2020-08-10

Welcome to Week 21!

Live opera is coming back! Not in the US but in several places in Europe. Here is American soprano Lisette Operosa (I had the pleasure of meeting her several years ago here in DC when she gave song recital) in Madrid singing 'addio del passato' from Verdi's La Traviata at the end of last month in Madrid: https://www.youtube.com/watch?v=2ldOBson0vw The ovation at the end of the aria was well deserved and she then starts in with the second verse that is customarily deleted in modern performances. For my loyal readers here is Ms. Operosa in the Act 1 'Sempre libera.'

Of course it had to happen at the Georgia school with packed hallways and very few masks being worn. The picture of the hallway went viral and as The Washington Post reports, nine people are now positive for COVID-19. Apartments and condos are changing a lot of things to deal effectively with the coronavirus. Here is an unflattering picture of vaccine company Inovio; it is never good when corporate leaders sell stock right after a big run up. How about all these college students who are coming into towns even though learning will be remote; will they follow public health guidelines and not put citizens who live there full time at risk? No masks in St. Tropez results in……yes, COVID-19 infections. Cases of COVID-19 rose sharply in the second half of July.

Is FDA Commissioner Stephen Hahn caught between a rock and a hard place? The New York Times weights in. Here is an interactive showing what happens to viral particles on the NYC subway.

STAT have a story on a possible Dickensian winter if COVID-19 is not brought to heel.

Something is either wrong with the preprint servers or all COVID-19 research has stopped. I suspect it is the former as I have not seen any new posts in the past two days. I assume this will all get ironed out and there will be a deluge of new papers over the next couple of days. Because of this, and the fact medical journals do not publish over the weekend, the reading for today is sparse as you can see.

MODELING

- Mandates for mask use in public during the recent COVID-19 pandemic, worsened by global shortage of commercial supplies, have led to widespread use of homemade masks and mask alternatives. It is assumed that wearing such masks reduces the likelihood for an infected person to spread the disease, but many of these mask designs have not been tested in practice. We have demonstrated a simple optical measurement method to evaluate the efficacy of masks to reduce the transmission of respiratory droplets during regular speech. In proof-of-principle studies, we compared a variety of commonly available mask types and observed that some mask types approach the performance of standard surgical masks, while some mask alternatives, such as neck fleece or bandanas, offer very little protection. Our measurement setup is inexpensive and can be built and operated by non-experts, allowing for rapid evaluation of mask performance during speech, sneezing, or coughing. [**note: thanks to a loyal reader for this link! Good DIY work from these Duke researchers. Some good data here but download the PDF to see it in a more user-friendly manner. I was pleased to see that my fashion mask did quite**

well in this testing.]
https://advances.sciencemag.org/content/early/2020/08/07/sciadv.abd3083

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to explore the effectiveness of processed human amniotic fluid as a treatment for COVID-19. Past use of human amniotic products (i.e., membrane and fluid) has previously been FDA-approved as a human cells, Tissues, and Cellular and Tissue-Based Products (HCT/P) under 21 CFR 1271 for tissue injury; and has been used to reduce inflammation and fibrosis in patients with a variety of ailments. Given this, we hypothesize that intravenously (IV) administered processed sterile filtered amniotic fluid will reduce inflammation in COVID-19 patients, and improve secondary clinical outcomes. Specifically, we hypothesize that patients who receive IV administered hAF will see a 50% reduction in mean C-reactive protein levels following treatment. [**note: I really don't understand the rationale of this trial. If it does work, is there enough human amniotic fluid for broad scale treatment?**] NCT04497389

CLINICAL TRIAL RESULTS

- Nothing

DRUG DEVELOPMENT

- Nothing

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing

DIAGNOSTIC DEVELOPMENT

- Nothing.

>>

2020-08-11

Here is another live performance from Europe. French Soprano Julie Fuchs sings Deh vieni non tardar from Mozart's Marriage of Figaro. This is from last month with the National Opera Orchestra of Paris conducted by Philippe Jordan: https://www.youtube.com/watch?v=XSzO4QAyGjE

I thought that China would be the first country to license a COVID-19 vaccine but according to The Washington Post, Russia claim to have won the vaccine race. It's an adenovirus vector based vaccine and has not been tested in a large phase 3 study so it's curious why they would claim victory particularly as others are also working on the same approach. There is also a nice article on masks that references the Duke study I posted yesterday. Do watch the video as it is interesting. Alas, New Zealand's long streak of no COVID-19 cases has come to an end. However, if any country is able to do good track and tracing New Zealand should be the one.

The New York Times also reports on the Russian vaccine, saying it has been approved by the regulators there. Here is an interactive article inside one Houston Texas hospital. Is it safe to stay at a hotel? Hilton are partnering with Lysol to make sure; I'm not so sure.

The Guardian is documenting US healthcare workers whose lives were lost working to treat patients. A significant number were young.

STAT have an interesting article on whether monoclonal antibody treatment will have a major role to play in the COVID-19 pandemic. Lots of government money being spent on vaccine development but not mAbs. There is an article on UCSF research into nanobodies (also see the abstract below on the Chinese effort in this area).

The Lancet published a correspondence on the need for open public data standards and sharing in light of COVID-19. This has long been a problematic area in biomedical research necessitating a lot of work to create standard data models. This letter discusses digital health and COVID-19, a riff on the above letter.

Medscape have some comments from Moncef Slaoui, the head of the government's vaccine program, on the progress of getting a vaccine licensed. I find it interesting that an experimental use authorization will NOT be pursued but full licensure will be sought. Here is an article on chronic fatigue which may be one of the long term effects of COVID-19.

Annals of Internal Medicine have a commentary on the VA's efforts with virtual care during the pandemic.

Looks like some preprints now coming up on the server!!! Hurray!

MODELING

- Nothing new

NEWLY REGISTERED CLINICAL TRIALS

- Did not look

CLINICAL TRIAL RESULTS

- Unfortunately, no new results.

DRUG DEVELOPMENT

- A phase 2 trial conducted to further evaluate the immunogenicity and safety of a SARS-CoV-2 inactivated vaccine (CoronaVac). METHODS We conducted a randomized, double-blind, placebo-controlled trial to evaluate the optimal dose, immunogenicity and safety of the CoronaVac. A total of 600 healthy adults aged 18-59 years were randomly assigned to receive 2 injections of the trial vaccine at a dose of 3 μg/0.5 mL or 6 μg /0.5mL, or placebo on Day 0,14 schedule or Day 0,28 schedule. For safety evaluation, solicited and unsolicited adverse events were collected after each vaccination within 7 days and 28 days, respectively. Blood samples were taken for antibody assay. RESULTS CoronaVac was well tolerated, and no dose-related safety concerns were observed. Most of the adverse reactions fell in the solicited category and were mild in

severity. Pain at injection site was the most frequently reported symptoms. No Grade 3 adverse reaction or vaccine related SAEs were reported. CoronaVac showed good immunogenicity with the lower 3 μg dose eliciting 92.4% seroconversion under Day 0,14 schedule and 97.4% under Day 0,28 schedule. 28 days after two-dose vaccination, the Nab levels of individual schedules range from 23.8 to 65.4 among different dosage and vaccination schedules. CONCLUSIONS Favorable safety and immunogenicity of CoronaVac was demonstrated on both schedules and both dosages, which support the conduction of phase 3 trial with optimum schedule/dosage per different scenarios. [**note: this is the Sinovac vaccine from China and it looks pretty good in terms of immune response. It's an inactivated virus grown in Vero cells with aluminum hydroxide as the adjuvant. This is pretty established technology.**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20161216v1

