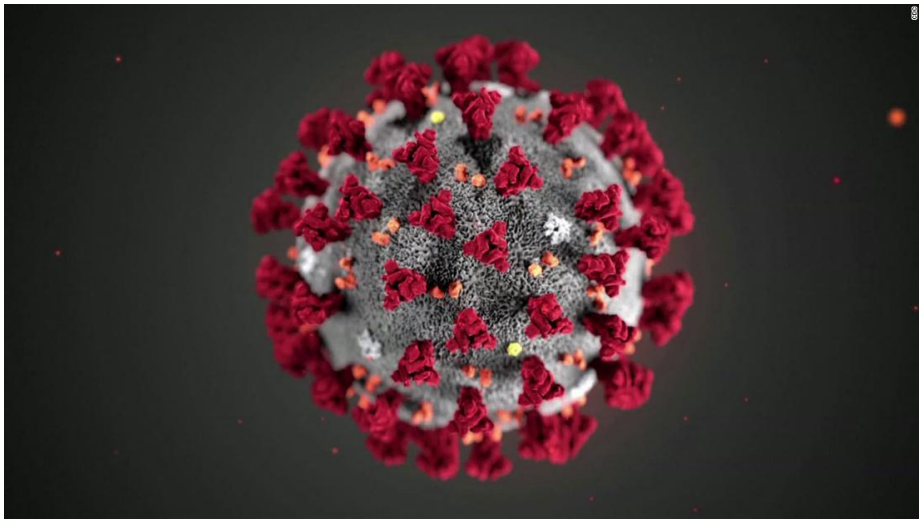
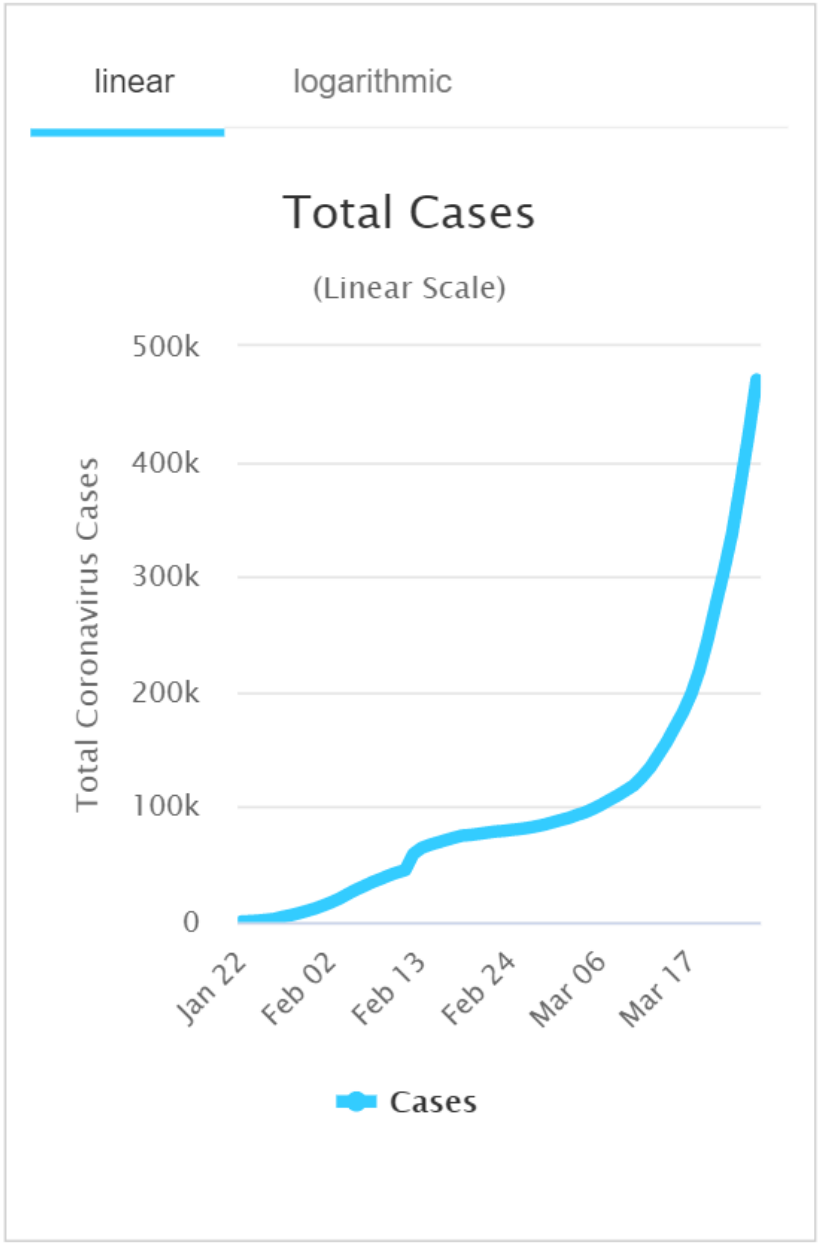
SARS-CoV-2, COVID-19, and Potential Small Molecule Therapeutics

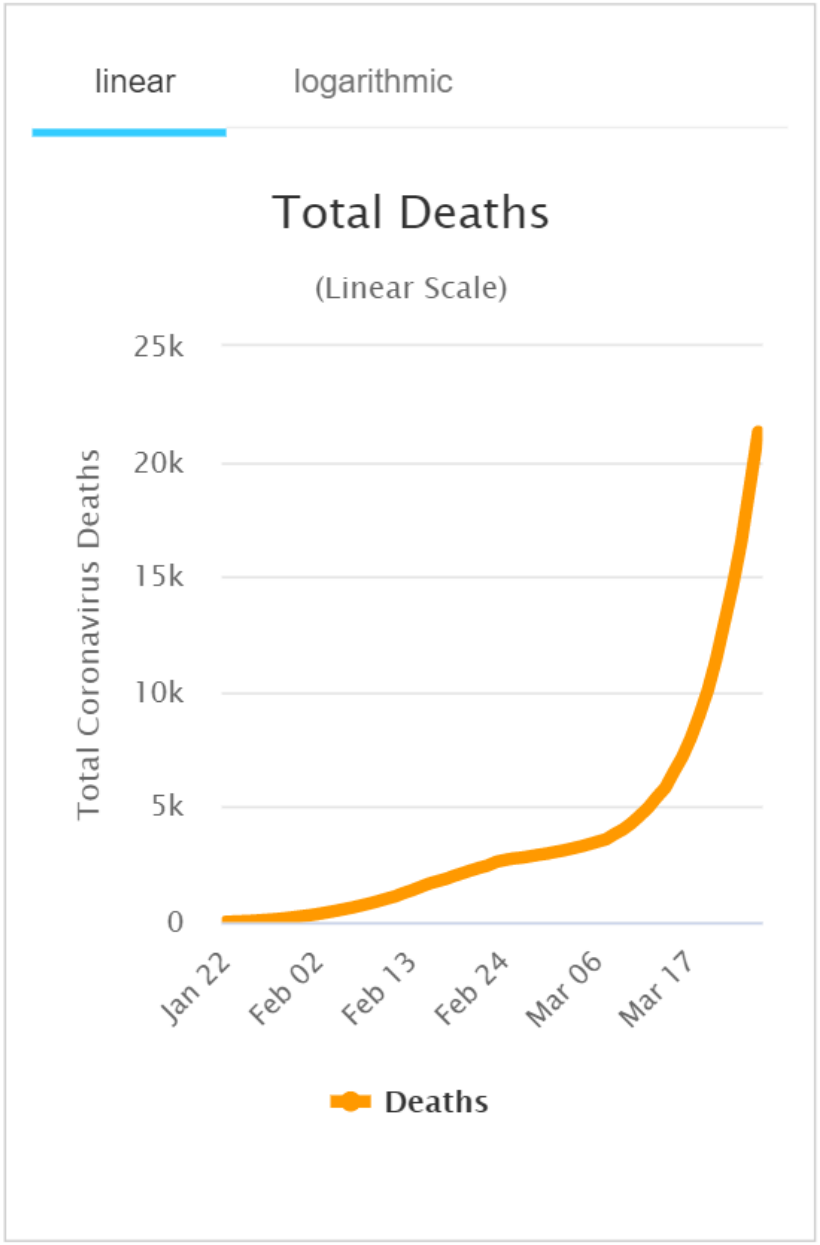


Matt Epplin

Burns Lab Group Meeting

03/27/20



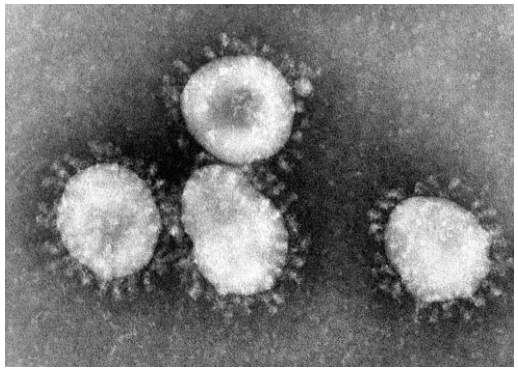


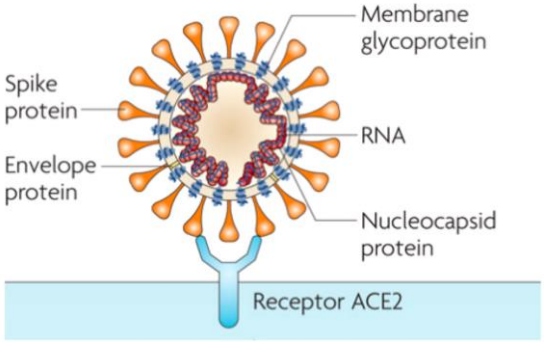
https://www.worldometers.info/coronavirus/#countries

