

## A SARS-CoV-2 antiviral therapy score card

Robert W. Shafer\*

Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford, CA, USA.

**Abstract:** The COVID-19 pandemic has unleashed an unprecedented effort to identify efficacious treatments for persons infected with SARS-CoV-2. As of September 2020, more than 750 completed, ongoing, or planned clinical trials of drugs intended to inhibit SARS-CoV-2 replication have been registered on the ClinicalTrials.gov or WHO International Clinical Trials Platform websites. Most of the treatments studied in these trials are repurposed licensed or investigational drugs targeting viral proteins or cellular pathways required for virus replication. The use of repurposed compounds is understandable because with the exception of monoclonal antibodies, it will be several months before novel SARS-CoV-2-specific drugs will be available for human testing. This editorial describes those compounds that I believe should be prioritized for clinical testing: *i*) viral RNA polymerase inhibitors including GS-441524, its prodrug remdesivir, and EIDD-2801; *ii*) entry inhibitors including monoclonal antibodies, ACE2 molecular decoys, and peptide fusion inhibitors; *iii*) parenteral and inhalational preparations of interferon  $\beta$  and  $\lambda$ ; and *iv*) inhibitors of host transmembrane protease serine 2 (TMPRSS2), endosomal trafficking, and pyrimidine synthesis. As SARS-CoV-2 is pandemic and as its most severe consequences result from a dysregulated immunological response to infection, the ideal therapies should be inexpensive and should be able to be administered to non-hospitalized persons at the time of their initial diagnosis.

**Keywords:** COVID-19, SARS-CoV-2, antiviral therapy

Although a SARS-CoV-2 vaccine will have the greatest impact on ending the COVID-19 pandemic, antiviral drugs are required to treat unvaccinated persons and vaccinated persons who do not develop protective immunity. Antiviral drugs for SARS-CoV-2 may also potentially be effective at treating severe endemic human coronavirus infections, MERS-CoV, and future pandemic coronaviruses. To prioritize licensed drugs and investigational compounds for COVID-19 clinical trials, it is necessary to compare their preclinical data and human pharmacokinetics. Compounds with little or no *in vitro* inhibitory activity will not be clinically efficacious while those with *in vitro* inhibitory activity may be clinically efficacious if their *in vitro* inhibition reflects physiologic conditions, sufficient inhibitory levels can be attained *in vivo*, and they are safe in humans. Regrettably, most clinical trials are studying drugs with minimal inhibitory *in vitro* activity or with a level of inhibitor activity unlikely to be attained with standard dosing. These include the chloroquine analogs, azithromycin, the HIV-1 protease inhibitors ritonavir-boosted lopinavir and darunavir, the anti-influenza drugs favipiravir, oseltamivir, and umifenovir, the antiparasitic drugs ivermectin and nitazoxanide, and the hepatitis C virus inhibitor sofosbuvir. Although there are more

than 100 clinical trials of convalescent plasma, few are randomized and/or sufficiently powered to yield meaningful quantitative results particularly as the levels of neutralizing antibodies in convalescent plasma are heterogeneous.

We have created a database to facilitate comparisons between candidate anti-SARS-CoV-2 compounds to help clinical investigators, public health officials, and funding agencies prioritize the most promising candidate drugs and investigational compounds for further development (<https://covdb.stanford.edu>) (1). The database classifies compounds according to four broad mechanisms of action: *i*) virus enzyme inhibitors; *ii*) virus entry inhibitors, *iii*) interferons, and *iv*) compounds targeting host processes. In this editorial, I describe promising compounds for the treatment of SARS-CoV-2 according to three main criteria: *i*) they act by a validated direct or indirect antiviral mechanism; *ii*) they display sub-micromolar inhibitor activity *in vitro*; and *iii*) they are likely to be safe and to have favorable pharmacokinetics in human subjects. Most of these compounds are being studied in clinical trials, although the numbers of trials for these compounds are far fewer than those for the less promising compounds described in the previous paragraph.