- The ongoing COVID-19 pandemic is associated with substantial morbidity and mortality. While much has been learned in the first months of the pandemic, many features of COVID-19 pathogenesis remain to be determined. For example, anosmia is a common presentation and many patients with this finding show no or only minor respiratory signs. Studies in animals experimentally infected with SARS-CoV-2, the cause of COVID-19, provide opportunities to study aspects of the disease not easily investigated in human patients. COVID-19 severity ranges from asymptomatic to lethal. Most experimental infections provide insights into mild disease. Here, using K18-hACE2 mice that we originally developed for SARS studies, we show that infection with SARS-CoV-2 causes severe disease in the lung, and in some mice, the brain. Evidence of thrombosis and vasculitis was detected in mice with severe pneumonia. Further, we show that infusion of convalescent plasma (CP) from a recovered COVID-19 patient provided protection against lethal disease. Mice developed anosmia at early times after infection. Notably, while treatment with CP prevented significant clinical disease, it did not prevent anosmia. Thus K18-hACE2 mice provide a useful model for studying the pathological underpinnings of both mild and lethal COVID-19 and for assessing therapeutic interventions. [**note: these Iowa researchers have a mouse model for COVID-19 study. Interesting that convalescent plasma prevented lung disease but not anosmia.]**
https://www.biorxiv.org/content/10.1101/2020.08.07.242073v1
- We here reported Nanobody (Nb) phage display libraries derived from four camels immunized with the SARS-CoV-2 spike receptor-binding domain (RBD), from which 381 Nbs were identified to recognize SARS-CoV-2-RBD. Furthermore, seven Nbs were shown to block interaction of human angiotensin converting enzyme 2 (ACE2) with SARS-CoV-2-RBD-variants, bat-SL-CoV-WIV1-RBD and SARS-CoV-1-RBD. Among the seven candidates, Nb11-59 exhibited the highest activity against authentic SARS-CoV-2 with ND50 of 0.55 μg/mL. Nb11-59 can be produced on a large-scale in Pichia pastoris, with 20 g/L titer and 99.36% purity. It also showed good stability profile, and nebulization did not impact its stability. Overall, Nb11-59 might be a promising prophylactic and therapeutic molecule against COVID-19, especially through inhalation delivery. [**note: from China, isolation of a high neutralizing nanobody that might be useful in inhalation delivery. A marked advantage is the ease of production using a genetically engineered yeast. IIRC, Phillips 66 were one of the first companies to engineer this yeast strain.**]
https://www.biorxiv.org/content/10.1101/2020.08.09.242867v1
- SARS-CoV-2 is responsible for COVID-19, resulting in the largest pandemic in over a hundred years. After examining the molecular structures and activities of hepatitis C viral inhibitors and

comparing hepatitis C virus and coronavirus replication, we previously postulated that the FDA-approved hepatitis C drug EPCLUSA (Sofosbuvir/Velpatasvir) might inhibit SARS-CoV-2. We subsequently demonstrated that Sofosbuvir triphosphate is incorporated by the relatively low fidelity SARS-CoV and SARS-CoV-2 RNA-dependent RNA polymerases (RdRps), serving as an immediate polymerase reaction terminator, but not by a host-like high fidelity DNA polymerase. Other investigators have since demonstrated the ability of Sofosbuvir to inhibit SARS-CoV-2 replication in lung and brain cells; additionally, COVID-19 clinical trials with EPCLUSA and with Sofosbuvir plus Daclatasvir have been initiated in several countries. SARS-CoV-2 has an exonuclease-based proofreader to maintain the viral genome integrity. Any effective antiviral targeting the SARS-CoV-2 RdRp must display a certain level of resistance to this proofreading activity. We report here that Sofosbuvir terminated RNA resists removal by the exonuclease to a substantially higher extent than RNA terminated by Remdesivir, another drug being used as a COVID-19 therapeutic. These results offer a molecular basis supporting the current use of Sofosbuvir in combination with other drugs in COVID-19 clinical trials. [**note: it will be interesting to see the clinical research for sofosbuvir in light of this data.**]
https://www.biorxiv.org/content/10.1101/2020.08.07.242156v1

- The SARS coronavirus 2 main protease 3CLpro tailor cuts various essential virus proteins out of long poly-protein translated from the virus RNA. If the 3CLpro is inhibited, the functional virus proteins cannot form and the virus cannot replicate and assemble. Any compound that inhibits the 3CLpro is therefore a potential drug to end the pandemic. Here we show that the diffraction power of 3CLpro crystals is effectively destroyed by Ebselen. It appears that Ebselen may be a widely available, relatively cost effective way to eliminate the SARS coronavirus 2. [**note: there are a couple of trials going on and this may be a low cost drug therapy *if* it works.]**
https://www.biorxiv.org/content/10.1101/2020.08.10.244525v1
- Neutralizing agents against SARS-CoV-2 are urgently needed for treatment and prophylaxis of COVID-19. Here, we present a strategy to rapidly identify and assemble synthetic human variable heavy (VH) domain binders with high affinity toward neutralizing epitopes without the need for high-resolution structural information. We constructed a VH-phage library and targeted a known neutralizing site, the angiotensin-converting enzyme 2 (ACE2) binding interface of the trimeric SARS-CoV-2 Spike receptor-binding domain (Spike-RBD). Using a masked selection approach, we identified 85 unique VH binders to two non-overlapping epitopes within the ACE2 binding site on Spike-RBD. This enabled us to systematically link these VH domains into multivalent and bi-paratopic formats. These multivalent and bi-paratopic VH constructs showed a marked increase in affinity to Spike (up to 600-fold) and neutralization potency (up to 1400-fold) on pseudotyped SARS-CoV-2 virus when compared to the standalone VH domains. The most potent binder, a trivalent VH, neutralized authentic SARS-CoV-2 with half-minimal inhibitory concentration (IC50) of 4.0 nM (180 ng/mL). A cryo-EM structure of the trivalent VH bound to Spike shows each VH domain bound an RBD at the ACE2 binding site, explaining its increased neutralization potency and confirming our original design strategy. Our results demonstrate that targeted selection and engineering campaigns using a VH-phage library can enable rapid assembly of highly avid and potent molecules towards therapeutically important protein interfaces. [**note: more good work from UCSF. These are interesting approaches to creating neutralizing molecules for treatment.**]
https://www.biorxiv.org/content/10.1101/2020.08.08.242511v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The COVID-19 pandemic affects millions of people worldwide with a rising death toll. The causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), uses its nonstructural protein 1 (Nsp1) to redirect host translation machinery to the viral RNA by binding to the ribosome and suppressing cellular, but not viral, protein synthesis through yet unknown mechanisms. We show here that among all viral proteins, Nsp1 has the largest impact on host viability in the cells of human lung origin. Differential expression analysis of mRNA-seq data revealed that Nsp1 broadly alters the transcriptome in human cells. The changes include repression of major gene clusters in ribosomal RNA processing, translation, mitochondria function, cell cycle and antigen presentation; and induction of factors in transcriptional regulation. We further gained a mechanistic understanding of the Nsp1 function by determining the cryo-EM structure of the Nsp1-40S ribosomal subunit complex, which shows that Nsp1 inhibits translation by plugging the mRNA entry channel of the 40S. We also determined the cryo-EM structure of the 48S preinitiation complex (PIC) formed by Nsp1, 40S, and the cricket paralysis virus (CrPV) internal ribosome entry site (IRES) RNA, which shows that this 48S PIC is nonfunctional due to the incorrect position of the 3' region of the mRNA. Results presented here elucidate the mechanism of host translation inhibition by SARS-CoV-2, provide insight into viral protein synthesis, and furnish a comprehensive understanding of the impacts from one of the most potent pathogenicity factors of SARS-CoV-2. [**note: fascinating finding about the impact of another SARS-CoV-2 protein. This one redirects the host cell to selectively process the viral RNA by binding to the ribosome and suppressing cellular but not viral protein synthesis.**] https://www.biorxiv.org/content/10.1101/2020.08.09.243451v1

DIAGNOSTIC DEVELOPMENT

- Nothing today

>>

2020-08-12

What could be finer than some chamber music by Beethoven? There really is not a good alternative answer for me. While the string quartets are often exalted, he wrote a bunch of other fine stuff as well. Here is the Archduke piano trio, op. 97. with members from the Camerata Pacifica: https://www.youtube.com/watch?v=ezRNLXBbB8Q Let us take a moment to remember Trini Lopez who just passed as a result of COVID-19. He was a huge hit in the US during my teen years, singing a variety of songs. Here is perhaps his greatest hit, 'If I had a Hammer,': https://www.youtube.com/watch?v=Kp1z8EzZ5Hs

US COVID-19 STATISTICS - **Infection Rate: 1.5%; CFR: 3.1%** (IR no change%; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post confirms what we all know, 'coronavirus fatigue is gripping America!' Here is an article on Dr. Peter Marks of FDA's Center for Biologics Evaluation and Review. He among others will make the call on when a COVID-19 vaccine is ready. University of North Carolina is opening with in class

instruction. I hope they can pull it off without much disruption. A you know, I have been following the actions of Purdue University under its president Mitch Daniels. Here is his back to school message. I look forward to the experiences of this large land-grant university. Post columnist Monica Hesse suggests treating mask wearing as part of the school dress code, "if you can punish a teenage girl for spaghetti straps, you can enforce a mask mandate!" The Big Ten cancels all fall sports including football. The Pac-12 follows suit. Where do these people come from? This Florida sheriff bans the wearing of masks by his deputies and people visiting his office, stating "We can debate and argue all day of why and why not. The fact is, the amount of professionals that give the reason why we should, I can find the exact same amount of professionals that say why we shouldn't (*wear masks*)."