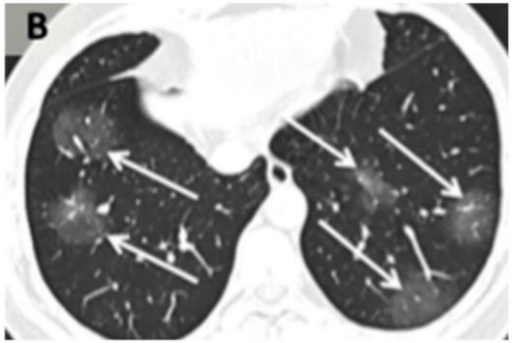
- ***SARS-CoV-2***: the Genbank name for the causative agent (virus) behind the illness
  - Stands for Severe Acute Respiratory Syndrome – CoronaVirus – 2
  - Shares 96% sequence homology with the SARS-CoV of the early 2000s
  - Originally designated 2019-nCoV
  - Same class as Middle East Respiratory Syndrome – CoronaVirus (MERS-CoV)
- ***COVID-19***: the name for the disease caused by SARS-CoV-2

Zhou, P.; *et al*. *Nature*, **2020**, *579*, 270-273.

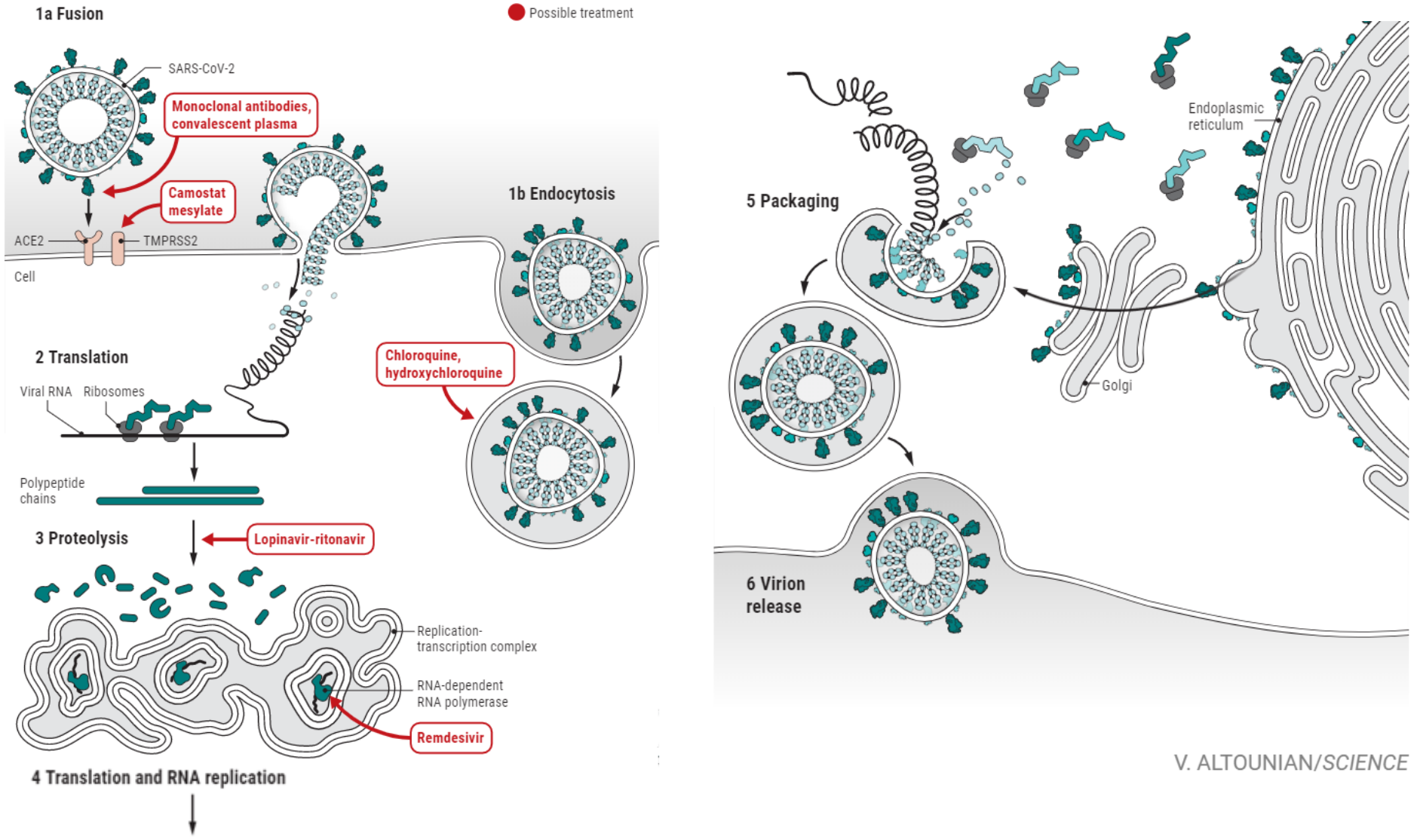
- Large (~30k base pairs), single-stranded positive-sense RNA virus
- Encapsulated by membrane envelope
  - Contains “spike” (S) glycoproteins, giving the crown-like appearance
- Four subtypes: alpha, beta, gamma, and delta
  - Beta-class includes SARS-CoV, MERS-CoV, and SARS-CoV-2
- Beta CoVs attack lower respiratory system causing viral pneumonia
  - Appear to also infect heart, liver, kidney, and gastrointestinal system
- Leads to death in ~1-2% of cases showing symptoms





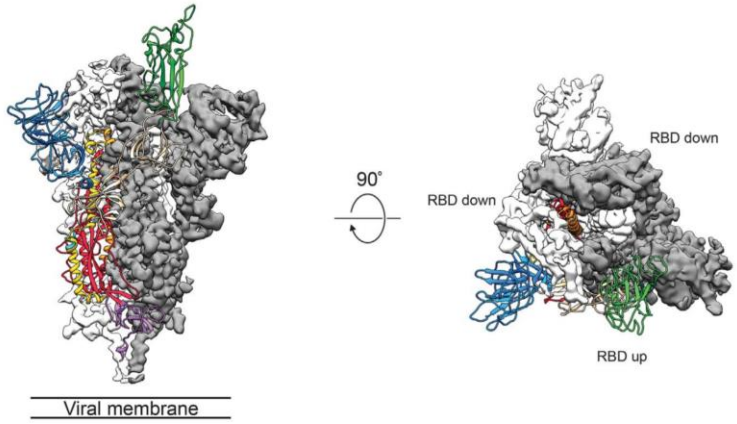


Fehr, A.R.; *et al. Methods Mol. Biol.*, **2015**, 1-23.
Wu, J.T.; *et al. Nature Medicine*, **2020**, doi: https://doi-org.stanford.idm.oclc.org/10.1038/s41591-020-0822-7.
Liu, C.; *et al. ACS Cent. Sci.*, doi: https://dx.doi.org/10.1021/acscentsci.0c00272 (2020).



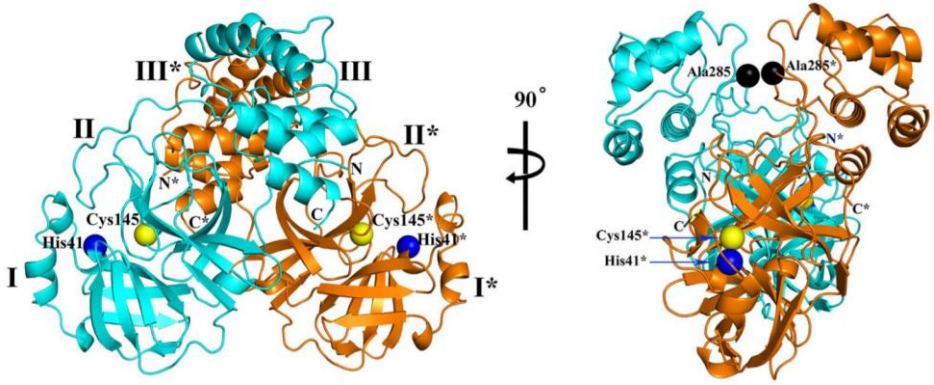
Kupferschmidt, K.; *et al*. *Science*, **2020**, *367*, 1412-1413.

