Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
COVID-19 is a devastating global pandemic around the world. While the majority of infected cases appear mild, in some cases individuals present respiratory complications with possible serious lung damage. There are no specific treatments for COVID-19 as yet, though a number are under evaluation, including experimental antivirals. Sofosbuvir, the clinically approved anti-hepatitis C virus (HCV) drug, is also capable of suppressing other families of positive-strand RNA viruses; Flaviviridae and Togaviridae. Coronaviruses are a family of positive-strand RNA viruses with conserved polymerase, so SARS-CoV-2 RdRp is very likely to be effectively inhibited by sofosbuvir. More importantly, sofosbuvir is safe and well tolerated at 400 mg daily in a 24 week therapeutic regimen. Sofosbuvir active metabolite, however, shows an extremely high intracellular stability so, it is hypothesized that SARS-CoV-2 infection could also be susceptible to sofosbuvir and we were convinced to design and run a clinical trial to evaluate the effect of sofosbuvir 400 mg (in combination with velpatasvir 100 mg, as add-on treatment, in addition to standard of care) on the COVID-19. However, we believe that this manuscript/correspondence should be made available to the international scientific community as soon as possible, with the help of this esteemed journal. © 2020 IMSS. Published by Elsevier Inc.

Key Words: Drug repurposing, Sofosbuvir, COVID-19, SARS-CoV-2.
Human pathogenic coronaviruses (SARS-CoV and SARS-CoV-2) bind to their target cells through angiotensin-converting enzyme 2 (ACE2), which is expressed by epithelial cells of the lung, intestine, kidney, and blood vessels (1–3). The ability of SARS-CoV2 to enter and infect the human nervous system, based on the strong expression of the ACE2 target throughout the brain (4), should be also considered. However, despite decades of extensive research, there are no specific/effective therapies approved by the US. Food and Drug Administration (FDA) for serious coronavirus infections such as SARS, MERS, and now COVID-19. In vitro and limited clinical data suggest potential benefit for chloroquine and hydroxychloroquine. Nevertheless, FDA on March 27th, 2020, issued an emergency authorization for experimental coronavirus treatment using these anti-malarial drugs. On the other hand, although in vitro and limited clinical data suggest potential benefit for Kaletra (Lopinavir; Ritonavir), and its actual role in the treatment of COVID-19 is still unclear, some preclinical data suggested potential benefit. However, more recent data has failed to confirm Kaletra efficacy for COVID-19 treatment (5) (https://www.elsevier.com/__data/assets/pdf_file/0007/988648/COVID-19-Drug-Therapy_Mar-2020.pdf). While the COVID-19 outbreak continues to spread around the world, the absence of a clinically proven antiviral therapy is a serious challenge for the treatment of severe COVID-19 cases (6).

According to (7,8), SARS, MERS and SARS-CoV-2 coronaviruses, like HCV and the flaviviridae (9), are positive-sense single-strand RNA viruses and these viruses share a similar replication mechanism requiring a RNA-dependent RNA polymerase (RdRp). So, there is a strong possibility that Sofosbuvir, Ribavirin, AZT (and other HCV/HIV nucleoside/nucleotide analogues such as Remdesivir) can tightly bind to SARS-CoV-2 RdRp. In a recent in silico (prenliminary) study, sequence analyses as well as homology modeling were used to build a new SARS-nCoV RdRp model which then targeted by anti-polymerase drugs, including the approved drugs Sofosbuvir and Ribavirin (10). The docking scores suggested possible eligibilities of Sofosbuvir, Ribavirin, (and Remdesivir) as potent drugs against the new coronavirus. These theoretical data needed to be confirmed by the experimental observations. Using polymerase extension experiments, in vitro, Chien M, et al. also demonstrated that the biologically activate triphosphate forms of four well-known nucleotide/nucleoside analogue anti-viral (anti-HCV/HBV, anti-HIV/AIDS) drugs; Sofosbuvir (Figure 1), Tenofovir alafenamide, Alovudine, and AZT were incorporated by RNA-dependent RNA polymerase (RdRp) enzymes of SARS-CoV as well as SARS-CoV-2, and permanently blocked further incorporation (further polymerase extension was terminated). They considered all these compounds as permanent/strong terminators for the SARS-CoV-2 RdRp (7,8). Due to widely availability of these FDA approved drugs (Sofosbuvir, Tenofovir and AZT), they expressed hope that the drugs would be more evaluated quickly in laboratory and clinical trials for COVID-19 treatment. But the above researchers (Chien M, et al.) could not offer one (or more) of them as the best RdRp inhibitor(s) to introduce to the scientific community.