The New York Times has a piece on COVID-19 cytokine storm; it is an interesting story of how rheumatologists began to focus on treating sever COVID-19 with drugs that tamped down on the immune response. Here is an overview of the age-related mortality in Florida; it's not just the old who are dying. Things did not turn out well for the Georgia school district that opened last week. This Times story discusses the lack of phase 3 trial data for the Russian COVID-19 vaccine.

The Telegraph discusses the current outbreak of COVID-19 in New Zealand. These two ER docs in the US discuss life on the COVID-19 front line.

STAT discuss ongoing mental and neurological effects from COVID-19. Here is a good review of the UCSF llama nanobody research.

The Lancet have an interesting single patient case study of pseudotumor cerebri syndrome associated with multiinflammatory syndrome in a 14 year old girl. Here is a commentary on the need for forward planning regarding disaster-related mass gatherings amidst a pandemic. The 1Day Sooner Research Team has a correspondence piece on the ethics of COVID-19 challenge trials.

Nature are publishing the results of the Pfizer phase 1/2 vaccine trial. Here is a first hand account of a UK public health researcher's bout with COVID-19 and the prolonged symptoms she continues to experience. Nature also cover the Russian vaccine safety question.

MODELING

- Significant progress has already been made in development and testing of SARS-CoV-2 vaccines, and Phase III clinical trials have begun for 6 novel vaccine candidates to date. These Phase III trials seek to demonstrate direct benefits of a vaccine on vaccine recipients. However, vaccination is also known to bring about indirect benefits to a population through the reduction of virus circulation. The indirect effects of SARS-CoV-2 vaccination can play a key role in reducing case counts and COVID-19 deaths. To illustrate this point, we show through simulation that a vaccine with strong indirect effects has the potential to reduce SARS-CoV-2 circulation and COVID-19 deaths to a greater extent than an alternative vaccine with stronger direct effects but weaker indirect effects. Protection via indirect effects may be of particular importance in the context of this virus, because elderly individuals are at an elevated risk of death but are also less likely to be directly protected by vaccination due to immune senescence. We therefore encourage ongoing data collection and model development aimed at evaluating the indirect effects of forthcoming SARS-CoV-2 vaccines. [**note: some interesting conjecture about indirect effects from a COVID-19 vaccine. It will be important to track elderly individuals response to**

the vaccine in terms of immune response.]
https://www.medrxiv.org/content/10.1101/2020.08.07.20170456v1

NEWLY REGISTERED CLINICAL TRIALS

- COVID-19 outcomes are worse in male patients. Androgen signaling, therefore, is a target for clinical exploration. TMPRSS2 is a membrane protease required for COVID pathogenesis that is regulated by androgens. Blocking TMPRSS2 with bicalutamide may reduce viral replication and improve the clinical outcome. Therefore, the study proposes to test bicalutamide at 150 mg oral daily dosing in a double-blind placebo-controlled randomized trial in male patients with early symptomatic COVID-19 disease. [**note: this is for all the male readers. I can't find any *in vitro* data that suggests this is a proteinase inhibitor.**] NCT04509999
- This is a Phase 3, multi-center, randomized, double blind, placebo controlled, clinical study of bucillamine (2 dosage levels) in patients with mild-moderate COVID-19. Patients will be randomized 1:1:1 to receive bucillamine 100 mg 3 times a day (TID), bucillamine 200 mg TID or placebo TID for up to 14 days. After the first interim analysis when a single dose is selected, patients will then be randomized 2:1 to the selected bucillamine dose or placebo The study will be overseen by an independent Data and Safety Monitoring Board (DSMB). Up to 10 centers in the United States will conduct this study. Up to 1000 patients will be enrolled in this study. Patients will participate in the study approximately 45 days. [**note: company is Revive Therapeutics.**] NCT04504734
- The purpose of this study is to assess safety and clinical efficacy of rivaroxaban in people with mild Coronavirus Disease 2019 who are at increased risk of disease progression. [**note: study sponsor is The Gates Foundation.**] NCT04504032
- The VOICE-COVID study will evaluate the concordance of screening for symptoms of COVID-19 using a voice based device (Amazon Alexa) compared to manual screening by a study coordinator for individuals entering the Cardiology/Heart Failure clinic at the McGill University Health Centre. [**note: paging Siri, why haven't you started a clinical trial?**] NCT04508972
- This is a placebo-controlled, double blind, randomized, Phase II dose escalation study intended to evaluate the potential safety and efficacy of tenecteplase for the treatment of COVID-19 associated respiratory failure. The hypothesis is that administration of the drug, in conjunction with heparin anticoagulation, will improve patients' clinical outcomes. [**note: this is an investigator sponsored study with a Genentech drug**] NCT04505592

CLINICAL TRIAL RESULTS

- Background Coronavirus disease 2019 (COVID-19) due to infection with SARS-CoV-2 causes substantial morbidity. Tocilizumab, an interleukin-6 receptor antagonist, might improve outcomes by mitigating inflammation. Methods We conducted a retrospective study of patients admitted to the University of Washington Hospital system with COVID-19 and requiring supplemental oxygen. Outcomes included clinical improvement, defined as a two-point reduction in severity on a 6-point ordinal scale or discharge, and mortality within 28 days. We used Cox proportional-hazards models with propensity score inverse probability weighting to compare outcomes in patients who did and did not receive tocilizumab. Results We evaluated 43 patients who received tocilizumab and 45 who did not. Patients receiving tocilizumab were younger with fewer comorbidities but higher baseline oxygen requirements. Tocilizumab

treatment was associated with reduced CRP, fibrinogen, and temperature, but there were no meaningful differences in Cox models of time to clinical improvement (adjusted hazard ratio [aHR], 0.92; 95% CI, 0.38-2.22) or mortality (aHR, 0.57; 95% CI, 0.21-1.52). A numerically higher proportion of tocilizumab-treated patients had subsequent infections, transaminitis, and cytopenias. Conclusions Tocilizumab did not improve outcomes in hospitalized patients with COVID-19. However, this study was not powered to detect small differences, and there remains the possibility for a survival benefit. [**note: small sample date from Univ of Washington on once-promising drug tocilizumab showing as have others that it likely does not work.**] https://www.medrxiv.org/content/10.1101/2020.08.05.20169060v1

- Neutrophilia and high levels of proinflammatory cytokines and other mediators of inflammation are common finds in patients with severe acute respiratory syndrome due to COVID-19. By its action on leukocytes, we propose colchicine as an intervention worthy of being tested. Objective. To evaluate whether the addition of colchicine to standard treatment for COVID-19 results in better outcomes. Methods. We present the interim analysis of a single-center randomized, double-blinded, placebo controlled clinical trial of colchicine for the treatment of moderate to severe COVID-19, with 38 patients allocated 1:1 from April 11 to July 06, 2020. Colchicine regimen was 0.5 mg thrice daily for 5 days, then 0.5 mg twice daily for 5 days. The first dose was 1.0 mg whether body weight was ≥ 80 kg. Endpoints. The primary endpoints were the need for supplemental oxygen; time of hospitalization; need for admission and length of stay in intensive care units; and death rate and causes of mortality. As secondary endpoints, we assessed: serum C-reactive protein, serum Lactate dehydrogenase and relation neutrophil to lymphocyte of peripheral blood samples from day zero to day 7; the number, type, and severity of adverse events; frequency of interruption of the study protocol due to adverse events; and frequency of QT interval above 450 ms. Results. Thirty-five patients (18 for Placebo and 17 for Colchicine) completed the study. Both groups were comparable in terms of demographic, clinical and laboratory data at baseline. Median (and interquartile range) time of need for supplemental oxygen was 3.0 (1.5-6.5) days for the Colchicine group and 7.0 (3.0-8.5) days for Placebo group (p = 0.02). Median (IQR) time of hospitalization was 6.0 (4.0-8.5) days for the Colchicine group and 8.5 (5.5-11.0) days for Placebo group (p = 0.03). At day 2, 53% vs 83% of patients maintained the need for supplemental oxygen, while at day 7 the values were 6% vs 39%, in the Colchicine and Placebo groups, respectively (log rank; p = 0.01). Hospitalization was maintained for 53% vs 78% of patients at day 5 and 6% vs 17% at day 10, for the Colchicine and Placebo groups, respectively (log rank; p = 0.01). One patient per group needed admission to ICU. No recruited patient died. At day 4, patients of Colchicine group presented significant reduction of serum C-reactive protein compared to baseline (p < 0.001). The majority of adverse events were mild and did not lead to patient withdrawal. Diarrhea was more frequent in the Colchicine group (p = 0.17). Cardiac adverse events were absent. Discussion. *The use of colchicine reduced the length of supplemental oxygen therapy and the length of hospitalization. Clinical improvement was in parallel with a reduction on serum levels of C-reactive protein. The drug was safe and well tolerated. Colchicine may be considered a beneficial and not expensive option for COVID-19 treatment. Clinical trials with larger numbers of patients should be conducted to further evaluate the efficacy and safety of colchicine as an adjunctive therapy for hospitalized patients with moderate to severe COVID-19.* [**note: this is the first data I have seen**

on colchicine. There are some trials going on and clearly more data on whether this finding holds up are needed.] https://www.medrxiv.org/content/10.1101/2020.08.06.20169573v1