## Viral polymerase inhibitors

Remdesivir is a delayed chain terminator phosphoramidate prodrug of a 1'-cyano-substituted adenine C-nucleoside analogue (GS-441524) with high nanomolar SARS-CoV-2 inhibitory activity *in vitro* (2). It reduces viral replication and lung pathology in mice and rhesus macaques when administered shortly after infection (2,3). In placebo-controlled randomized clinical trial, its intravenous administration led to a significant reduction in time to recovery from 15 to 11 days ( $p < 0.001$ ) and a non-statistically significant reduction in day 14 mortality of 11.9% vs. 7.1% ( $p = 0.06$ ) (4). Ongoing trials are examining its safety and efficacy when administered subcutaneously or *via* inhalation. It has been suggested, however, that the parent compound GS-441524 may actually have multiple advantages over remdesivir including the ability to be administered orally, more favorable pharmacokinetics, and a less complicated synthesis (5). EIDD-2801 is a nucleoside analog viral mutagen, which like remdesivir has high nanomolar SARS-CoV-2 inhibitory activity (6). It reduces SARS-CoV and MERS-CoV replication and lung pathology in a mouse model and is currently being evaluated in two Phase II clinical trials.

## Entry inhibitors (monoclonal antibodies [mAbs])

More than thirty research groups have identified mAbs that neutralize virus infection by binding to the SARS-CoV-2 spike protein. These studies characterize neutralizing mAbs according to one or more of the following properties: *i*) the mAb concentrations required for virus neutralization; *ii*) the mAb sequence; *iii*) the 3-dimensional structure of the mAb bound to SARS-CoV-2 spike; and *iv*) the level of protection in animal model challenge studies. One pair of mAbs identified in these studies, REGN10933 and REGN10987 possess sub-nanomolar inhibitor activity and bind to non-overlapping ACE2-competing SARS-CoV-2 receptor binding domain epitopes (7,8). The combination also reduces virus replication and lung pathology in Syrian hamsters and rhesus macaques (9). The combination of REGN10933 plus REGN10987 and another mAb LY3819253 are in Phase III trials for the prevention and treatment of SARS-CoV-2 infection. Seven other mAbs are in Phase I trials including AZD7442, BII-196, CT-P59, JS016, SCTA01, STI-1499, and TY027. However, as of early September 2020, there are no publications describing LY3819253 or any of these other mAbs.

## Interferons

Interferon- $\alpha$ , interferon- $\beta$ , and interferon- $\lambda$  each inhibit SARS-CoV-2 by 90-99% at the relatively low concentrations of approximately 100 international units/mL (10-13). Inhalational interferon- $\alpha$  and parenteral

interferon- $\beta$  have been associated with modest reductions in disease severity and/or virus levels in two small open-label randomized clinical trials (13,14). An inhaled formulation of interferon- $\beta$  has been reported in the news to reduce the odds of developing severe disease or death in a blinded randomized control trial of 220 patients, but the study has not yet been published (SNG016; <https://www.isrctn.com/ISRCTN14241621>). There are currently four planned or ongoing Phase II or III placebo-controlled trials of parenteral or inhaled interferon- $\beta$  and four planned or ongoing Phase II placebo-controlled trials of parenteral interferon- $\lambda$ .

## Host-acting compounds

Camostat and nafamostat are serine protease inhibitors that inhibit human transmembrane protease 2 (TMPRSS2) which appears to be required to cleave the SARS-CoV-2 spike protein thus priming it for host cell fusion (15-18). Both drugs are used in Japan for the treatment of pancreatitis while nafamostat is also used as an anticoagulant and for the treatment of disseminated intravascular coagulation. Although nafamostat has > 10-fold greater TMPRSS2 enzymatic and SARS-CoV-2 antiviral inhibitory activity than camostat, it is thought to be associated with greater toxicity. Camostat is being studied in two blinded and two open label randomized controlled trials totaling about 900 patients. Nafamostat is being studied in three small randomized open label trials totaling about 200 patients.