- Structural “spike” (S) protein mediates host cell invasion via angiotensin-converting enzyme 2 (ACE2)



S protein crystal structure

- Non-structural RNA-dependent RNA polymerase (RdRp), coronavirus main protease (3CLpro), and papain-like protease (RLpro) then assist in viral replication

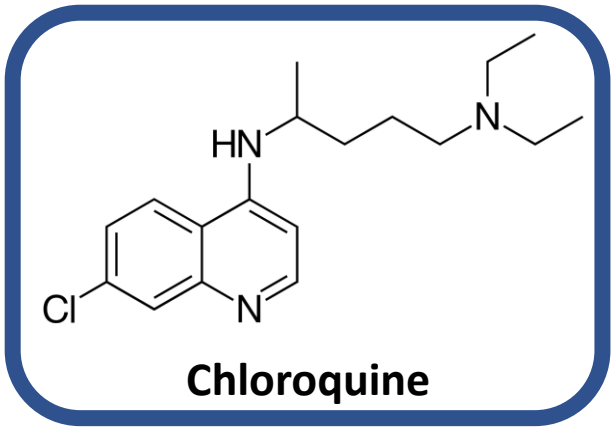


3CLpro crystal structure

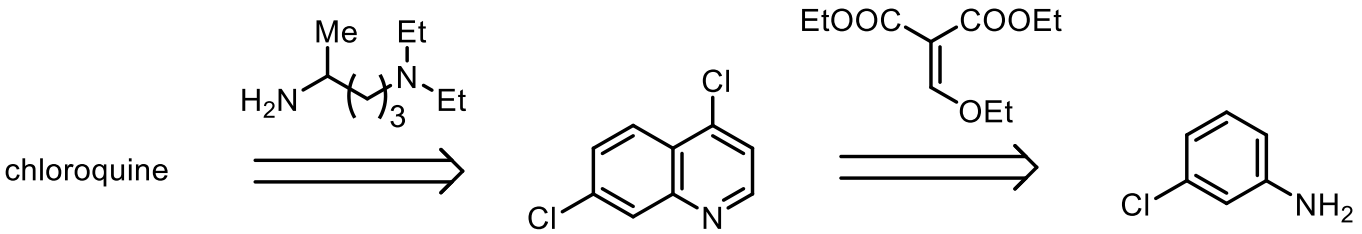
Zhang, L.; *et al*. *Science*, 10.1126/science.abb3405 (2020).
Wrapp, D.; *et al*. *Science*, **2020**, *367*, 1260-1263.

- Antibodies - Dosing external antibodies already targeted at part of virus
  - Immunoglobulin therapy
    - Infusing the plasma (containing the antibodies) of previously sick patients into newly sick patients
    - On March 24th, FDA approved this strategy for emergency situations
  - Monoclonal antibodies
    - Several epitopes (i.e. exposed region of a protein) to target including sections of the S protein
    - Counted at least 18 currently in development
- Vaccines – Harnesses internal immune system to target virus
  - "Traditional" vaccines (e.g. compromised whole pathogens, pathogen surface protein, etc.)
    - Clinically established, but slower to develop/produce
  - RNA/DNA vaccines
    - Injection of RNA/DNA of antigen so cells directly produce antibodies
    - Fast and scalable, but no FDA-approved therapies

- Member of the 4-aminoquinolone class of anti-malarials
  - First synthesized in the 1930s as a derivative of quinine
  - Most widely used anti-malarial historically



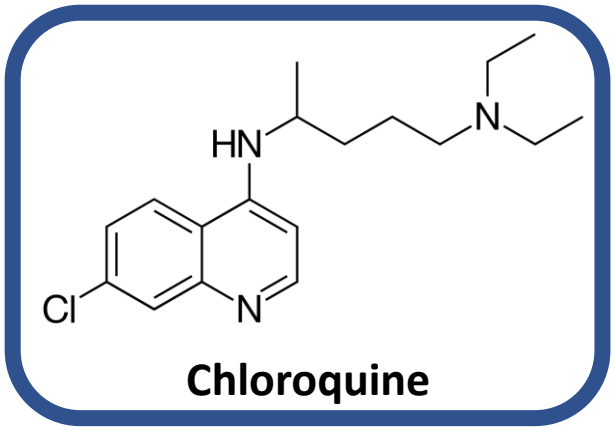
- Synthesized industrially from 3-chloroaniline

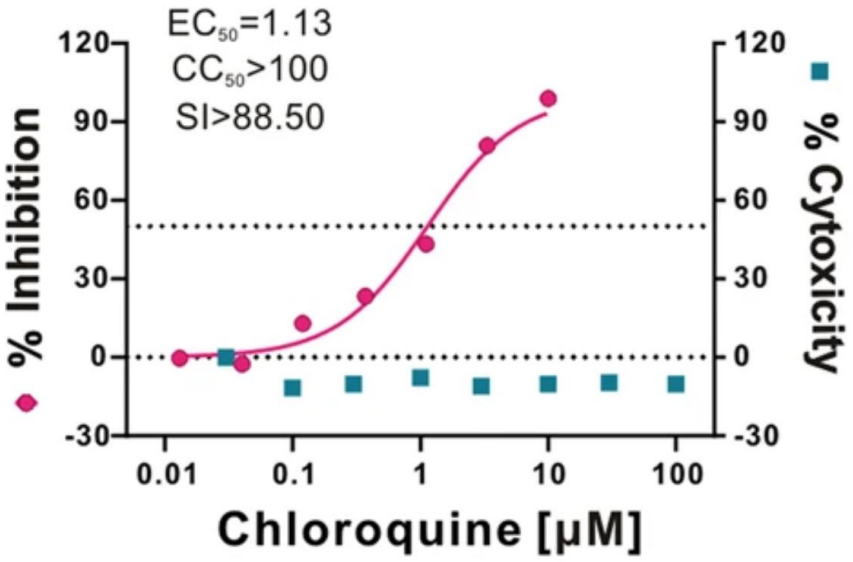


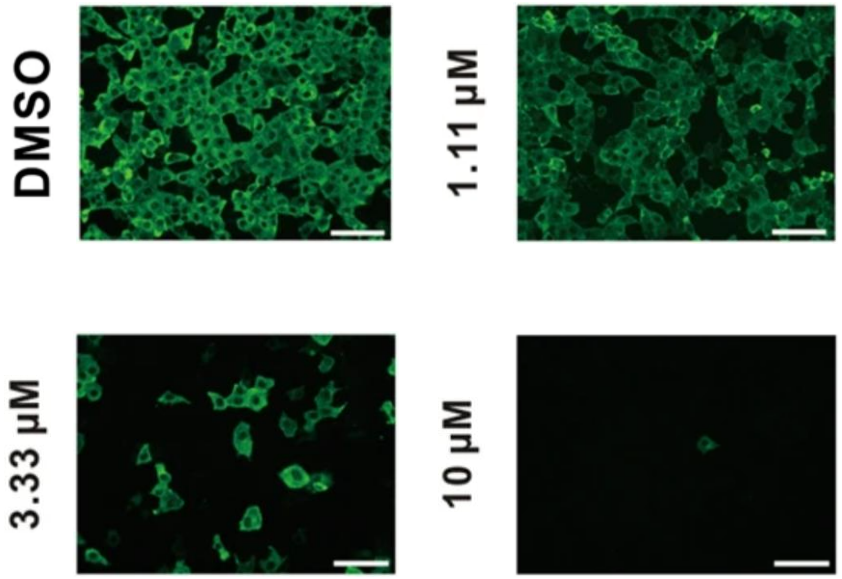
- Mechanism of action still unclear
  - Well-established to accumulate in lysosomes
    - Increase in lysosome pH prevents viral release
  - Known to bind purine and disrupt DNA/RNA synthesis
  - Also binds zinc, increasing conc. intracellularly, inhibiting RNA polymerase

Schrezenmeier, E.; *et al*. *Nat. Rev. Rheum.*, **2020**, *16*, 155-166.
Vardanyan, R. S.; *et al*. *Synth. Ess. Drugs*, **2006**, 559-582.
Xue, J.; *et al*. *PLoS ONE* **2014**, *9*, e109180.
te Velthuis, A. J.; *et al*. *PLoS One* **2010**, *6*, e1001176.

- Chinese group screened FDA-approved drugs against SARS-CoV-2 *in vitro*
  - Found chloroquine to be 1 µM inhibitor of SARS-CoV-2 infected Vero E6 cells

