**(3): p. 851-6.
- 171 6. Wagstaff, K.M., et al., *An AlphaScreen(R)-based assay for high-throughput screening*
172 *for specific inhibitors of nuclear import*. Journal of biomolecular screening, 2011.
173 **16**(2): p. 192-200.
- 174 7. Mastrangelo, E., et al., *Ivermectin is a potent inhibitor of flavivirus replication*
175 *specifically targeting NS3 helicase activity: new prospects for an old drug*. The
176 Journal of antimicrobial chemotherapy, 2012.
- 177 8. Kosyna, F.K., et al., *The importin alpha/beta-specific inhibitor Ivermectin affects HIF-*
178 *dependent hypoxia response pathways*. Biol Chem, 2015. **396**(12): p. 1357-67.
- 179 9. van der Watt, P.J., et al., *Targeting the Nuclear Import Receptor Kpnbeta1 as an*
180 *Anticancer Therapeutic*. Mol Cancer Ther, 2016. **15**(4): p. 560-73.
- 181 10. Yang, S.N.Y., et al., *The broad spectrum antiviral ivermectin targets the host nuclear*
182 *transport importin alpha/beta1 heterodimer*. Antiviral Res, 2020: p. 104760.
- 183 11. Caly, L., K.M. Wagstaff, and D.A. Jans, *Nuclear trafficking of proteins from RNA*
184 *viruses: Potential target for anti-virals?* Antiviral research, 2012. **95**: p. 202-206.
- 185 12. Jans, D.A., A.J. Martin, and K.M. Wagstaff, *Inhibitors of nuclear transport*. Curr Opin
186 Cell Biol, 2019. **58**: p. 50-60.
- 187 13. Lv, C., et al., *Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance*
188 *into the nucleus and proliferation of the virus in vitro and vivo*. Antiviral Res, 2018.
189 **159**: p. 55-62.
- 190 14. Ketkar, H., et al., *Lack of efficacy of ivermectin for prevention of a lethal Zika virus*
191 *infection in a murine system*. Diagn Microbiol Infect Dis, 2019. **95**(1): p. 38-40.
- 192 15. Yamasmith, E., et al., *Efficacy and Safety of Ivermectin against Dengue Infection: A*
193 *Phase III, Randomized, Double-blind, Placebo-controlled Trial*, in *he 34th Annual*
194 *Meeting The Royal College of Physicians of Thailand- 'Internal Medicine and One*
195 *Health'*. 2018: Chonburi, Thailand.
- 196 16. Rowland, R.R., et al., *Intracellular localization of the severe acute respiratory*
197 *syndrome coronavirus nucleocapsid protein: absence of nucleolar accumulation*
198 *during infection and after expression as a recombinant protein in vero cells*. J Virol,
199 2005. **79**(17): p. 11507-12.
- 200 17. Timani, K.A., et al., *Nuclear/nucleolar localization properties of C-terminal*
201 *nucleocapsid protein of SARS coronavirus*. Virus Res, 2005. **114**(1-2): p. 23-34.

- 202 18. Wulan, W.N., et al., *Nucleocytoplasmic transport of nucleocapsid proteins of*
203 *enveloped RNA viruses*. Front Microbiol, 2015. **6**: p. 553.
- 204 19. Hiscox, J.A., et al., *The coronavirus infectious bronchitis virus nucleoprotein localizes*
205 *to the nucleolus*. J Virol, 2001. **75**(1): p. 506-12.
- 206 20. Wurm, T., et al., *Localization to the nucleolus is a common feature of coronavirus*
207 *nucleoproteins, and the protein may disrupt host cell division*. J Virol, 2001. **75**(19): p.
208 9345-56.
- 209 21. Frieman, M., et al., *Severe acute respiratory syndrome coronavirus ORF6 antagonizes*
210 *STAT1 function by sequestering nuclear import factors on the rough endoplasmic*
211 *reticulum/Golgi membrane*. J Virol, 2007. **81**(18): p. 9812-24.
- 212 22. Dong, L., S. Hu, and J. Gao, *Discovering drugs to treat coronavirus disease 2019*
213 *(COVID-19)*. Drug Discov Ther, 2020. **14**(1): p. 58-60.
- 214 23. Elfiky, A.A., *Anti-HCV, nucleotide inhibitors, repurposing against COVID-19*. Life Sci,
215 2020. **248**: p. 117477.
- 216 24. Gordon, C.J., et al., *The antiviral compound remdesivir potently inhibits RNA-*
217 *dependent RNA polymerase from Middle East respiratory syndrome coronavirus*. J
218 Biol Chem, 2020.
- 219 25. Li, G. and E. De Clercq, *Therapeutic options for the 2019 novel coronavirus (2019-*
220 *nCoV)*. Nat Rev Drug Discov, 2020. **19**(3): p. 149-150.
- 221 26. Wang, M., et al., *Remdesivir and chloroquine effectively inhibit the recently emerged*
222 *novel coronavirus (2019-nCoV) in vitro*. Cell Res, 2020. **30**(3): p. 269-271.
- 223 27. Buonfrate, D., et al., *Multiple-dose versus single-dose ivermectin for Strongyloides*
224 *stercoralis infection (Strong Treat 1 to 4): a multicentre, open-label, phase 3,*
225 *randomised controlled superiority trial*. Lancet Infect Dis, 2019. **19**(11): p. 1181-1190.
- 226 28. Navarro, M., et al., *Safety of high-dose ivermectin: a systematic review and meta-*
227 *analysis*. J Antimicrob Chemother, 2020. **75**(4): p. 827-834.
- 228 29. Nicolas, P., et al., *Safety of oral ivermectin during pregnancy: a systematic review*
229 *and meta-analysis*. Lancet Glob Health, 2020. **8**(1): p. e92-e100.
- 230 30. Ono, N., et al., *Measles viruses on throat swabs from measles patients use signaling*
231 *lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor*. J
232 Virol, 2001. **75**(9): p. 4399-401.
- 233 31. Corman, V.M., et al., *Detection of 2019 novel coronavirus (2019-nCoV) by real-time*
234 *RT-PCR*. Euro Surveill, 2020. **25**(3).
- 235