Apilimod has been found in several drug screens to inhibit SARS-CoV-2 with high selectivity at concentrations below 100 nM (19,20). It inhibits PIKfyve, an enzyme involved in the formation of a membrane protein required for the endosomal trafficking of SARS-CoV-2 and other viruses (21,22). It has been studied in humans in several clinical trials and been found to be safe and well tolerated. A theoretical concern with the use of apilimod for treating viral infections, however, is that it may interfere with T cell antigen presentation (23). Apilimod is being studied for the treatment of mild SARS-CoV-2 infections in one randomized placebo-controlled Phase II trial.

PTC299 is an inhibitor of dihydroorotate dehydrogenase (DHODH), a rate limiting enzyme in the pyrimidine biosynthesis pathway (24). DHODH inhibitors are therapeutic targets for autoimmune diseases and viral infections (25,26). PTC299 has been found to be safe and have favorable pharmacokinetics in more than 300 human subjects. In one study, it demonstrated low nanomolar SARS-CoV-2 inhibitory activity and a high selectivity index (27). As both viral replication and cytokine overproduction depend on pyrimidine synthesis, DHODH inhibition may have a dual role in COVID-19 treatment. DHODH inhibitors are expected to be synergistic with viral polymerase inhibitors as both interfere with viral genomic copying

and transcription (26). There is one Phase II/III trial of PTC299 for patients with severe COVID-19. Three other DHODH inhibitors have been studied *in vitro* and/or are in COVID-19 clinical trials including leflunomide, brequinar, and IMU-838.

### Other compounds

Soluble recombinant human ACE2 has been studied as a treatment for acute respiratory distress syndrome (ARDS) in humans; it has also been reported to protect mice from developing SARS-CoV-1-associated ARDS (28,29). It inhibits SARS-CoV-2 spike binding at nanomolar concentrations in several cell lines (30,31). There are two ongoing Phase II trials of an intravenous commercial rhACE2 preparation. Recombinant ACE2-IgG is also highly active *in vitro* but has not yet been studied in humans (31). Likewise, two highly potent lipopeptide fusion inhibitors have been described – HR2P-EK1C4 (32) and IPB03 (33) – but are not yet being studied clinically.

Ciclesonide is an inhaled corticosteroid that may interfere with membrane trafficking by binding directly to nsp-3 or nsp-4 or indirectly through a host protein. It is one of the few compounds reported to exert evolutionary pressure on SARS-CoV-2 in that it selects for SARS-CoV-2 mutations during *in vitro* passage (34). Although it inhibits SARS-CoV-2 at low micromolar levels, higher inhibitory levels may be attained clinically through the inhalational route. Niclosamide, a licensed anti-parasitic drug with sub-micromolar activity but poor oral bioavailability, is being studied in a Phase I trial in which it will be administered intramuscularly (35).

### Conclusions

The current paradigm of drug development involves competing research laboratories and companies engaged in compound screening, drug optimization, preclinical studies, pharmacokinetics, and clinical trials. The process is vertically but not horizontally integrated. It is opaque to most stakeholders because it is often difficult to obtain the necessary data to compare different treatment approaches during their development. In this editorial, I've outlined those drugs and compounds that I believe should currently be prioritized pending the development of more targeted SARS-2-specific antivirals. The ability to horizontally integrate the development of new therapeutics is required to expedite their development during a pandemic.

### References

1. Tzou PL, Tao K, Nouhin J, Rhee SY, Hu BD, Pai S, Parkin N, Shafer RW. Coronavirus antiviral research database (CoV-RDB): an online database designed to facilitate comparisons between candidate anti-coronavirus