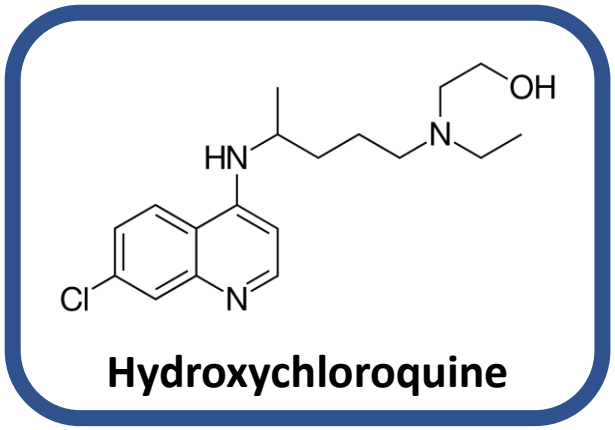




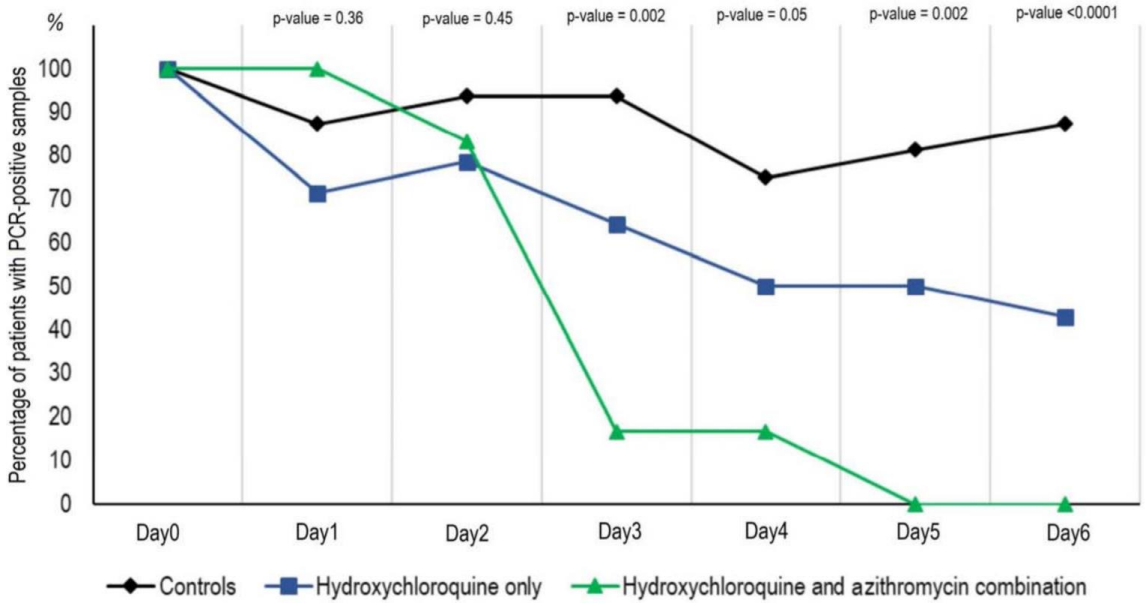


Wang, M.; *et al*. *Nat. Cell Res.*, **2020**, *30*, 269-271.

- French group ran open-label, non-randomized phase II trial in 36 patients
  - Used hydroxychloroquine (HCQ) due to clinical outcomes/availability
  - Several issues: 1) small trial 2) open-label 3) pass-fail criteria 4) treatment-group dropouts



Hydroxychloroquine



- Chinese group ran 30 patient study where 13/15 in HCQ group recovered after 7 days while 14/15 in control recovered

Gautret, P.; *et al. Intl. J. Antimicrobial Agents*, In Press 17 March **2020**.
Chen, J.; *et al. J. Zhej. Univ.* In Press 3 March **2020**.

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Trial record **33 of 94** for: hydroxychloroquine | Recruiting, Not yet recruiting, Active, not recruiting, Enrolling by invitation Studies

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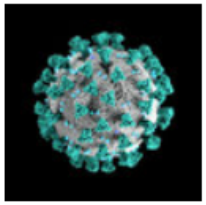
Hydroxychloroquine Chemoprophylaxis in Healthcare Personnel in Contact With COVID-19 Patients (PHYDRA Trial) (PHYDRA)



Coronavirus Live Updates

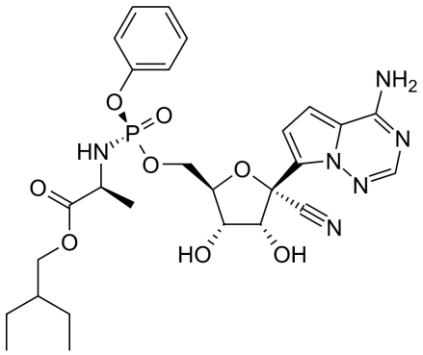
THE CORONAVIRUS CRISIS

Man Dies, Woman Hospitalized After Taking Form Of Chloroquine To Prevent COVID-19

March 24, 2020 · 4:20 AM ET

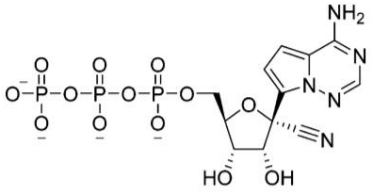
SCOTT NEUMAN

- *C*-nucleotide prodrug originally developed in 2016 by Gilead for Ebola virus
  - Inhibits viral replication by incorporating into RNA and interfering with RdRp (RNA polymerase)
  - Highly conserved across viruses
    - UNC group showed MERS-CoV replication in human lung 2B4 cells inhibited at ~30 nM remdesivir *in vitro* (right)



Remdesivir

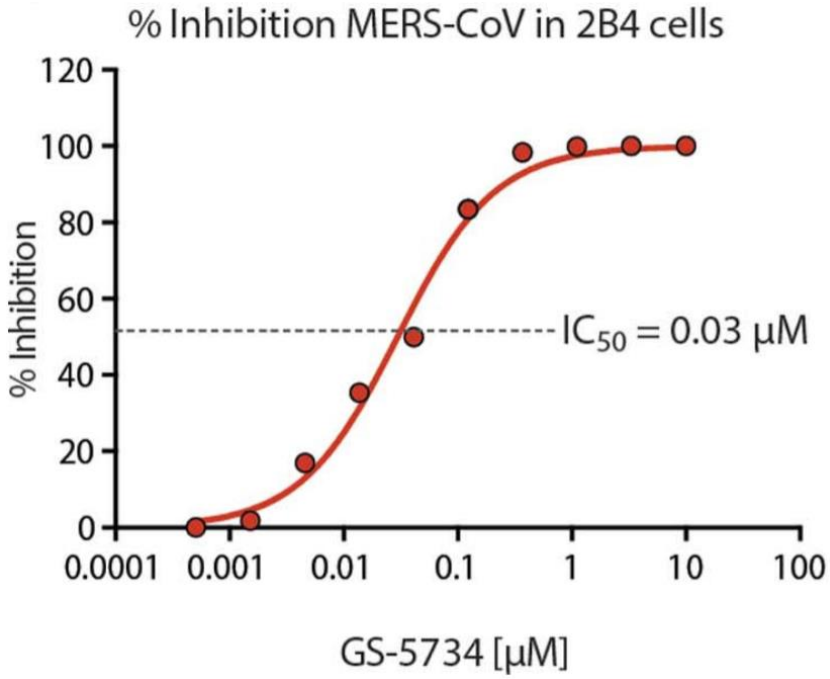
Table 2. Inhibition of RSV Polymerase, HCV Polymerase, and Human Polymerases by 4tp



4tp

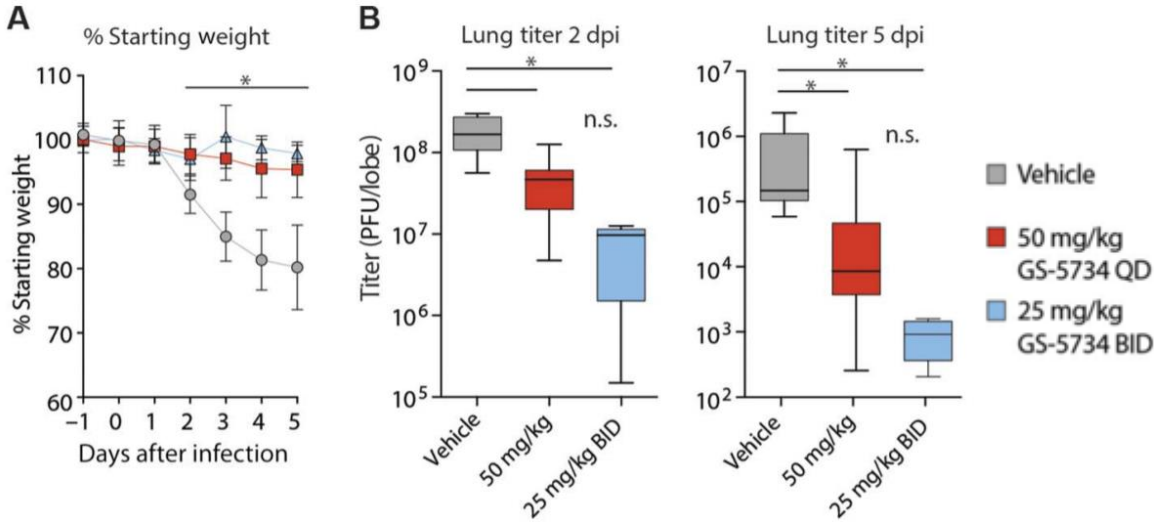
enzyme	**4tp** IC_{50} (μM)	**4tp** SNI[a] rate (%)
RSV RdRp	1.1	
HCV RdRp	5	
POLRMT	>200	6
RNA Pol II	>200	
DNA Pol α	>200	
DNA Pol β	>200	
DNA Pol γ	>200	0

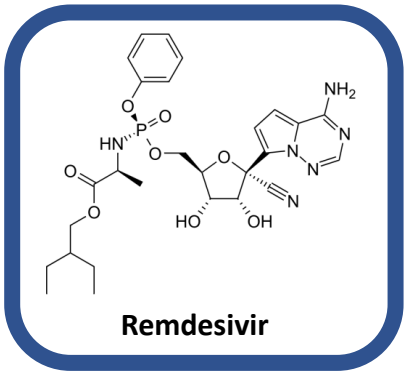
[a]SNI = single nucleotide incorporation.