Sofosbuvir is a clinically approved drug with potent antiviral effects against hepatitis C virus (HCV) with

![Figure 1](https://example.com/figure1.png)
diverse genotypes (11). The acid dissociation constant \((pK_a)\) of sofosbuvir is around 9.3 and as the class 3 compound possess high-solubility and low-permeability (12). The phosphorylation of sofosbuvir within the host cell (hepatocyte) converts it to the active form, nucleoside triphosphate, which terminates RNA replication in the nascent viral genome through the competition with the nucleotides of virus (11). Sofosbuvir exhibits potent antiviral activities, >90%, even against liver cirrhosis, as well as prior null response to ribavirin and IFN (13). Moreover, sofosbuvir is fast response and estimated that during the mean 0.8 and 2 d it exerts it 99 and 99.9% potency (14). Sofosbuvir offers high healing rate, low side effects, significant efficacy, short administration period good tolerability, and potent resistance defense (15) (e.g. FISSION (16), POSITRON (17), FUSION (18), PHOTON-1 (18). It is reported that the patients receiving combination of sofosbuvir and ribavirin with or without interferon-\(\alpha\) did not express primary sofosbuvir resistance mutation in NS5B, S282T (15,19). Moreover, sofosbuvir exerts pan-genotypic antiviral effects against HCV genotypes 1–6 (20).

This antiviral drug does not interfere with major drug metabolizing enzymes, such as the cytochrome P450 system, therefore has low drug-drug interactions, including with antiretrovirals, opioid substitution therapy, and calcineurin inhibitors, which is beneficial for treatment of who have traditionally been difficult to treat. Sofosbuvir has rapid clinical development due to its high efficacy and safety profile (21). Moreover, sofosbuvir can be prescribed in an oral single daily dose due to its promising pharmacokinetic profile. The bioavailability of sofosbuvir is high with maximum plasma concentrations \((C_{\text{max}})\) at \(\approx 0.5–2\ h\) in the oral administration (22).

It is interesting that Sofosbuvir, the clinically approved anti-hepatitis C virus (HCV) drug, has the ability to suppress different families of viruses. Different research groups have demonstrated that this safe drug inhibits the replication of flaviviruses (including ZIKV and DENV, and yellow fever virus (YFV) (23–28). Moreover, Ferreira AC, et al. (9) observed that, beyond members of the Flaviviridae family, sofosbuvir also inhibits chikungunya virus replication. They found that sofosbuvir was three times more selective in inhibiting Chikungunya virus (CHIKV) production and also was 25% less cytotoxic, in human hepatoma cells, than ribavirin. CHIKV is a member of the Togaviridae family has a positive-sense single-stranded RNA genome and its RdRp enzyme (is coded by NsP4 gene), as with other RNA polymerases from positive-sense RNA viruses (29), has well-conserved motifs. In this regard, since coronaviruses are a family of enveloped positive-strand RNA viruses with conserved polymerase, SARS-CoV-2 RdRp is very likely to be effectively inhibited by sofosbuvir. So, we hypothesized that SARS-CoV-2 infection could also be susceptible to this drug.

Prior to the discovery of sofosbuvir, a variety of nucleoside analogs had been examined as anti-hepatitis C (HCV) treatments, but this exhibited relatively low potency. Owing to the substrate specificity of the kinases, the activation of nucleoside analogues often proceeds insufficiently. The design of sofosbuvir, avoids this slow step by building the first phosphate group into the structure of the drug during synthesis. Moreover, additional groups are attached to this phosphate (to temporarily mask the negative charges of the phosphate group, thereby) facilitating entry of the pro-drug into the infected cell (30). Although hepatic cells have the most effective system for removing sofosbuvir phosphate protection, functional assays have revealed that other cells, relevant to SARS-CoV-2 infection, also activate sofosbuvir (9,31).

One of principle cell types productively infected by CHIKV are epithelial cells and variety of non-human and human epithelial cell lines (32). As mentioned above, sofosbuvir is (metabolized to the active drug and) capable of efficient controlling of this type of virus inside the epithelial cells. Furthermore, sofosbuvir appears to be highly active against ZIKV in human neuroepithelial stem cells (24,26) Antiviral activity of sofosbuvir against Zika virus or SARS-CoV-2 depends on the (cellular uptake and) intracellular enzymatic processing of the compound (33) and correlates with the intracellular concentration of the active triphosphate metabolite of the drug. As indicated in Figure 1, active triphosphate metabolite is produced by intracellular nucleoside-diphosphate-kinases (NDKs). The question may be raised: is NDK enzyme present in alveolar epithelial type II cells and the other cells, infected by SARS-CoV-2?