- Symptom screening is a widely deployed strategy to mitigate the COVID-19 pandemic and many public health authorities are mandating its use by employers for all employees in the workplace. While symptom screening has the benefit of reducing the number of infected individuals in the workplace, it raises some inherently difficult privacy issues as a traditional approach requires the employer to collect symptom data from each employee which is essentially medical information. In this paper, we describe a system to implement Cryptographic Anonymous Symptom Screening (CASS) which allows for individuals to perform COVID symptom screening anonymously while avoiding the privacy issues of traditional approaches. In the system, individuals report their symptoms without any identifying information and are issued a completion certificate. This certificate contains a cryptographic code which certifies that the certificate was obtained from the screener after reporting no symptoms. The codes can be verified using a cryptographic algorithm which is publicly available. A standard cryptography approach to implement such a system would be to use digital signatures. Unfortunately, standard digital signatures have some limitations for this application in that the signatures are often hundreds of characters long and if the signature contains the name of the individual, then there is also a risk of compromising privacy. In our approach, we develop and utilize a relaxed digital signature scheme to provide 16 character long codes and handle names using equivalence classes which helps preserve privacy. Both of these extensions technically compromise the security but in a way that is negligible for this application. Our system can either serve the function of standard symptom screening system approaches for employees, but can also extend symptom screening to non-employees such as visitors or customers. In this case, the system can be utilized in retail, restaurants and schools to ensure that everyone in the physical space, including employees, customers, visitors and students have performed symptom screening. [**note: the issue of individual confidentiality of health information in a pandemic was not one I put high up on the list of things to be worried about. However, this UCLA group has away around this via cryptographic digital signatures.**]
https://www.medrxiv.org/content/10.1101/2020.08.06.20169839v1
- The role of hydroxychloroquine (HCQ) and azithromycin in the treatment of COVID-19 and its effect on SARS-CoV-2 viral clearance is not known. Methods: This is a retrospective observational study to assess the effect of HCQ and Azithromycin on duration from symptom onset to negative SARS-CoV-2 PCR using nasopharyngeal swab in hospitalized patient with COVID-19. Eighty-five patients were included in the study, 65 in HCQ (Hydroxychloroquine + Azithromycin) and 20 in non-HCQ group. Measurement of duration from symptom onset to negative PCR and effect of gender, age and disease severity on time to viral clearance was measured. Results: Median time to negative PCR in HCQ group was 23 days (IQR: 9, Mean 24+8, N=65) compared with non-HCQ group, 19 days (IQR: 8, Mean 18+6, N=20), ($p < 0.05$). Forty-one (63%) patients in HCQ group and all patients (100%) in non-HCQ group had mild disease. Multivariate regression model ($F=6.8$, $P<0.002$, $R2=0.20$) shows that being in HCQ group would delay the time to negative PCR by 7 days (95%CI: 2-12) and with every year increase in the age, the time to negative PCR would be delayed by 0.12 days (95%CI: 0.017-0.22). Among HCQ sub-groups, gender and disease severity had no effect on duration (p 0.142 and 0.156 respectively) but older patients >60 year had longer duration compared to patients <60 year of age although

p value did not reach significance (p 0.073). Median time to negative PCR in mild-HCQ group (23 days, IQR: 9, Mean 23+8, N=41) was longer when compared with non-HCQ group (p <0.05). On day 28, all patients in non-HCQ group had negative PCR while only 50/65 (77%) were negative in HCQ group. Conclusion: Hydroxychloroquine (HCQ) and azithromycin delay SARS-CoV-2 virus clearance in hospitalized patients with COVID-19 and it is correlated with older age. Larger studies are needed to confirm this finding. [**note: more negative data on HCQ, this time from Saudi Arabia. The wooden stakes are at the ready for Zombie attack.**]
https://www.medrxiv.org/content/10.1101/2020.08.05.20151027v1

- The evidence that BCG (bacille Calmette-Guerin) vaccine may increase the ability of the immune system to fight off pathogens other than tuberculosis has been studied in the past. This nonspecific immunity gained our interest, especially after initial reports of less cases in countries with universal BCG vaccination. In hopes of possible protective immunity, all staff of the Emirates International Hospital (United Arab Emirates) were offered a booster BCG vaccine in early March 2020. All the hospital staff were then tested for Covid-19 infection by the end of June 2020. Methodology : We divided the subjects into two groups: booster vaccinated, versus unvaccinated. The rate of Covid-19 infection was compared between the groups. Criteria included all staff who were offered the vaccine. Results: 71 subjects received the booster vaccination. This group had zero cases of positive COVID 19 infection. 209 subjects did not receive the vaccination, with 18 positive PCR confirmed COVID 19 cases The infection rate in the unvaccinated group was 8.6% versus zero in the booster vaccinated group. (Fishers exact test p-value=0.004). Conclusion : Our findings demonstrated the potential effectiveness of the booster BCG vaccine, specifically the booster in preventing Covid-19 infections in an elevated-risk healthcare population. [**note: this is the first paper I've seen with results on BCG vaccine as a prophylactic. This is a small sample size and there could be confounders as to why the vaccinated group performed better (zero COVID-19 cases) compared to the control group. As I am fond of saying, TIWWDCT. There are clinical trials underway to study this.**]
https://www.medrxiv.org/content/10.1101/2020.08.10.20172288v1
- Angiotensin receptor blockers (ARBs) have been postulated as tentative pharmacological agents to treat Covid-19-induced lung inflammation. Trial design. This trial is a parallel group, randomized, two arm, open label, multicenter superiority trial with 1:1 allocation ratio. Methods. Participants included patients who were 18 years of age or older and who had been hospitalized with confirmed Covid-19 with 4 or fewer days since symptom onset. Exclusion criteria included intensive care unit admission prior to randomization and use of angiotensin receptor blocker or angiotensin converting enzyme inhibitors at admission. Participants in the treatment arm received telmisartan 80 mg bid during 14 days plus standard care. Participants in the control arm received standard care alone. Primary outcome was to achieve significant reductions in plasma levels of C-reactive protein in telmisartan treated Covid-19 patients at day 5 and 8 after randomization. Key secondary outcomes included time to discharge evaluated at 15 days after randomization and admission to ICU and death at 15- and 30-days post randomization. We present here a preliminary report. Results. A total of 78 patients were included in the interim analysis, 40 in the telmisartan and 38 in the control groups. CRP levels at day 5 in the control group were 51.1 +/- 44.8 mg/L (mean +/- SD; n=28) and in the telmisartan group were 24.2 +/- 31.4 mg/L (mean +/- SD; n=32, p<0.05). At day 8, CRP levels were 41.6 +/- 47.6 mg/L (mean +/- SD; n=16) and 9.0 +/- 10.0 mg/L (mean +/- SD; n=13, p < 0.05) in the control

and telmisartan groups, respectively. Also, analysis of time to discharge by Kaplan-Meier method showed that telmisartan treated patients had statistically significant lower time to discharge (median time to discharge control group=15 days; telmisartan group=9 days). No differences were observed for ICU admission or death. No significant adverse events related to telmisartan were reported. Conclusions. *In the present preliminary report, despite the small number of patients studied, ARB telmisartan, a well-known inexpensive safe antihypertensive drug, administered in high doses, demonstrates anti-inflammatory effects and improved morbidity in hospitalized patients infected with SARS -CoV-2, providing support for its use in this serious pandemia* (NCT04355936). [**note: this is a preliminary report from Buenos Aires on the use of telmisartan in the treatment of COVID-19. Again there are small numbers here but it appears there is a treatment effect. There are trials on other sartans going on as well.**] https://www.medrxiv.org/content/10.1101/2020.08.04.20167205v1

DRUG DEVELOPMENT

- AT-527, an orally administered double prodrug of a guanosine nucleotide analog, has been shown previously to be highly efficacious and well tolerated in HCV-infected subjects. Herein we report the potent *in vitro* activity of AT-511, the free base form of AT-527, against several coronaviruses, including SARS-CoV-2, the causative agent of COVID-19. In normal human airway epithelial (HAE) cell preparations, the average concentration of AT-511 required to inhibit replication of SARS-CoV-2 by 90% (EC_{90}) was 0.5 µM, very similar to the EC_{90} for AT-511 against HCoV-229E, HCoV-OC43 and SARS-CoV in Huh-7 cells. No cytotoxicity was observed for AT-511 in any of the antiviral assays up to the highest concentration tested (100 µM). Surprisingly, AT-511 was 30-fold less active against MERS-CoV. This differential activity may provide a clue to the apparent unique mechanism of action of the guanosine triphosphate analog formed from AT-527. [**note: this is from Atea Pharma, a company working on small molecule antivirals. The compound is currently in a Phase 2 trial.**] https://www.biorxiv.org/content/10.1101/2020.08.11.242834v2