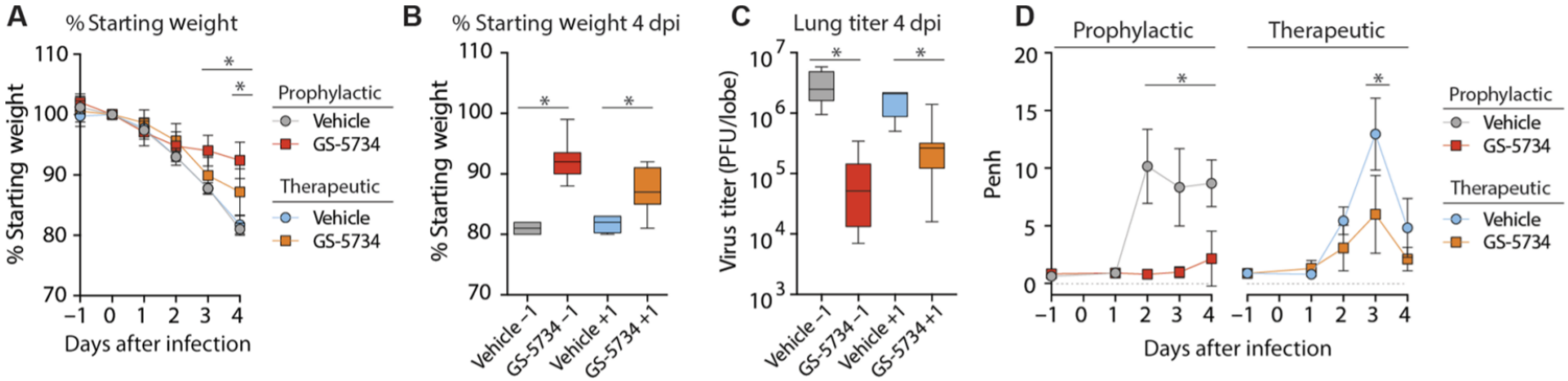
Siegel, D.; *et al. J. Med. Chem.* **2017**, *60*, 1648-1661.
Sheahan, T. P.; *et al. Sci. Trans. Med.* **2017**, *9*, eaal3653.

- Demonstrated efficacy prophylactically in mice *in vivo*





- And (maybe less convincingly) therapeutically

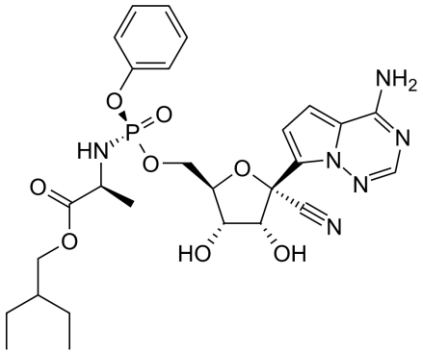


Sheahan, T. P.; *et al*. *Sci. Trans. Med.*, **2017**, *9*, eaal3653.

RNA Polymerase Inhibitors – Remdesivir

In vivo data promising enough to instigate several phase II and III clinical trials

1. Two sponsored by Gilead
   - Phase III, unblinded, no control (1), open-label, March
2. Two sponsored by China-Japan Friendship Hospital
   - Phase III, blinded, placebo controlled, February
3. One sponsored by the NIH
   - Phase II, blinded, placebo controlled, adaptive, February
4. One sponsored by INSERM (French NIH)
   - Phase III, unblinded, SoC controlled, adaptive, March



Remdesivir

Partly Stanford located!

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ClinicalTrials.gov

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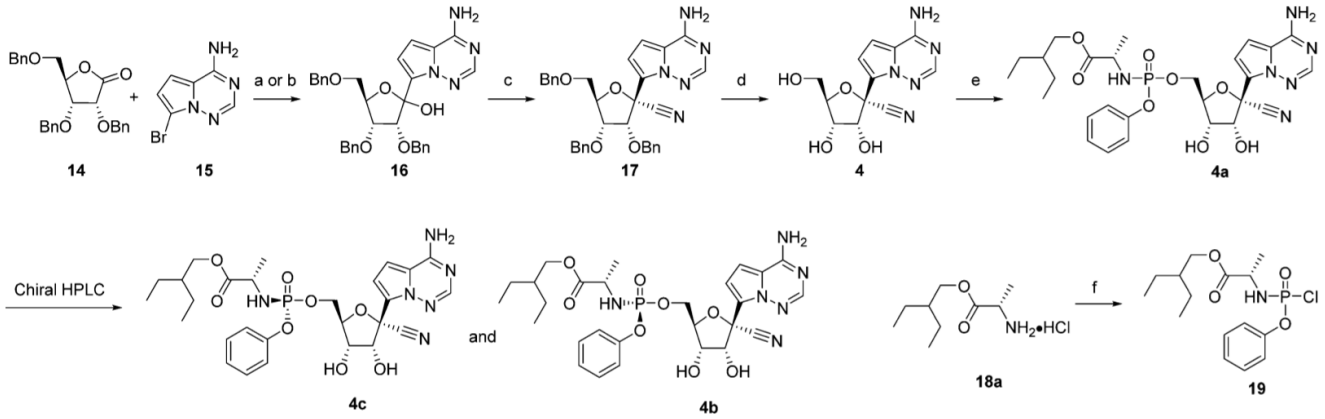
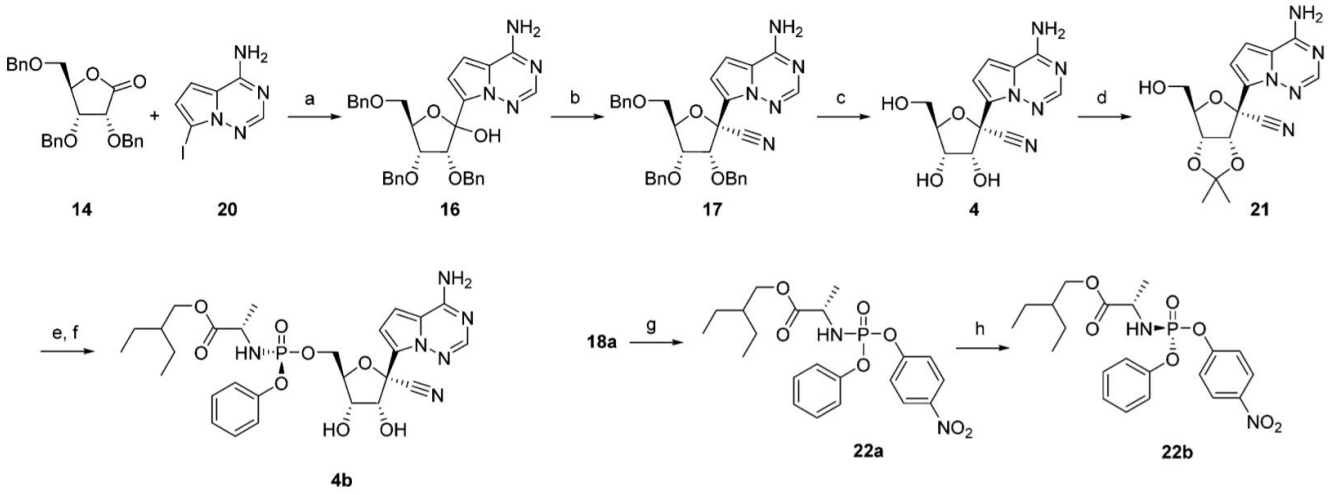
Row	Saved	Status	Study Title	Conditions	Interventions
1	☐	Recruiting NEW	Study to Evaluate the Safety and Antiviral Activity of **Remdesivir** (GS-5734™) in Participants With Severe Coronavirus Disease (COVID-**19**)	• COVID-**19**	• Drug: **Remdesivir** • Drug: Standard of Care
2	☐	Recruiting NEW	Study to Evaluate the Safety and Antiviral Activity of **Remdesivir** (GS-5734™) in Participants With Moderate Coronavirus Disease (COVID-**19**) Compared to Standard of Care Treatment	• COVID-**19**	• Drug: **Remdesivir** • Drug: Standard of Care
3	☐	Recruiting	Adaptive COVID-**19** Treatment Trial (ACTT)	• Corona Virus Infection	• Other: Placebo • Drug: **Remdesivir**
4	☐	Not yet recruiting NEW	Trial of Treatments for COVID-**19** in Hospitalized Adults	• Corona Virus Infection	• Drug: **Remdesivir** • Drug: Lopinavir/ritonavir • Drug: Interferon Beta-1A • Other: Standard of care
5	☐	Recruiting NEW	Adverse Events Related to Treatments Used Against Coronavirus Disease 2019	• Coronavirus • Iatrogenic Disease • Acute Kidney Injury • ARDS, Human	• Drug: Any drug used to treat Covid-**19**

Scheme 1. First Generation Synthesis of 4b[a]



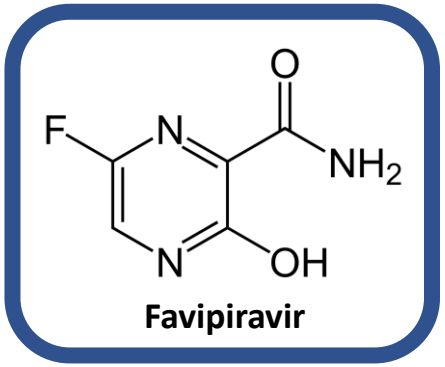
[a]Reagents and conditions: (a) *n*-BuLi, (TMS)Cl, THF, − 78 °C, 25%; (b) 1,2-bis(chlorodimethylsilyl)ethane, NaH, *n*-BuLi, THF, − 78 °C, 60%; (c) (TMS)CN, $BF_3{\cdot}Et_2O$, CH_2Cl_2, − 78 °C, 58% (89:11β-**17**/α); (d) BCl_3, CH_2Cl_2, − 78 °C, 74%; (e) **19**, NMI, $OP(OMe)_3$, 21%; (f) $OP(OPh)Cl_2$, Et_3N, CH_2Cl_2, 0 °C, 23%.