- Compounds. *Viruses*. 2020; 12:1006. doi:10.20944/preprints202007.0551.v1.
2. Pruijssers AJ, George AS, Schäfer A, *et al.* Remdesivir inhibits SARS-CoV-2 in human lung cells and chimeric SARS-CoV expressing the SARS-CoV-2 RNA polymerase in mice. *Cell Rep*. 2020; 32:107940.
3. Williamson BN, Feldmann F, Schwarz B, *et al.* Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. *Nature*. 2020; 585:273-276.
4. Beigel JH, Tomashek KM, Dodd LE, *et al.* Remdesivir for the treatment of Covid-19 - preliminary report [published online ahead of print. *N Engl J Med*. 2020; doi:10.1056/NEJMoa2007764.
5. Yan VC, Muller FL. Advantages of the parent nucleoside GS-441524 over remdesivir for Covid-19 treatment. *ACS Med Chem Lett*. 2020; 11:1361-1366.
6. 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:eabb5883.
7. Hansen J, Baum A, Pascal KE, *et al.* Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science*. 2020; 369:1010-1014.
8. Baum A, Fulton BO, Wloga E, *et al.* Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. *Science*. 2020; 369:1014-1018.
9. Baum A, Copin R, Ajithdoss D, *et al.* REGN-COV2 antibody cocktail prevents and treats SARS-CoV-2 infection in rhesus macaques and hamsters. *bioRxiv* 2020; doi:10.1101/2020.08.02.233320. <https://www.biorxiv.org/content/10.1101/2020.08.02.233320v1>
10. Felgenhauer U, Schoen A, Gad HH, Hartmann R, Schaubmar AR, Failing K, Drosten C, Weber F. Inhibition of SARS-CoV-2 by type I and type III interferons. *J Biol Chem*. 2020; doi: 10.1074/jbc.AC120.013788.
11. Mantlo E, Bukreyeva N, Maruyama J, Paessler S, Huang C. Antiviral activities of type I interferons to SARS-CoV-2 infection. *Antiviral Res*. 2020; 179:104811.
12. Vanderheiden A, Ralfs P, Chirkova T, *et al.* Type I and Type III IFN Restrict SARS-CoV-2 Infection of Human Airway Epithelial Cultures. *J Virol*. 2020; doi:10.1128/JVI.00985-20.
13. Zheng F, Zhou Y, Zhou Z, *et al.* SARS-CoV-2 clearance in COVID-19 patients with Novaferon treatment: a randomized, open-label, parallel-group trial. *Int J Infect Dis*. 2020; 99:84-91.
14. Hung IF, Lung KC, Tso EY, *et al.* Triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. *Lancet*. 2020; 395:1695-1704.
15. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Müller MA, Drosten C, Pöhlmann S. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell*. 2020; 181:271-280.e8.
16. Hoffmann M, Schroeder S, Kleine-Weber H, Müller MA, Drosten C, Pöhlmann S. Nafamostat mesylate blocks activation of SARS-CoV-2: new treatment option for COVID-19. *Antimicrob Agents Chemother*. 2020; 64:e00754-20.
17. Yamamoto M, Kiso M, Sakai-Tagawa Y, Iwatsuki-