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- New therapeutics are urgently needed to inhibit SARS-CoV-2, the virus responsible for the on-going Covid-19 pandemic. Nsp15, a uridine-specific endoribonuclease found in all coronaviruses, processes viral RNA to evade detection by RNA-activated host defense systems, making it a promising drug target. Previous work with SARS-CoV-1 established that Nsp15 is active as a hexamer, yet how Nsp15 recognizes and processes viral RNA remains unknown. Here we report a series of cryo-EM reconstructions of SARS-CoV-2 Nsp15. The UTP-bound cryo-EM reconstruction at 3.36 Å resolution provides molecular details into how critical residues within the Nsp15 active site recognize uridine and facilitate catalysis of the phosphodiester bond, whereas the apo-states reveal active site conformational heterogeneity. We further demonstrate the specificity and mechanism of nuclease activity by analyzing Nsp15 products using mass spectrometry. Collectively, these findings advance understanding of how Nsp15 processes viral RNA and provide a structural framework for the development of new therapeutics. [**note: here is research on the Nsp15 enzyme that helps it evade one of the bodies defense mechanisms.**] https://www.biorxiv.org/content/10.1101/2020.08.11.244863v1

DIAGNOSTIC DEVELOPMENT

- Mucosal immunity, including secretory IgA (sIgA), plays an important role in early defenses against respiratory pathogens. Salivary testing, the most convenient way to measure sIgA, has been used to characterize mucosal immune responses to many viral infections including SARS, MERS, influenza, HIV, and RSV. However, its role has not yet been characterized in the COVID-19 pandemic. Here, we report development and validation of a rapid immunoassay for measuring salivary IgA against the SARS-CoV-2 virus, and report quantitative results in both pre-COVID-19 and muco-converted subjects. Methods: We developed and refined a specific test for salivary IgA against SARS-CoV-2 on the Brevitest platform, a rapid immunoassay system designed for point-of-care use. A qualitative test was validated as per FDA guidelines with saliva obtained from subjects prior to the emergence of COVID-19, and from PCR-confirmed COVID-19 patients. We also generated a quantitative measure of anti-SARS-CoV-2 salivary IgA. Time taken for saliva self-collection was measured and its ease-of-use assessed. Results: We successfully validated a qualitative salivary assay for SARS-CoV-2 IgA antibodies, with positive and negative predictive values of 92% and 97%, respectively, and no observable cross-reactivity with any of seven potential confounders. Pre-COVID-19 saliva samples showed an 8-fold range of IgA concentrations, suggesting a broad continuum of natural antibody resistance against the novel virus, though at levels lower than that observed in COVID-19 PCR-confirmed subjects. Samples from muco-positive subjects also shown a ~9-fold variation in salivary IgA levels, with elevated salivary IgA observed beyond three months after onset of symptoms. We observed a correlation (r=0.4405) between salivary IgA levels and COVID-19 disease severity. In anecdotal observations, we observed individuals who exhibited antibodies early in the course of their disease, contemporaneously with a positive PCR test, as well as individuals who muco-converted despite no known direct exposure to a COVID-19 patient, no symptoms, and negative molecular and/or serum antibody tests. Salivary collection took 5-10 minutes, and was reported as being easy (mean of 1.1 on a scale of 1 to 10). Implications: Mucosal immunity, including secretory IgA, plays an important role in host defense against respiratory pathogens, and our early data suggest it may do so in COVID-19. Salivary IgA, an accessible marker of mucosal immunity, may be a useful indicator of several key parameters including individual and community immune response, disease severity, clinical risk, and herd immunity. The non-invasive nature and ease of saliva collection facilitates its potential use as a biomarker for ongoing patient assessment and management, as well as a community surveillance tool. By measuring mucosal immune responses directly and systemic immune responses indirectly, salivary IgA could be useful in developing and deploying a vaccine(s) against COVID-19. Quantitative IgA assessment could also potentially serve as a tool to segment the population into different risk categories and inform individual and collective decisions relating to appropriate activities and vaccine prioritization/delivery. These data reinforce the importance of further investigation into the role of mucosal immunity and IgA in host responses against COVID-19. [**note: this is an interesting development of a salivary test for secretory IgA which may a good marker for mucosal immunity. The company developing tis is Brevitest.**]
  https://www.medrxiv.org/content/10.1101/2020.08.07.20170258v1

>>

2020-08-13

For today's music selection let us go to a time just before the pandemic hit. Here is the Frankfurt Radio Symphony performing the Saint-Saëns cello concerto with soloist Gautier Capuçon under the baton of Alain Altinoglu. The performance was recorded on Valentines Day of this year: https://www.youtube.com/watch?v=pfBkzWNQTpI

It's CONTEST TIME!! Here is a real brain teaser. There are two small batch bourbon distilleries in Kentucky that each age their final barrels for the same length of time. Distillery #1 observes that 58% of the starting volume of bourbon that goes into the oak barrels is lost to evaporation as part of the aging process. Distillery #2 finds they have a loss of 138% of the final bottled volume. Assuming both companies sell the same number of bottles for the same price the year they reach the market, which distillery is more profitable? A nice prize to the first correct response. Loyal readers can send me a bottle of Booker's anytime they wish.

US COVID-19 STATISTICS - **Infection Rate: 1.6%; CFR: 3.1%** (IR up 0.1%; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

Politico asks whether there will be a superspreader event in a college town this fall. This is a pretty easy one to answer; yes of course there will. Let us hope there is only one.

The New York Times speculates that the true COVID-19 mortality has already surpassed 200,000. New Zealand has instituted a new lockdown to try to stop the small outbreak of COVID-19 in Auckland. Hollywood tries to restart the blockbuster machine in the midst of a pandemic. Should gyms and health clubs reopen?

The Washington Post also discusses Hollywood. I need them to get 'Better Call Saul' out soon as I want to find out what happens to Jimmy McGill aka Saul Goodman. If you have one of those masks with a valve on it, throw it in the trash as it does not work. I always thank the workers in the grocery stores I shop at and all of you should do as well! Sometimes, political leaders draw false analogies as Florida Governor Ron DeSantis clearly demonstrates; it is time to send a Seal Team after SARS-CoV-2! Finally, I was saddened to see Bergamo's home team Atalanta concede two very late goals and fall to Paris Saint Germain in the European Champions League. The hard-hit city deserved better on the night.

STAT covers the great American mask giveaway. Good to see that a 140-student charter school in Florida received 37,500 masks, about a year's supply for every student. Among recipients were Fortune 500 companies Chevron and Bristol Myers Squibb. More fodder for late-night talk show hosts. Why didn't policy makers teach people how to socialize safely I the time of coronavirus?

This JAMA article suggests there is an opportunity for further evolution of the field of precision public health. I'm not sure I buy this given we cannot even convince people to wear simple masks. "Walk before you run" is a good start. There is an assessment of Lupus Anticoagulant Positivity in patents with COVID-19 from the Montefirore Medical Center in the Bronx. JAMA also have a viewpoint on operational considerations on the American Academy of Pediatrics guidance for K-12 school reopenings as well as one on K-12 virtual schooling.

This New England Journal of Medicine letter from New Zealand researchers was a tad premature.

Derek Lowe is right on target about the Russian COVID-19 vaccine. Scroll down the drug development section for a nice new vaccine candidate from University of Washington. It may be too late for Operation Warp Speed but could turn out to be the best approach of all.