Scheme 2. Second Generation Synthesis of 4b[a]

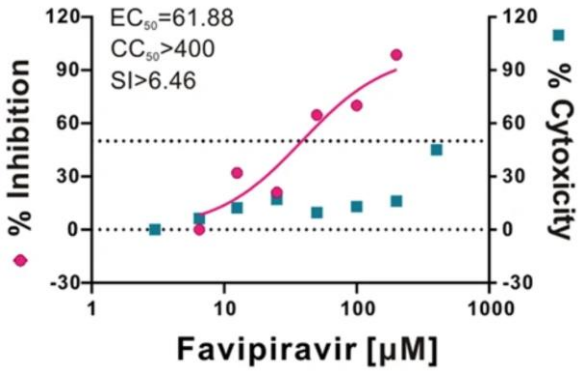


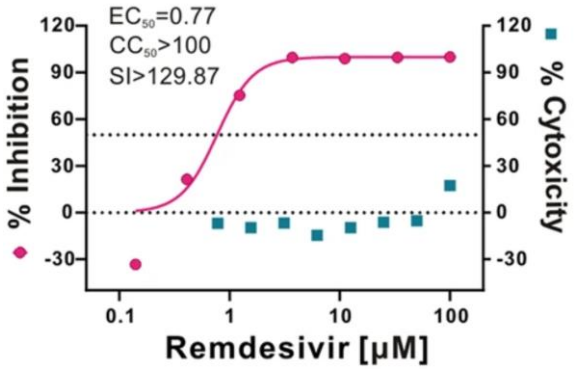
[a]Reagents and conditions: (a) TMSCl, PhMgCl, *i*-PrMgCl·LiCl, THF, − 20 °C, 40%; (b) TMSCN, TfOH, TMSOTf, CH_2Cl_2, − 78 °C, 85%; (c) BCl_3, CH_2Cl_2, − 20 °C, 86%; (d) 2,2-dimethoxypropane, H_2SO_4, acetone, rt, 90%; (e) **22b**, $MgCl_2$, $(i\text{-Pr})_2NEt$, MeCN, 50 °C, 70%; (f) 37% HCl, THF, rt, 69%; (g) $OP(OPh)Cl_2$, Et_3N, CH_2Cl_2, − 78 °C, then 4-nitrophenol, Et_3N, 0 °C, 80%; (h) $i\text{-Pr}_2O$, 39%.

- Another RNA polymerase inhibitor developed by Toyama Pharmaceuticals
- Unlike remdesivir, not potent inhibitor (62 µM) of SARS-CoV-2 *in vitro*



Favipiravir

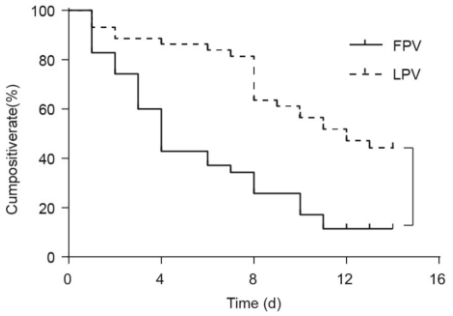




- However, significant improvement over Arbidol and Kaletra (effectively controls)

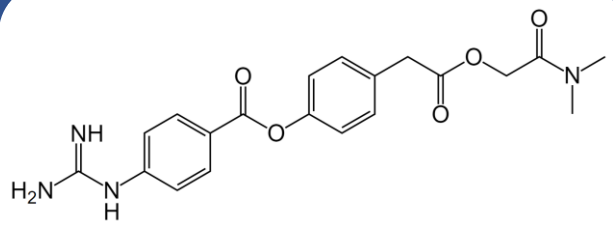
Table 2. Comparison of 7 day's clinical recovery rate of favipiravir and arbidol in COVID-19 patients.

Variables	Favipiravir group	Arbidol group	Rate ratio (95% CI)	P value
Total patients	**(N = 116)**	**(N = 120)**		0.1396
Recovered, n (%)	71 (61.21)	62 (51.67)	0.0954 (-0.0305, 0.2213)	
Ordinary patients	**(N = 98)**	**(N = 111)**		
Recovered, n (%)	70 (71.43)	62 (55.86)	0.1557 (0.0271, 0.2843)	0.0199
Critical patients	**(N = 18)**	**(N = 9)**		
Recovered, n (%)	1 (5.56)	0 (0.00)	0.0556 (-0.0503, 0.1614)	0.4712
Patients with hypertension and/or diabetes	**(N = 42)**	**(N = 35)**		
Recovered, n (%)	23 (54.76)	18 (51.43)	0.0333 (-0.1904, 0.2571)	0.7704



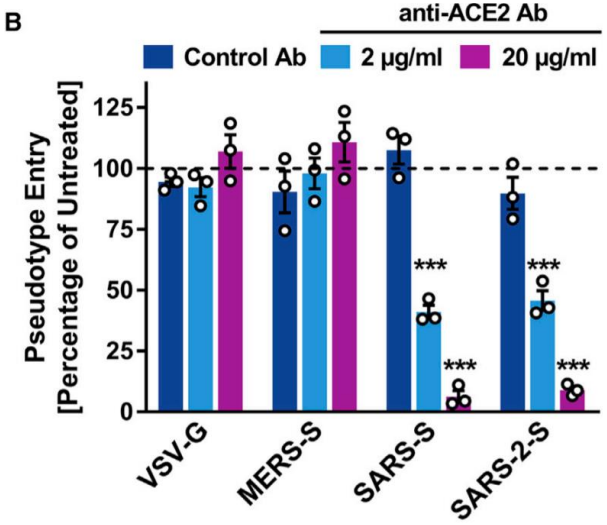
Wang, M.; *et al*. *Nat. Cell Res.*, **2020**, *30*, 269-271.
Chen, C.*; Huang, J.; *et al*. medRxiv preprint doi: https://doi.org/10.1101/2020.03.17.20037432.
Cai, Q.; *et al*. *Engineering*, https://doi.org/10.1016/j.eng.2020.03.007.

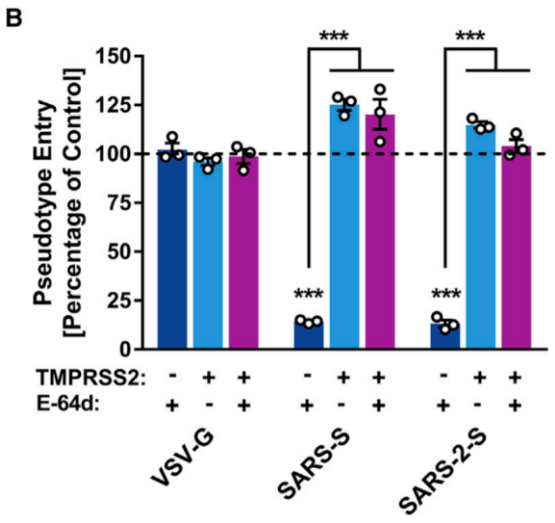
- Camostat is a serine protease inhibitor approved in Japan for chronic pancreatitis
  - Known to have actions on TMPRSS2 protease



Camostat

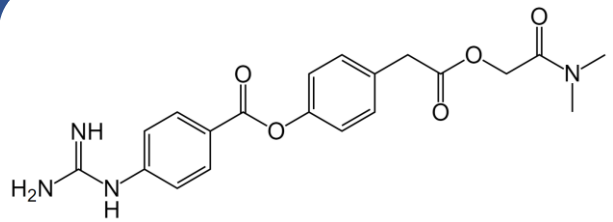
- Recent paper examined SARS-CoV-2 cellular entry
  - Demonstrated uses ACE2 (analogous to SARS-CoV) for binding to cell surface
  - And SARS-CoV-2 spike (S) protein requires priming by TMPRSS2 for binding



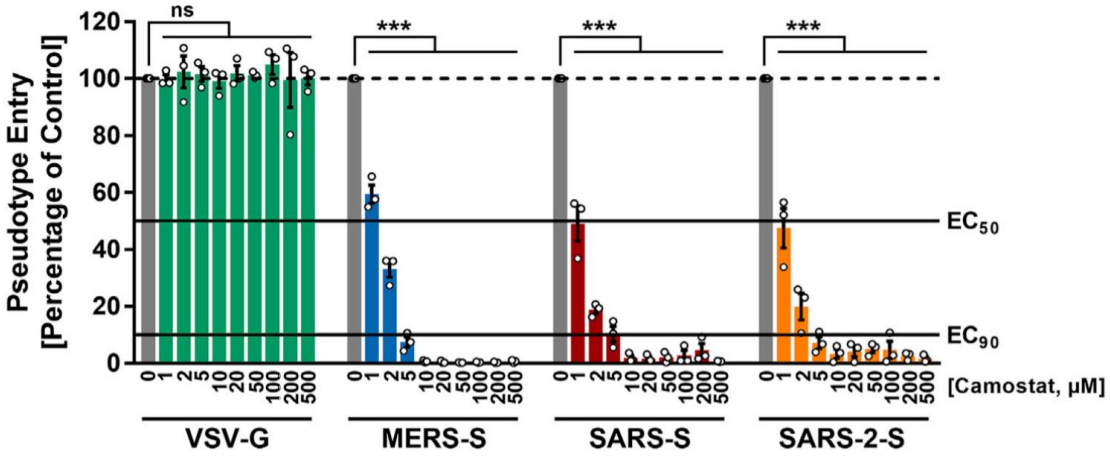


Hoffmann, M.; *et al*. *Cell*, **2020**, *181*, 1-10.