- Horimoto K, Imai M, Takeda M, Kinoshita N, Ohmagari N, Gohda J, Semba K, Matsuda Z, Kawaguchi Y, Kawaoka Y, Inoue JI. The anticoagulant nafamostat potently inhibits SARS-CoV-2 S protein-mediated fusion in a cell fusion assay system and viral infection *in vitro* in a cell-type-dependent manner. *Viruses*. 2020; 12:629.
18. Ko M, Jeon S, Ryu WS, Kim S. Comparative analysis of antiviral efficacy of FDA-approved drugs against SARS-CoV-2 in human lung cells. *J Med Virol*. 2020; doi:10.1002/jmv.26397.
  19. Riva L, Yuan S, Yin X, *et al.* Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. *Nature*. 2020; doi:10.1038/s41586-020-2577-1.
  20. Bouhaddou M, Memon D, Meyer B, *et al.* The Global Phosphorylation Landscape of SARS-CoV-2 Infection. *Cell*. 2020; 182:685-712.
  21. Ou X, Liu Y, Lei X, *et al.* Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun*. 2020; 11:1620.
  22. Kang YL, Chou YY, Rothlauf PW, Liu Z, Soh TK, Cureton D, Case JB, Chen RE, Diamond MS, Whelan SPJ, Kirchhausen T. Inhibition of PIKfyve kinase prevents infection by Zaire ebolavirus and SARS-CoV-2. *Proc Natl Acad Sci U S A*. 2020; 117:20803-20813.
  23. Baranov MV, Bianchi F, Schirmacher A, van Aart MAC, Maassen S, Muntjewerff EM, Dingjan I, Ter Beest M, Verdoes M, Keyser SGL, Bertozzi CR, Diederichsen U, van den Bogaart G. The Phosphoinositide Kinase PIKfyve Promotes Cathepsin-S-Mediated Major Histocompatibility Complex Class II Antigen Presentation. *iScience*. 2019; 11:160-177.
  24. Cao L, Weetall M, Trotta C, *et al.* Targeting of hematologic malignancies with PTC299, a novel potent inhibitor of dihydroorotate dehydrogenase with favorable pharmaceutical properties. *Mol Cancer Ther*. 2019; 18:3-16.
  25. Cheung NN, Lai KK, Dai J, Kok KH, Chen H, Chan KH, Yuen KY, Kao RYT. Broad-spectrum inhibition of common respiratory RNA viruses by a pyrimidine synthesis inhibitor with involvement of the host antiviral response. *J Gen Virol*. 2017; 98:946-954.
  26. Liu Q, Gupta A, Okesli-Armlovich A, Qiao W, Fischer CR, Smith M, Carette JE, Bassik MC, Khosla C. Enhancing the antiviral efficacy of RNA-dependent RNA polymerase inhibition by combination with modulators of pyrimidine metabolism. *Cell Chem Biol*. 2020; 27:668-677.e9.
  27. Luban J, Sattler R, Muhlberger E, *et al.* The DHODH Inhibitor PTC299 Arrests SARS-CoV-2 Replication and Suppresses Induction of Inflammatory Cytokines. *bioRxiv* 2020; doi:10.1101/2020.08.05.238394. <https://www.biorxiv.org/content/10.1101/2020.08.05.238394v1>
  28. Imai Y, Kuba K, Rao S, *et al.* Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature*. 2005; 436:112-116.
  29. Khan A, Benthin C, Zeno B, *et al.* A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care*. 2017; 21:234.
  30. Monteil V, Kwon H, Prado P, *et al.* Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell*. 2020; 181:905-913.e7.
  31. Lei C, Qian K, Li T, Zhang S, Fu W, Ding M, Hu S. Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig. *Nat Commun*. 2020; 11:2070.
  32. Xia S, Liu M, Wang C, *et al.* Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Res*. 2020; 30:343-355.
  33. Zhu Y, Yu D, Yan H, Chong H, He Y. Design of Potent Membrane Fusion Inhibitors against SARS-CoV-2, an Emerging Coronavirus with High Fusogenic Activity. *J Virol*. 2020; 94:e00635-20.
  34. Matsuyama S, Kawase M, Nao N, Shirato K, Ujike M, Kamitani W, Shimojima M, Fukushi S. The inhaled steroid ciclesonide blocks SARS-CoV-2 RNA replication by targeting viral replication-transcription complex in culture cells. *bioRxiv* 2020; doi:10.1101/2020.08.22.258459. <https://www.biorxiv.org/content/10.1101/2020.08.22.258459v1>
  35. Jeon S, Ko M, Lee J, Choi I, Byun SY, Park S, Shum D, Kim S. Identification of antiviral drug candidates against SARS-CoV-2 from FDA-approved drugs. *Antimicrob Agents Chemother*. 2020; 64:e00819-20.
- 
- Received September 1, 2020; Revised September 7, 2020; Accepted September 13, 2020.
- Released online in J-STAGE as advance publication September 20, 2020.
- \*Address correspondence to:*  
 Robert W. Shafer, Division of Infectious Diseases, Department of Medicine, Stanford University, Stanford, 1000 Welch Rd Ste 202, Palo Alto, CA 94304, USA.  
 E-mail: rshafer@stanford.edu