NDKs, encoded by NME (also named NM23) genes, are a family of multifunctional enzymes that are evolutionarily highly conserved among different species (from bacteria to humans), are found in all cells, and their activities maintain an equilibrium between the concentrations of different nucleoside triphosphates, so are the source of RNA and DNA precursors (except ATP), CTP for lipid synthesis, UTP for polysaccharide synthesis and GTP for protein elongation, signal transduction and microtubule polymerization (34).

Using isoform-specific antibodies, Muimo R, et al. suggested that NDK-A and NDK-B are present bound to the airway epithelial membranes (31). In an interesting study on pneumocyte and hepatic cell lines, Mumtaz R, et al. hypothesized that the conflicting results of sofosbuvir-mediated virus suppression, in different infected cell lines, could be explained by differences in intracellular processing of the pro-drug, leading to different concentration of the active triphosphate metabolite (33). The extent of drug uptake and cytosolic levels of metabolizing enzymes (such as NDK), involved in the metabolic activation of sofosbuvir in the infected cells, are two main determinants. Other important enzyme is carboxylesterase 1 (CES1) which is needed for activation of sofosbuvir. The hepatic cells
intracellular stability (triphosphate of the Sofosbuvir showed an extremely high
metabolically active triphosphate metabolites within lung epithelial
cells are low, though local lung inflammation may enhance
endothelial permeability and then improve epithelial uptake
of the drug in the disease state. Which feature of sofosbuvir
give hope? That may be intracellular stability of the
triphosphate metabolite. The surprising differences in stabil-
ity of nucleoside analogue triphosphates of nucleotide ana-
logues have been reported. It has been shown that the
intracellular stability of the Sofosbuvir showed an extremely high
intracellular stability (~35 h), which was also made respon-
sible for the significant and persistent anti-HCV effect of
active drug to inhibit NS5B-polymerase (37). Babusis D,
et al. also confirmed this observation and evaluated In vitro
metabolism of sofosbuvir and ribavirin in primary human he-
patocytes (38). They showed that activation was efficient for
two investigated drugs, but the triphosphate metabolite of so-
fosbuvir persisted with a half-life of 24 h while ribavirin
triphosphate had a short initial half-life of approximately
4 h. Considering phosphatases (on the opposite side of ki-
nases) in alveolar epithelial type II cells, in accordance with
the potent antiviral activity of sofosbuvir, the above state-
ments demonstrate that the intracellular triphosphate levels
achieved following sofosbuvir administration may equal or
exceed the inhibition constant (EC₅₀) for SARS-CoV-2
RdRp. Velpatasvir, which is other inhibitor of the NS5A pro-
tein of HCV, may inhibit SARS-CoV-2 RdRp enzyme (39).

Overall, although sofosbuvir is not currently listed as
potential option of COVID-19 drug therapy, till now (https://www.elsevier.com/__data/assets/pdf_file/0007/988648/COVID-19-Drug-Therapy_Mar-2020.pdf), the evidence on the
advantageous action of this drug on viral RdRp in vitro, stability
of triphosphate drug metabolite and “the least interaction
with standard of care” constituted the rationale to perform a
trial on patients with SARS-CoV-2 infection. Moreover, sofos-
buvir is safe and well tolerated at 400 mg daily in a 24 week
therapeutic regimen (9), clinically relevant drug-drug interac-
tions are rare and its important drug interaction is with ami-
darone, which causes bradycardia (Sovaldi [Sofosbuvir]:
Prescribing information. Foster City, CA: Gilead Sciences;
2013). Sofosbuvir presents an unusually clean safety profile for a nucleotide therapeutic. The safety profile of sofosbuvir,
either in combination with Peg-IFN and RBV or in combina-
tion with ribavirin alone in all-oral regimen, was excellent,
even in cirrhotic patients. Also, In vitro, sofosbuvir exhibits
cytotoxicity, mitochondrial toxicity, or bone marrow toxicity
dosed at multiples above the effective dose (40,41).

So, we were convinced to design and run a clinical trial
to evaluate the effect of sofosbuvir 400 mg (in combination
with velpatasvir 100 mg, as add-on treatment, in addition to
standard of care) on the COVID-19. The written research
proposal was submitted to the University research coun-
cil/ethics Committee, then the approved proposal regis-
tered in the Iranian Registry of Clinical trials (code: https://www.irc.ir/trial/46790). The clinical trial is
currently in process. In agreement with (9), we advocate
generic sofosbuvir beyond treatment of HCV-infected pa-
tients. As it represents a safe and effective antiviral option,
compared to similar anti-viral agents, we encourage clinical
investigators to consider these dual-component HCV drugs,
(velpatasvir/sofosbuvir), as re-purposed treatment against
SARS-CoV-2 infection. However, we believe that this
manuscript/correspondence should be made available to
the international community as soon as possible, with the
help of this esteemed journal.