- Hypothesis being that blocking TMPRSS2 activity could prevent viral entry
  - Spike protein of SARS-CoV-2 would not be properly primed for entry



Camostat



- Clinical trial in Denmark started March 25th
  - Randomized/blinded/placebo-controlled

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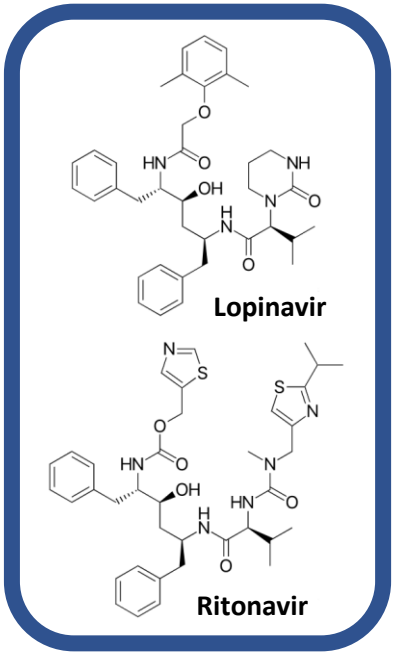
Row	Saved	Status	Study Title	Conditions	Interventions
1	☐	Not yet recruiting NEW	The Impact of Camostat Mesilate on COVID-19 Infection	• Corona Virus Infection	• Drug: Camostat Mesilate • Drug: Placebo oral tablet

Hoffmann, M.; *et al*. *Cell*, **2020**, *181*, 1-10.

- Both lopinavir/ritonavir (Kaletra, Abbvie) and darunavir (J&J) approved for treatment and prevention of HIV/AIDS
- Act as HIV-1 protease inhibitors
  - However, little to no conservation between CoV and HIV proteases
  - Would have to hypothesize there were other unknown mechanisms of action inhibiting viruses



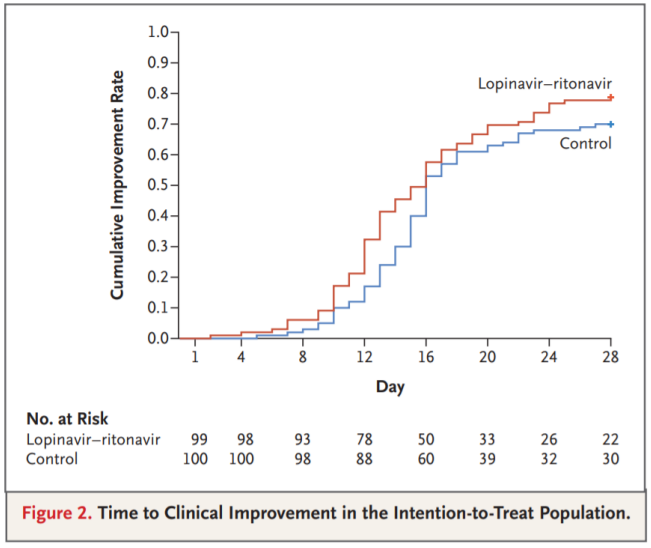
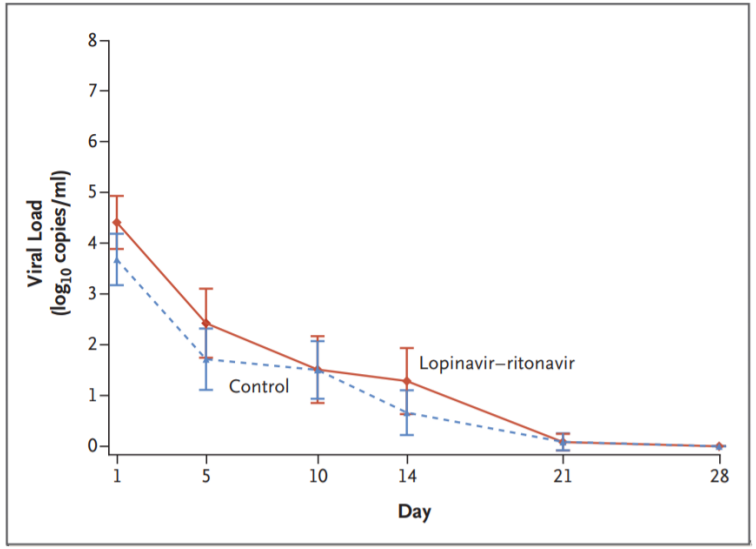
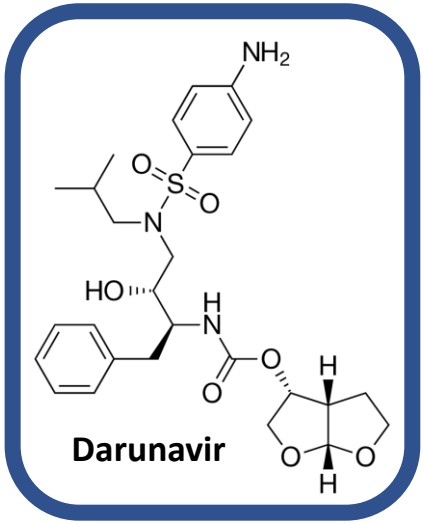
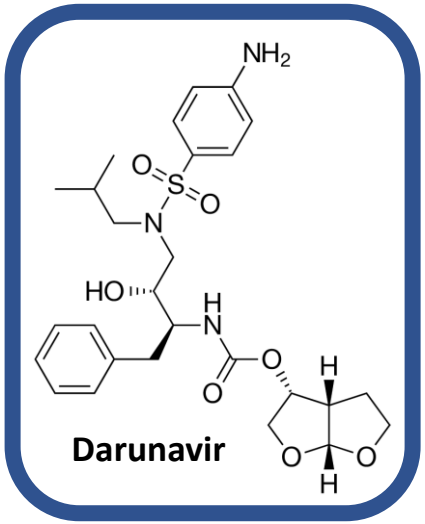


Figure 2. Time to Clinical Improvement in the Intention-to-Treat Population.



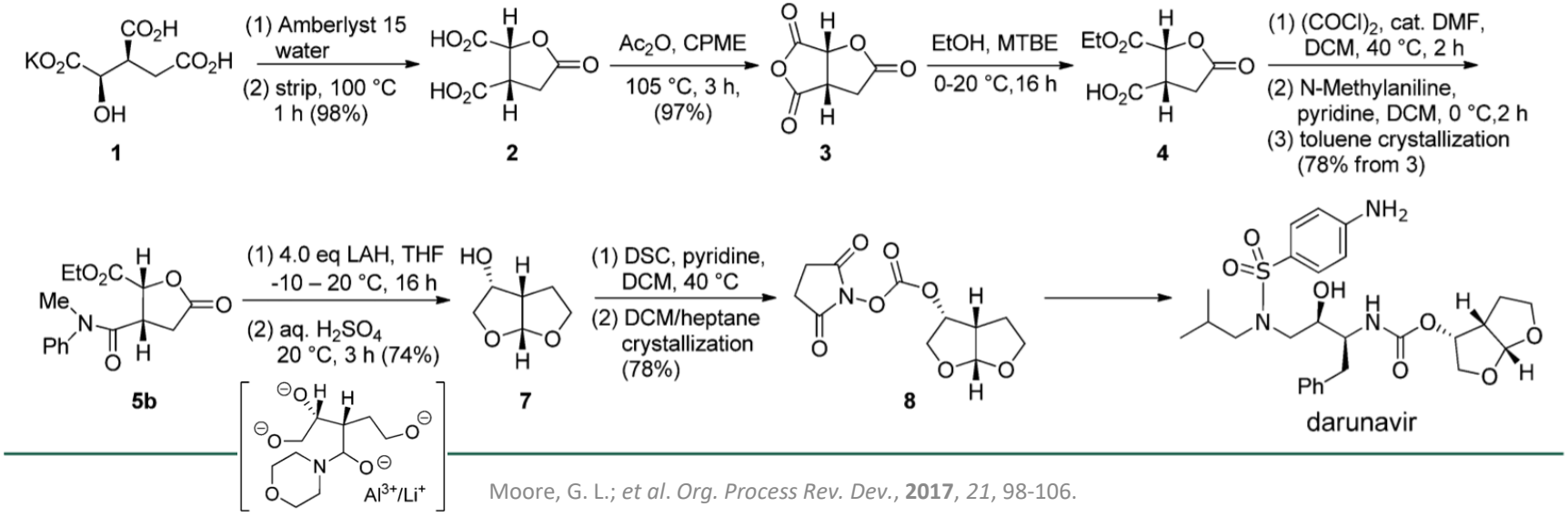


Cao, B.; *et al*. *NEJM*, DOI: 10.1056/NEJMoa2001282 (2020).