MODELING

- Australia recorded its first case of COVID-19 in late January 2020. On 22 March 2020, amid increasing daily case numbers, the Australian Government implemented lockdown restrictions to help flatten the curve. Our study aimed to understand the impact of lockdown restrictions on sexual and reproductive health. Here we focus on sexual practices. Methods: An online survey was open from the 23 April 2020 to the 11 May 2020. Participants were recruited online via social media and other networks and were asked to report on their sexual practices in 2019 and during lockdown. Logistic regression was used to calculate the difference (including 95% confidence intervals) in the proportion of sex practices between time periods. Results: Of the 1187 who commenced the survey, 965 (81.3%) completed it. Overall 70% were female and 66.3% were aged 18 to 29 years. Most (53.5%) reported less sex during lockdown than in 2019. Compared with 2019, participants were more likely to report sex with a spouse (35.3% vs 41.7%; difference=6.4%; 95%CI: 3.6, 9.2) and less likely to report sex with a girl/boyfriend (45.1% vs 41.8%; diff=-3.3%; 95%CI: -7.0, -0.4) or with casual hook-up (31.4% vs 7.8%; 95%CI:-26.9, -19.8). Solo sex activities increased, 14.6% (123/840) reported using sex toys more often and 26.0% (218/838) reported masturbating more often. Dating app use decreased during lockdown compared with 2019 (42.1% vs 27.3%; difference= -14.8%; 95%CI: -17.6, -11.9). Using dating apps for chatting/texting (89.8% vs 94.5%; diff=4.7%; 95%CI:1.0, 8.5) and for setting up virtual dates (2.6% vs 17.2%; diff=14.6%; 95%CI:10.1, 19.2) increased during lockdown. Conclusion: Although significant declines in sexual activity during lockdown were reported, people did not completely stop engaging in sexual activities during the pandemic, highlighting the importance of ensuring availability of normal sexual and reproductive health services during global emergencies. [**note: it's official sexual proclivities during a pandemic can be reduced to a set of statistics! Someone had to do this research and why not the Australians?]** https://www.medrxiv.org/content/10.1101/2020.08.10.20171348v1
- wheather children are easily susceptible to SARS-CoV-2 infection is still a debated question and a currently a hot topic, particularly in view of important decisions on school opening. For this reason, we decide to describe preliminary data showing the prevalence of anti-SARS-CoV-2 IgG in children with known household exposure to SARS-CoV-2. Interestingly, our report shows that household transmission of SARS-CoV-2 is high in both adults and children, with similar rates of SARS-CoV-2 IgG in all age groups, including the younger children. A total of 44 out of 80 household contacts (55%) of index patients had anti SARS-CoV-2 IgG. In particular, 16 (59,26%) adult partners had IgG antibodies compared with 28 (52,83%) of pediatric contacts ($P > 0.05$). Among the pediatric population, children ≥ 5 years of age had similar probability of having SARS-CoV-2 IgG (21/39, 53.8%) compared with those < 5 years (7/14, 50%) ($P > 0.05$). Adult partners and children also had a probability of having SARS-CoV-2 IgG. Interestingly, 35.7% of children and 33.3% of adults with SARS-CoV-2 IgG were previously diagnosed as COVID-19 cases. Since this evidence of high rate of IgG in children exposed to SARS-CoV-2 has public health implication, with this comment we highlight the need of establishing appropriate guidelines for

school opening and other social activities related to childhood. [**note: this is an interesting but small serology study of households in Italy. Kids do get COVID-19 and do generate antibodies.**] https://www.medrxiv.org/content/10.1101/2020.08.10.20169912v1

- A model of the distribution of respiratory droplets and aerosols by Lagrangian turbulent air-flow is developed and used to show how the SARS-CoV-2 Coronavirus can be dispersed by the breathing of an infected person. It is shown that the concentration of viruses in the exhaled cloud can increase to infectious levels with time, in a confined space where the air re-circulates. The model is used to analyze the air-flow and SARS-CoV-2 Coronavirus build-up in a restaurant in Guangzhou, China [16, 17]. It is concluded that the outbreak of Covid-19 in the restaurant in January 2020, is due to the build-up of the airborne droplets and aerosols carrying the SARS-CoV-2 Coronavirus and could not have been prevented by standard air-conditioning. [**note: here is another model for droplets/aerols in confined spaces. This one is based on Langrangian fluid dynamics of turbulent air-flow! There is some really cool math in this paper.**] https://www.medrxiv.org/content/10.1101/2020.08.11.20173195v1
- A long-standing question in infectious disease dynamics is the role of transmission heterogeneities, particularly those driven by demography, behavior and interventions. Here we characterize transmission risk between 1,178 SARS-CoV-2 infected individuals and their 15,648 close contacts based on detailed contact tracing data from Hunan, China. We find that 80% of secondary transmissions can be traced back to 14% of SARS-CoV-2 infections, indicating substantial transmission heterogeneities. Regression analysis suggests a marked gradient of transmission risk scales positively with the duration of exposure and the closeness of social interactions, after adjusted for demographic and clinical factors. Population-level physical distancing measures confine transmission to families and households; while case isolation and contact quarantine reduce transmission in all settings. Adjusted for interventions, the reconstructed infectiousness profile of a typical SARS-CoV-2 infection peaks just before symptom presentation, with ~50% of transmission occurring in the pre-symptomatic phase. *Modelling results indicate that achieving SARS-CoV-2 control would require the synergistic efforts of case isolation, contact quarantine, and population-level physical distancing measures, owing to the particular transmission kinetics of this virus*. [**note: this model comes from China and shows pretty much what we already know in terms of public health measures.**] https://www.medrxiv.org/content/10.1101/2020.08.09.20171132v1

NEWLY REGISTERED CLINICAL TRIALS

- Hey, I checked on this yesterday!!!

CLINICAL TRIAL RESULTS

- Remdesivir has been granted emergency use authorization for treatment of severe COVID-19. Remdesivir's pricing is based on a presumed reduction of hospital length of stay (LOS) by four days. But the Adaptive COVID-19 Treatment Trial (ACTT-1) that suggested this treatment benefit excluded patients who were expected to be discharged within 72 hours. Perhaps as a result, median time to recovery was unusually long in both arms of the study (15 days vs 11 days). Remdesivir requires a 5-day inpatient stay, so patients who would otherwise be discharged in fewer than 5 days may remain hospitalized to complete treatment while patients who would be discharged between 5 and 8 days, would only have potential reductions in their hospital LOS of

0-3 days. In a retrospective analysis of 1643 adults with severe COVID-19 admitted to Columbia University Medical Center and the Allen community hospital between March 9, 2020 and April 23, 2020, median hospital LOS was 7 (3-14) days. Five-hundred and eighty-six patients (36%) had a LOS of 1-4 days, 384 (23%) had a LOS of 5-8 days, and 673 (41%) were hospitalized for greater than or equal to 9 days. Remdesivir treatment may not provide the LOS reductions that the company relied on when pricing the therapy: 36% of the cohort would need to have LOS prolonged to receive a 5-day course, and only 41% of patients in our cohort had LOS of 9 days or more, meaning they could have their LOS shortened by 4 days and still receive a full Remdesivir course. Further investigation of shorter treatment courses and programs to facilitate outpatient intravenous Remdesivir administration are needed. [**note: perhaps Gilead is pricing remdesivir too high]** https://www.medrxiv.org/content/10.1101/2020.08.10.20171637v1

- Passive antibody transfer is a longstanding treatment strategy for infectious diseases that involve the respiratory system. In this context, human convalescent plasma has been used to treat coronavirus disease 2019 (COVID-19), but the efficacy remains uncertain. Objective: To explore potential signals of efficacy of COVID-19 convalescent plasma. Design: Open-label, Expanded Access Program (EAP) for the treatment of COVID-19 patients with human convalescent plasma. Setting: Multicenter, including 2,807 acute care facilities in the US and territories. Participants: Adult participants enrolled and transfused under the purview of the US Convalescent Plasma EAP program between April 4 and July 4, 2020 who were hospitalized with (or at risk of) severe or life threatening acute COVID-19 respiratory syndrome. Intervention: Transfusion of at least one unit of human COVID-19 convalescent plasma using standard transfusion guidelines at any time during hospitalization. Convalescent plasma was donated by recently-recovered COVID-19 survivors, and the antibody levels in the units collected were unknown at the time of transfusion. Main Outcomes and Measures: Seven and thirty-day mortality. Results: The 35,322 transfused patients had heterogeneous demographic and clinical characteristics. This cohort included a high proportion of critically-ill patients, with 52.3% in the intensive care unit (ICU) and 27.5% receiving mechanical ventilation at the time of plasma transfusion. The seven-day mortality rate was 8.7% [95% CI 8.3%-9.2%] in patients transfused within 3 days of COVID-19 diagnosis but 11.9% [11.4%-12.2%] in patients transfused 4 or more days after diagnosis ($p<0.001$). Similar findings were observed in 30-day mortality (21.6% vs. 26.7%, $p<0.0001$). Importantly, a gradient of mortality was seen in relation to IgG antibody levels in the transfused plasma. For patients who received high IgG plasma (>18.45 S/Co), seven-day mortality was 8.9% (6.8%, 11.7%); for recipients of medium IgG plasma (4.62 to 18.45 S/Co) mortality was 11.6% (10.3%, 13.1%); and for recipients of low IgG plasma (<4.62 S/Co) mortality was 13.7% (11.1%, 16.8%) ($p=0.048$). This unadjusted dose-response relationship with IgG was also observed in thirty-day mortality ($p=0.021$). The pooled relative risk of mortality among patients transfused with high antibody level plasma units was 0.65 [0.47-0.92] for 7 days and 0.77 [0.63-0.94] for 30 days compared to low antibody level plasma units. Conclusions and Relevance: The relationships between reduced mortality and both earlier time to transfusion and higher antibody levels provide signatures of efficacy for convalescent plasma in the treatment of hospitalized COVID-19 patients. This information may be informative for the treatment of COVID-19 and design of randomized clinical trials involving convalescent plasma. Trial Registration: ClinicalTrials.gov Identifier: NCT04338360 [**note: preliminary results from the**

