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

• 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

Liu, C.; et al. ACS Cent. Sci., doi: https://dx.doi.org/10.1021/acscentsci.0c00272 (2020).
SARS-CoV-2 and COVID-19 Background

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

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

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

• 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

- Chloroquine

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

Hydroxychloroquine Chemoprophylaxis in Healthcare Personnel in Contact With COVID-19 Patients (PHYDRA Trial) (PHYDRA)
Man Dies, Woman Hospitalized After Taking Form Of Chloroquine To Prevent COVID-19

March 24, 2020 · 4:20 AM ET
• 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)

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

<table>
<thead>
<tr>
<th>enzyme</th>
<th>4tp IC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>4tp SNI* rate (%)</th>
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<td>RSV RdRp</td>
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<td>POLRMT</td>
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<tr>
<td>DNA Pol α</td>
<td>&gt;200</td>
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</tr>
<tr>
<td>DNA Pol β</td>
<td>&gt;200</td>
<td></td>
</tr>
<tr>
<td>DNA Pol γ</td>
<td>&gt;200</td>
<td>0</td>
</tr>
</tbody>
</table>

*SNI = single nucleotide incorporation.

• Demonstrated efficacy prophylactically in mice *in vivo*

![](image)

• And (maybe less convincingly) therapeutically

![](image)
**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

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**Remdesivir**

RNA Polymerase Inhibitors – Remdesivir

Partly Stanford located!
RNA Polymerase Inhibitors – Remdesivir

Scheme 1. First Generation Synthesis of 4b

Reagents and conditions: (a) n-ButLi, (TMS)Cl, THF, $-78^\circ C$, 25%; (b) 1,2-bis(chlorodimethylsilyl)ethane, NaH, n-ButLi, THF, $-78^\circ C$, 60%; (c) (TMS)CN, BF$_3$-Et$_2$O, CH$_2$Cl$_2$, $-78^\circ C$, 58% (89:11/$\beta$-$17/\alpha$); (d) BCl$_3$, CH$_2$Cl$_2$, $-78^\circ C$, 74%; (e) 19, NMI, OP(OMe)$_3$, 21%; (f) OP(OPh)Cl$_2$, Et$_3$N, CH$_2$Cl$_2$, 0 $^\circ C$, 23%.

Scheme 2. Second Generation Synthesis of 4b

Reagents and conditions: (a) TMSCl, PhMgCl, i-PrMgCl-LiCl, THF, $-20^\circ C$, 40%; (b) TMSCN, TfOH, TMSOTf, CH$_2$Cl$_2$, $-78^\circ C$, 85%; (c) BCl$_3$, CH$_2$Cl$_2$, $-20^\circ C$, 86%; (d) 2,2-dimethoxypropane, H$_2$SO$_4$, acetone, rt, 90%; (e) 22b, MgCl$_2$, (i-Pr)$_2$NEt, MeCN, 50 $^\circ C$, 70%; (f) 37% HCl, THF, rt, 69%; (g) OP(OPh)Cl$_2$, Et$_3$N, CH$_2$Cl$_2$, $-78^\circ C$, then 4-nitrophenol, Et$_3$N, 0 $^\circ C$, 80%; (h) i-Pr$_2$O, 39%.
• Another RNA polymerase inhibitor developed by Toyama Pharmaceuticals

• Unlike remdesivir, not potent inhibitor (62 μM) of SARS-CoV-2 in vitro

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

Chen, C.*; Huang, J.; et al. medRxiv preprint doi: https://doi.org/10.1101/2020.03.17.20037432
Camostat is a serine protease inhibitor approved in Japan for chronic pancreatitis
  - Known to have actions on TMPRSS2 protease

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

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

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

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.

Organic Process Research & Development

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

- 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

• **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
• 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”

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