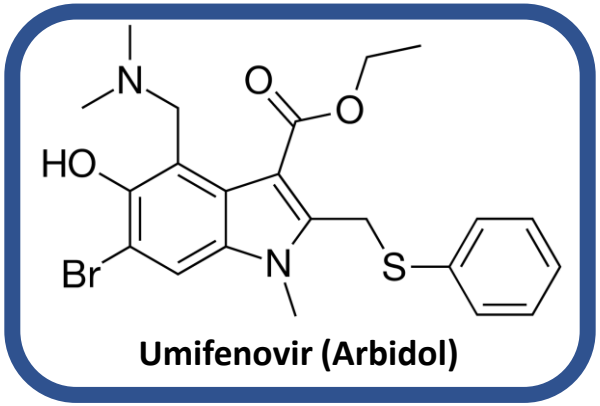
Organic Process Research & Development Article

Scheme 2. Optimization of the Synthetic Process from Isocitrate to (3*R*,3a*S*,6a*R*)-Hexahydrofuro[2,3-*b*]furan-3-ol

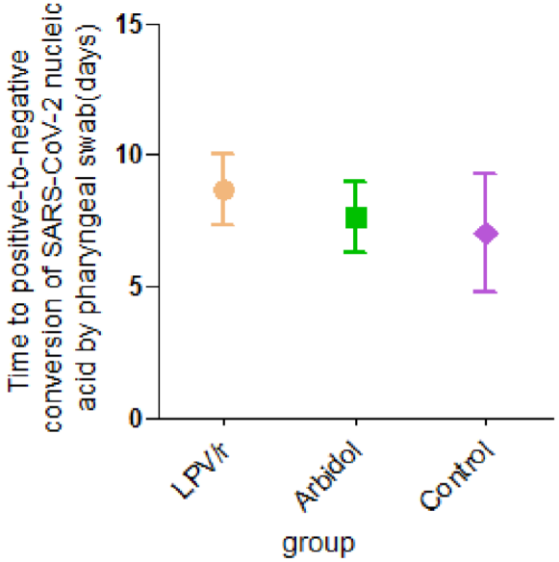


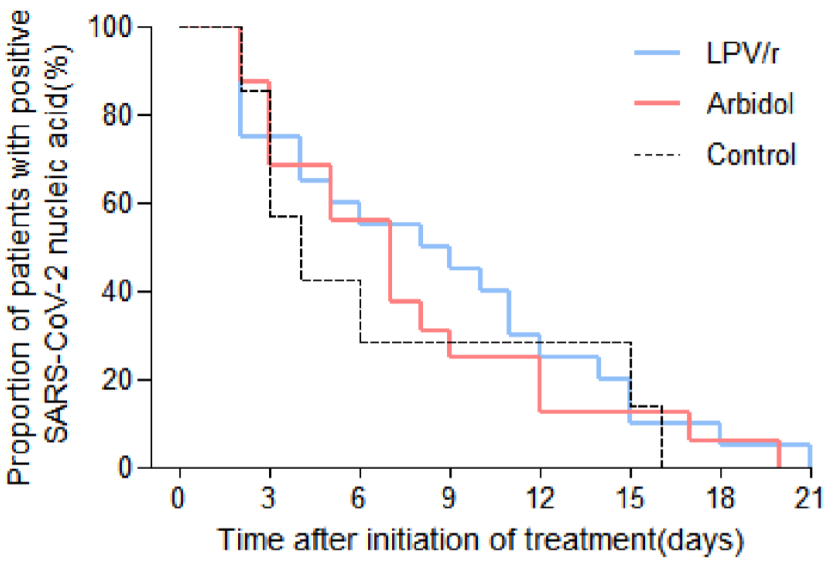
Moore, G. L.; *et al. Org. Process Rev. Dev.*, **2017**, *21*, 98-106.

- Oral influenza drug approved in Russia and China
  - Some evidence it affects viral entry, but MoA generally unknown
- And limited clinical evidence it's effective in humans for flu
  - Also appears to be ineffective against SARS-CoV-2



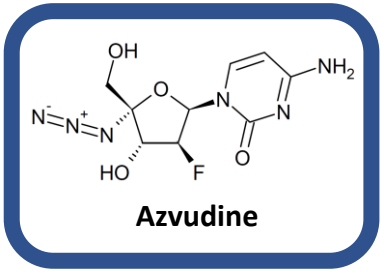
Umifenovir (Arbidol)



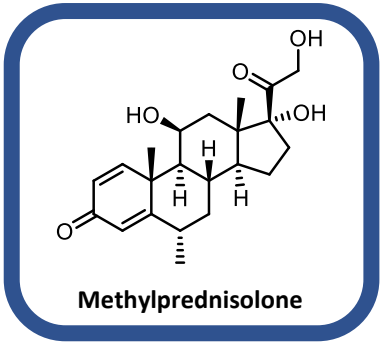


Li Y.*; Xie, Z.*; Lin, W.*; Cai W.*; *et al*. medRxiv preprint doi: https://doi.org/10.1101/2020.03.19.20038984 (2020).

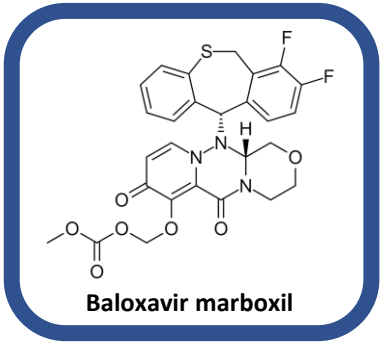
- Azvudine
  - Nucleoside reverse transcriptase inhibitor (NRTI)
  - CoVs not known to contain or use reverse transcriptase so not much hope here
- Methylprednisolone
  - Steroid-based nuclear receptor inhibitor
  - Some evidence anti-inflammatory effects can improve mortality in patients with severe pneumonia
  - Would just be managing symptoms
- Baloxavir marboxil
  - Prodrug approved for influenza as a cap-dependent endonuclease inhibitor
  - Found nothing suggesting endonuclease conserved between SARS-CoV-2 and influenza
- Oseltamivir (Tamiflu)
  - Prodrug approved for influenza as neuraminidase inhibitor
  - Ineffective against SARS



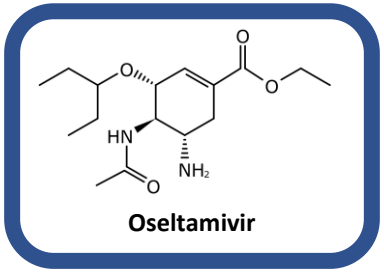
Azvudine



Methylprednisolone



Baloxavir marboxil



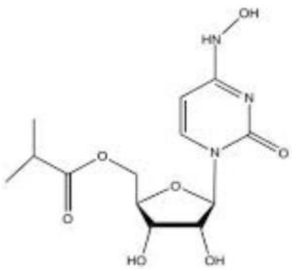
Oseltamivir

Stern, A.; *et al*. *Cochrane Database Syst. Rev.*, **2017**, *12*, CD007720.

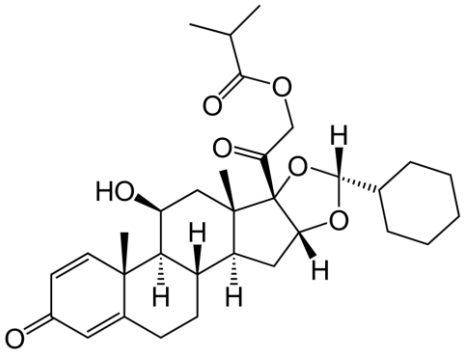
- Overall, some early evidence suggesting certain classes of small molecules could be effective against SARS-Cov-2
  - However, all trials have been very small (<50 people) to this point
- Data compelling enough (HCQ, remdesivir, favipiravir?) to follow up with larger trials
  - Already ongoing
- Additional trials and production will take significant amounts of time
  - Likely months at an absolute minimum
  - Will this pandemic be past us by the time a useful therapeutic hits the market?
- Of course, no one knows how long this will last (or if it will reappear) so it's possible longer-term efforts could pay future dividends

- EIDD-2801
  - RNA polymerase inhibitor (similar to Remdesivir)
  - Can be taken orally, but safety profile not established like Remdesivir so a long way to go
  - Starting phase I clinical trials “within weeks”

EIDD-2801



- Ciclesonide
  - FDA-approved glucocorticoid for asthma
  - Recent BioRxiv suggesting it targets viral protein nsp15, involved in RNA replication
  - One clinical trial started in Japan



https://cen-acs-org.stanford.idm.oclc.org/biological-chemistry/infectious-disease/Emory-discovered-antiviral-poised-COVID/98/i12

Matsuyama, S.; *et al*. *bioRxiv* **doi:** https://doi.org/10.1101/2020.03.11.987016 (2020).