Mayo Clinic study on the use of convalescent plasma. Early transfusion and high antibody titer make a difference.] https://www.medrxiv.org/content/10.1101/2020.08.12.20169359v1

DRUG DEVELOPMENT

- The raging COVID-19 pandemic caused by SARS-CoV2 has infected millions of people and killed several hundred thousand patients worldwide. Currently, there are no effective drugs or vaccines available for treating coronavirus infections. In this study, we have focused on the SARS-CoV2 helicase (Nsp13), which is critical for viral replication and the most conserved non-structural protein within the coronavirus family. Using homology modeling and molecular dynamics approaches, we generated structural models of the SARS-CoV2 helicase in its apo- and ATP/RNA-bound conformations. We performed virtual screening of ~970,000 chemical compounds against the ATP binding site to identify potential inhibitors. Herein, we report docking hits of approved human drugs targeting the ATP binding site. Importantly, two of our top drug hits have significant activity in inhibiting purified recombinant SARS-CoV-2 helicase, providing hope that these drugs can be potentially repurposed for the treatment of COVID-19. [**note: I've lost track of all the potential drug targets but here is another one. I'm not sure how much faith I put in these types of studies of which there are now many and we have yet to see a compound brought forward into clinical trials. You have to read the paper to see which drugs they think may have potential and I'll save you the job. The two top hits are cepharanthine and lumacaftor.**]
https://www.biorxiv.org/content/10.1101/2020.08.09.243246v1
- ACE2 is the main receptor of SARS-CoV-2 S1 protein and mediates viral entry into host cells. Herein, the membrane nanoparticles prepared from ACE2-rich cells are discovered with potent capacity to block SARS-CoV-2 infection. The membrane of human embryonic kidney-239T cell highly expressing ACE2 is screened to prepare nanoparticles. The nanomaterial termed HEK-293T-hACE2 NPs contains 265.1 ng mg^{-1} of ACE2 on the surface and acts as a bait to trap SARS-CoV-2 S1 in a dose-dependent manner, resulting in reduced recruitment of the viral ligand to host cells. Interestingly, SARS-CoV-2 S1 can translocate to the cytoplasm and affect the cell metabolism, which is also inhibited by HEK-293T-hACE2 NPs. Further studies reveal that HEK-293T-hACE2 NPs can efficiently suppress SARS-CoV-2 S pseudovirions entry into human proximal tubular cells and block viral infection with a low half maximal inhibitory concentration. Additionally, this biocompatible membrane nanomaterial is sufficient to block the adherence of SARS-CoV-2 D614G-S1 mutant to sensitive cells. Our study demonstrates a easy-to-achieve membrane nano-antagonist for curbing SARS-CoV-2, which enriches the existing antiviral arsenal and provides new possibilities to treat COVID-19. [**note: these Chinese researchers have come up with the classic "bait and switch" approach to virus control. Nanoparticles with ACE2 on the surface can trap SARS-CoV-2 in a dose dependent manner. Whether this can be scaled up as a therapeutic is not known.**]
https://www.biorxiv.org/content/10.1101/2020.08.12.247338v1
- One strategy for implementing cell-based immunity involves the use of chimeric antigen receptor (CAR) technology. Unlike CAR T cells, which need to be developed using primary T cells derived from COVID-19 patients with lymphopenia, clinical success of CAR NK cell immunotherapy is possible through the development of allogeneic, universal, and off-the-shelf CAR-NK cells from a third party, which will significantly broaden the application and reduce

costs. Here, we develop a novel approach for the generation of CAR-NK cells for targeting SARS-CoV-2. CAR-NK cells were generated using the scFv domain of CR3022 (henceforth, CR3022-CAR-NK), a broadly neutralizing antibody for SARS-CoV-1 and SARS-CoV-2. CR3022-CAR-NK cells can specifically bind to RBD of SARS-CoV-2 and pseudotyped SARS-CoV-2 S protein, and can be activated by pseudotyped SARS-CoV-2-S viral particles in vitro. Further, CR3022-CAR-NK cells can specifically kill pseudo-SARS-CoV-2 infected target cells. Thus, off-the-shelf CR3022-CAR-NK cells may have the potential to treat patients with severe COVID-19 disease. [**note: here is a neat approach by a Rutgers group to generating off the shelf NK cells to target SARS-CoV-2 infected cells.**] https://www.biorxiv.org/content/10.1101/2020.08.11.247320v1

- Here, we describe the structure-based design of self-assembling protein nanoparticle immunogens that elicit potent and protective antibody responses against SARS-CoV-2 in mice. The nanoparticle vaccines display 60 copies of the SARS-CoV-2 spike (S) glycoprotein receptor-binding domain (RBD) in a highly immunogenic array and induce neutralizing antibody titers roughly ten-fold higher than the prefusion-stabilized S ectodomain trimer despite a more than five-fold lower dose. Antibodies elicited by the nanoparticle immunogens target multiple distinct epitopes on the RBD, suggesting that they may not be easily susceptible to escape mutations, and exhibit a significantly lower binding:neutralizing ratio than convalescent human sera, which may minimize the risk of vaccine-associated enhanced respiratory disease. The high yield and stability of the protein components and assembled nanoparticles, especially compared to the SARS-CoV-2 prefusion-stabilized S trimer, suggest that manufacture of the nanoparticle vaccines will be highly scalable. These results highlight the utility of robust antigen display platforms for inducing potent neutralizing antibody responses and have launched cGMP manufacturing efforts to advance the lead RBD nanoparticle vaccine into the clinic. [**note: this is the first new vaccine approach in a couple of weeks and it is a very good approach. The misce data indicate that it produces a 10 fold higher neutralizing activity at a more than five-fold lower dose level of antigen. I'm glad to see this kind of research as we are going to need multiple vaccine candidates to figure out which one is best. I would put this one right up there near the top. The abstract notes a manufacturing effort and I hope they can get this into the clinic in a timely manner.**]
  https://www.biorxiv.org/content/10.1101/2020.08.11.247395v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Individuals infected by the virus exhibited different degrees of symptoms, the basis of which remains largely unclear. Currently, though convalescent individuals have been shown with both cellular and humoral immune responses, there is very limited understanding on the immune responses, especially adaptive immune responses, in patients with severe COVID-19. Here, we examined 10 blood samples from COVID-19 patients with acute respiratory distress syndrome (ARDS). The majority of them (70%) mounted SARS-CoV-2-specific humoral immunity with production of neutralizing antibodies. However, compared to healthy controls, the percentages and absolute numbers of both NK cells and CD8+ T cells were significantly reduced, accompanied with decreased IFNg expression in CD4+ T cells in peripheral blood from severe patients. Most notably, we failed in detecting SARS-CoV-2-specific IFNg production by peripheral blood lymphocytes from these patients. Our work thus indicates that COVID-19 patients with severe symptoms are associated with defective cellular immunity, which not only provides

insights on understanding the pathogenesis of COVID-19, but also has implications in developing an effective vaccine to SARS-CoV-2. [**note: small numbers here, but this Chinese study suggests that defective cellular immunity is associated with severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.08.10.20171371v1