Acknowledgments
The authors gratefully acknowledge the Research Council of Ker-
manshah University of Medical Sciences.

References
1. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and
diabetes mellitus at increased risk for COVID-19 infection? Lancet
Respir Med 2020;8:e21.
2. Wan Y, Shang J, Graham R, et al. Receptor recognition by the novel
coronavirus from Wuhan: an analysis based on decade-long structural
studies of SARS coronavirus. J Virol 2020;94:1–9. https://doi.org/
10.1128/JVI.00127-20. e00127-20.
3. Gracia-Ramos AE. Is the ACE2 Overexpression a Risk Factor for
COVID-19 Infection? Arch Med Res 2020;51:345–346. https://
doi.org/10.1016/j.arcmed.2020.03.011.
4. Kabbani N, Olds JL. Does COVID19 infect the brain? If so, smokers
might be at a higher risk. Mol Pharmacol 2020;97:351–353.
5. Cao B, Wang Y, Wen D, et al. A Trial of Lopinavir-Ritonavir in
Adults Hospitalized with Severe Covid-19. N Engl J Med, 2020;
https://doi.org/10.1056/NEJMoa2001282. [Epub ahead of print].
6. Rahimi F, Abadi ATB. Practical Strategies Against the Novel Coro-
navirus and COVID-19—the Imminent Global Threat. Arch Med Res
2020;51:280–281. https://doi.org/10.1016/j.arcmed.2020.03.005.
7. Chien M, Anderson TK, Jockusch S, et al. Nucleotide Analogues as
Inhibitors of SARS-CoV-2 Polymerase. bioRxiv, 2020; https://
doi.org/10.1101/2020.03.18.997585.
8. Ju J, Li X, Kumar S, et al. Nucleotide Analogues as Inhibitors of
SARS-CoV Polymerase. bioRxiv, 2020; https://doi.org/10.1101/
2020.03.12.989186.
9. Ferreira AC, Reis PA, de Freitas CS, et al. Beyond members of the
Flaviviridae family, sofosbuvir also inhibits chikungunya virus repli-
cation. Antimicrob Agents Chemother 2019;63. https://doi.org/
10.1128/AAC.00127-20. e00127-20.
10. Elfiky AA. Anti-HCV, nucleotide inhibitors, repurposing against
COVID-19. Life Sci 2020;248:117477.
11. Rodríguez-Torres M. Sofosbuvir (GS-7977), a pan-genotype, direct-
acting antiviral for hepatitis C virus infection. Expert Rev Anti Infect
Ther 2013;11:1269–1279.
12. Amidon GL, Lennernäs H, Shah VP, et al. A theoretical basis for a
biopharmaceutic drug classification: the correlation of in vitro drug
product dissolution and in vivo bioavailability. Pharm Res 1995;12:413–420.

13. Gentile I, Marcolało AE, Buonomo AR, et al. The discovery of sofosbuvir: a revolution for therapy of chronic hepatitis C. Expert Opin Drug Discov 2015;10:1363–1377.

14. Guedj J, Pang PS, Denning J, et al. Analysis of hepatitis C viral kinetics during administration of two nucleotide analogues: sofosbuvir (GS-7977) and GS-0938. Antivir Ther 2014;19:211–220.

15. Svarovskaia E, Dvory H, Hebner C, et al. No Resistance Detected in four Phase 3 Clinical Studies in HCV Genotype 1-6 of Sofosbuvir+Ribavirin with or without Peginterferon: 1843. Hepatology 2013:58.

16. Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. N Engl J Med 2013;368:1878–1887.

17. Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. N Engl J Med 2013;368:1867–1877.

18. Naggie S, Sulkowski M, Lalezari J, et al. All-oral therapy with sofosbuvir plus ribavirin for the treatment of HCV genotype 1, 2, and 3 infection in patients co-infected with HIV (PHOTO-N-1). 21st Conference on Retroviruses and Opportunistic Infections (CROI) 2014.

19. Svarovskaia E, Dvory-Sobol H, Gontcharova V, et al. Comprehensive Resistance Testing in Patients Who Relapsed after Treatment with Sofosbuvir (GS-7977)-Containing Regimens in Phase 2 Studies: 753. Hepatology 2012:56.