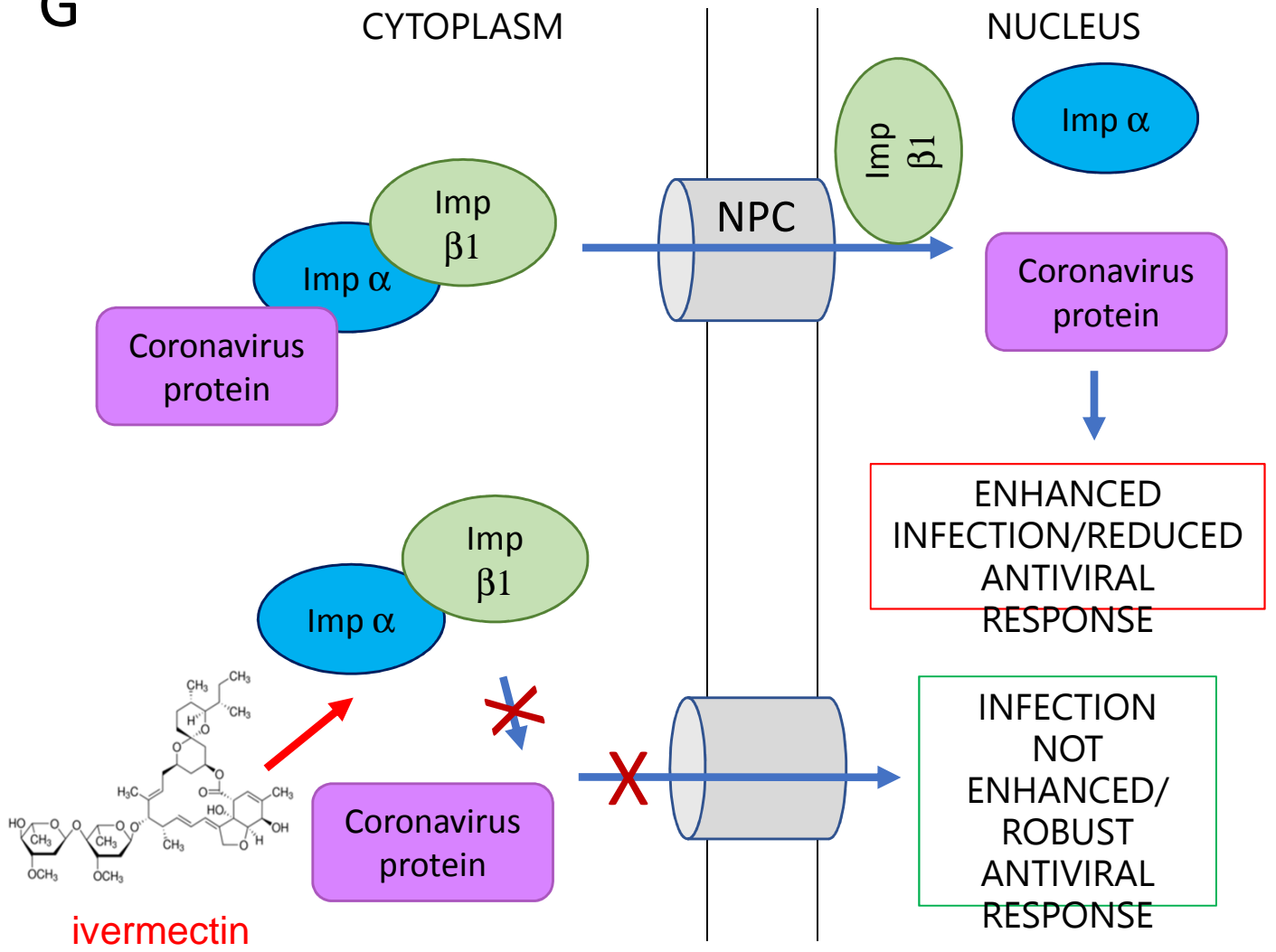
237 **Figure 1: Ivermectin is a potent inhibitor of the SARS-CoV-2 clinical isolate**
238 **Australia/VIC01/2020.** Vero/hSLAM cells were infected with SARS-CoV-2 clinical
239 isolate Australia/VIC01/2020 (MOI = 0.1) for 2 h prior to addition of vehicle (DMSO) or
240 Ivermectin at the indicated concentrations.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

255

A**B**

C**D****E****F**

G



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.

Journal Pre-proof