20. Hebner C, Lee Y-J, Han B, et al. In Vitro Pan-genotypic and Combination Activity of Sofosbuvir (GS-7977) in Stable Replicon Cell Lines: 1875. Hepatology 2012:56.

21. Mariño Z, van Bömmel F, Forns X, et al. New concepts of sofosbuvir-based treatment regimens in patients with hepatitis C. Gut 2014;63:207–215.

22. Kirby BJ, Symonds WT, Kearney BP, et al. Pharmacokinetic, pharmacodynamic, and drug-interaction profile of the hepatitis C virus NS5B polymerase inhibitor sofosbuvir. Clin Pharmacokinet 2015;54:677–690.

23. Ferreira AC, Zaverucha-do-Valle C, Reis PA, et al. Sofosbuvir protects Zika virus-infected mice from mortality, preventing short-and long-term sequelae. Sci Rep 2017;7:1–9.

24. Bullard-Feibelman KM, Govero J, Zhu Z, et al. The FDA-approved drug sofosbuvir inhibits Zika virus infection. Antiviral Res 2017;137:134–140.

25. Sacramento CQ, De Melo GR, De Freitas CS, et al. The clinically approved antiviral drug sofosbuvir inhibits Zika virus replication. Sci Rep 2017;7:40920.

26. Onorati M, Li Z, Liu F, et al. Zika virus disrupts phospho-TBK1 localization and mitosis in human neuroepithelial stem cells and radial glia. Cell Rep 2016;16:2576–2592.

27. Xu H-T, Colby-Germinario SP, Hassounah SA, et al. Evaluation of Sofosbuvir (β-D-2’-deoxy-2’-α-fluoro-2’-β-C-methyluridine) as an inhibitor of Dengue virus replication. Sci Rep 2017;7:11–4.

28. De Freitas CS, Higa LM, Sacramento CQ, et al. Yellow fever virus is susceptible to sofosbuvir both in vitro and in vivo. PLoS Negl Trop Dis 2019;13:e0007072.

29. Cheng G, Tian Y, Doehle B, et al. In vitro antiviral activity and resistance profile characterization of the hepatitis C virus NS5A inhibitor ledipasvir. Antimicrob Agents Chemother 2016;60:1847–1853.

30. Murakami E, Tolstyk T, Bao H, et al. Mechanism of activation of PSI-7851 and its diastereoisomer PSI-7977. J Biol Chem 2010;285:34337–34347.

31. Muiño R, Crawford RM, Mehta A. Nucleoside diphosphate kinase A as a controller of AMP-kinase in airway epithelia. J Bioenerg Biomembr 2006;38:181.

32. Tang BL. The cell biology of C hikungunya virus infection. Cell Microbiol 2012:14:1354–1363.

33. Muntaz N, Jimmerson LC, Bushman LR, et al. Cell-line dependent antiviral activity of sofosbuvir against Zika virus. Antiviral Res 2017;146:161–163.

34. Boissan M, Schlattner U, Lacombe M-L. The NDPK/NME super-family: state of the art. Lab Invest 2018;98:164–174.

35. Mutch E, Nave R, McCracken N, et al. The role of esterases in the metabolism of ciclesonide to desisobutyryl-ciclesonide in human tissue. Biochem Pharmacol 2007;73:1657–1664.

36. McCracken N, Blain P, Williams F. Nature and role of xenobiotic metabolizing esterases in rat liver, lung, skin and blood. Biochem Pharmacol 1993;45:31–36.

37. Meiér C. Nucleoside diphosphate and triphosphate prodrugs—An unsolvable task? Antivir Chem Chemother 2017:25:69–82.

38. Babusis D, Curry MP, Kirby B, et al. Sofosbuvir and ribavirin liver pharmacokinetics in patients infected with hepatitis C virus. Antimicrob Agents Chemother 2018:62. https://doi.org/10.1128/AAC.02587-17.

39. Chen YW, Yiu C-PB, Wong K-Y. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CL pro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. F1000research 2020:9:129. https://doi.org/10.12688/f1000research.22457.2.

40. Lam AM, Murakami E, Espiritu C, et al. PSI-7851, a pronucleotide of β-D-2’-deoxy-2’-fluoro-2’-C-methyluridine monophosphate, is a potent and pan-genotype inhibitor of hepatitis C virus replication. Antimicrob Agents Chemother 2010;54:3187–3196.

41. Mangia A, Piazzolla V. Overall efficacy and safety results of sofosbuvir-based therapies in phase II and III studies. Dig Liver Dis 2014:46:S179–S185.