- Melanoma differentiation-associated gene-5 (MDA5) acts as a cytoplasmic RNA sensor to detect viral dsRNA and mediates type I interferon (IFN) signaling and antiviral innate immune responses to infection by RNA viruses. Upon recognition of viral dsRNA, MDA5 is activated with K63-linked polyubiquitination and then triggers the recruitment of MAVS and activation of TBK1 and IKK, subsequently leading to IRF3 and NF-κB phosphorylation. Great numbers of symptomatic and severe infections of SARS-CoV-2 are spreading worldwide, and the poor efficacy of treatment with type I interferon and antiviral agents indicates that SARS-CoV-2 escapes from antiviral immune responses via an unknown mechanism. Here, we report that SARS-CoV-2 nonstructural protein 8 (NSP8) acts as an innate immune suppressor and inhibits type I IFN signaling to promote infection of RNA viruses. It downregulates the expression of type I IFNs, IFN-stimulated genes and proinflammatory cytokines by binding to MDA5 and impairing its K63-linked polyubiquitination. Our findings reveal that NSP8 mediates innate immune evasion during SARS-CoV-2 infection and may serve as a potential target for future therapeutics for SARS-CoV-2 infectious diseases. [**note: another immune suppressor protein from the virus!**] https://www.biorxiv.org/content/10.1101/2020.08.12.247767v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of coronavirus disease 2019 (COVID-19), it binds to angiotensin-converting enzyme 2 (ACE2) to enter into human cells. The expression level of ACE2 potentially determine the susceptibility and severity of COVID-19, it is thus of importance to understand the regulatory mechanism of ACE2 expression. Tripartite motif containing 28 (TRIM28) is known to be involved in multiple processes including antiviral restriction, endogenous retrovirus latency and immune response, it is recently reported to be co-expressed with SARS-CoV-2 receptor in type II pneumocytes; however, the roles of TRIM28 in ACE2 expression and SARS-CoV-2 cell entry remain unclear. This study showed that knockdown of TRIM28 induces ACE2 expression and increases pseudotyped SARS-CoV-2 cell entry of A549 cells and primary pulmonary alveolar epithelial cells (PAEpiCs). In a co-culture model of NK cells and lung epithelial cells, our results demonstrated that NK cells inhibit TRIM28 and promote ACE2 expression in lung epithelial cells, which was partially reversed by depletion of interleukin-2 and blocking of granzyme B in the co-culture medium. *Furthermore, TRIM28 knockdown enhanced interferon-γ (IFN-γ)-induced ACE2 expression through a mechanism involving upregulating IFN-γ receptor 2 (IFNGR2) in both A549 and PAEpiCs. Importantly, the upregulated ACE2 induced by TRIM28 knockdown and co-culture of NK cells was partially reversed by dexamethasone in A549 cells but not PAEpiCs. Our study identified TRIM28 as a novel regulator of ACE2 expression and SARS-CoV-2 cell entry*. [**note: some more good information from China on a human regulatory factor that regulates viral entry.]** https://www.biorxiv.org/content/10.1101/2020.08.12.247825v1

DIAGNOSTIC DEVELOPMENT

- Most current tests for SARS-CoV-2 are based on RNA extraction followed by quantitative reverse-transcription PCR assays that involve a separate RNA extraction and qPCR reaction for each sample with a fixed cost and reaction time. While automation and improved logistics can

increase the capacity of these tests, they cannot exceed this lower bound dictated by one extraction and reaction per sample. Multiplexed next generation sequencing (NGS) assays provide a dramatic increase in throughput, and hold the promise of richer information on viral strains and host immune response. Here, we establish a significant improvement of existing RNA-seq detection protocols. Our workflow, ApharSeq, includes a fast and cheap RNA capture step, that is coupled to barcoding of individual samples, followed by sample-pooling prior to the reverse transcription, PCR and massively parallel sequencing. Thus, only one step is performed before pooling hundreds of barcoded samples for subsequent steps and further analysis. We characterize the quantitative aspects of the assay, and test ApharSeq on dozens of clinical samples in a robotic workflow. Our proposed workflow is estimated to reduce costs by 10-50 fold, labor by 5-100 fold, automated liquid handling by 5-10 fold, and reagent requirements by 100-1000 fold compared to existing testing methods. [**note: good work from Israel on an improved pool technique for rt-PCR testing**]
https://www.medrxiv.org/content/10.1101/2020.08.08.20170746v1

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

2020-08-14

A loyal reader writes that I should feature the Donizetti aria 'Una furtiva lagrima' in honor the Bergamo Atalanta football club as that Italian city was Donizetti's birthplace. Of course I always honor my reader's requests but this time I do the reader one better! Here is Peruvian tenor, Juan Diego Florez from his 2015 Salzburg Festival recital. He accompanies himself on guitar in Malgueña, then sings Granada before exiting. The crowd won't stand for this and bring him back for a spectacular encore, Donizetti's Ah! mes amis….pour mon ame" from the Daughter of the Regiment. Florez handles the 9 high Cs as if they are nothing: https://www.youtube.com/watch?v=x5aLNi632MY I don't want to slight my reader, so here is Una furtiva lagrima from L'elisir d'amore:
https://www.youtube.com/watch?v=_NPshURqqyA

Yesterday's contest appeared difficult, but it really is not. The correct answer is that both distilleries make the exact same amount of money as the losses to evaporation are equal. In one case, evaporation is presented as a percent of initial volume while the other case it is of final-bottled volume. The way the data are presented makes it look like the second distiller has a larger loss. The puzzle comes from Carl Bergstrom and Jevin West book, "Calling Bullshit: the Art of Scepticism in a Data-Driven World." One reader pointed me to Blanton's Gold Edition, another small batch Kentucky bourbon, that previously was only made for the export market but is coming to the US. However, at $120/bottle, I will wait for someone to gift me (maybe I need to start of GoFundMe page for this though that would likely not be allowed).

US COVID-19 STATISTICS - **Infection Rate: 1.6%; CFR: 3.1%** (IR no change; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

The New York Times has an article on how contact tracing on an Indian reservation is saving lives. Interestingly, the use of a pulse oximeter turned out to be quite useful. Let us not forget all the children who tragically lost one or more parents. Questions remain as New York City schools open up in a month. One third of US users of TikTok may be under the age of 14; oh the horror, the horror (of course they are too young to read the Conrad novela)! Finally, finally, finally, yes, frozen food is not likely to

cause COVID-19. You can safety put those Tater Tots™ in the oven to munch on with your hamburgers tonight!

The Washington Post tells you how to care for your facemask. Make sure you buy a fine Procter & Gamble detergent so that I, as a P&G shareholder, can benefit. The Post is following the NY Times lead and tracking vaccine development. The article is flawed as it does not mention the Novavax protein subunit vaccine that is produced in a baculovirus cell culture system. As a senior citizen (and it pains me to say this😂) I am most interested in studies on this age group. We know that old folks have less robust immune systems. Look at how they jacked up the recent GSK shingles vaccine with a robust adjuvant. It's my own humble opinion that for this age group, an adjuvant protein base vaccine might be the best one. If all the vaccines were available today, that is the one I would take. This is the perfect time for F. Scott Fitzgerald to return from the grave and document these times. Nursing homes are being hit hard again which is baffling as we know how to control infections (or maybe we should know). Will the relaunch of cruise ships in Europe work out? We will find out.

The Guardian discusses the surge in COVID-19 cases among children. Here are some of the "safety" features in some US classrooms. One group of US companies is doing well in the pandemic.

Seriously, what is wrong with this country.

STAT has an opinion article on how COVID-19 anosmia reveals about how the mind works. COVID-19 trials may not be diverse enough.

The Lancet has a correspondence that breaks down mortality by sex difference and age.

Derek Lowe talks about nanobodies. I think the technology is cool and needs to go into the clinic ASAP.

MODELING

- SARS-CoV-2 can persist on surfaces, suggesting that surface-based transmission might be important for this pathogen. We find that fomites may be a substantial source of risk, particularly in schools and child daycares. Combining surface cleaning and decontamination with strategies to reduce pathogen shedding on surfaces can help mitigate this risk. [**note: this may be true for daycare and primary schools but I don't think it is generally applicable outside those settings.**] https://www.medrxiv.org/content/10.1101/2020.08.10.20171629v1

NEWLY REGISTERED CLINICAL TRIALS

- Again, I didn't spend any time to find the 1-2 newly registered trials. I'll try later on today.

CLINICAL TRIAL RESULTS

- Since the beginning of the SARS-CoV-2 pandemic, COVID-19 has appeared as a unique disease with unconventional tissue and systemic immune features. While COVID-19 severe forms share clinical and laboratory aspects with various pathologies such as hemophagocytic lymphohistiocytosis, sepsis or cytokine release syndrome, their exact nature remains unknown. This is severely impeding the ability to treat patients facing severe stages of the disease. To this aim, we performed an in-depth, single-cell RNA-seq analysis of more than 150.000 immune cells

isolated from matched blood samples and broncho-alveolar lavage fluids of COVID-19 patients and healthy controls, and integrated it with clinical, immunological and functional ex vivo data. We unveiled an immune signature of disease severity that correlated with the accumulation of naive lymphoid cells in the lung and an expansion and activation of myeloid cells in the periphery. Moreover, we demonstrated that myeloid-driven immune suppression is a hallmark of COVID-19 evolution and arginase 1 expression is significantly associated with monocyte immune regulatory features. Noteworthy, we found monocyte and neutrophil immune suppression loss associated with fatal clinical outcome in severe pa-tients. Additionally, our analysis discovered that the strongest association of the patients clinical outcome and immune phenotype is the lung T cell response. We found that patients with a robust CXCR6+ effector memory T cell response have better outcomes. This result is line with the rs11385942 COVID-19 risk allel, which is in proximity to the CXCR6 gene and suggest effector memory T cell are a primary feature in COVID-19 patients. By systemically quantifying the viral landscape in the lung of severe patients, we indeed identified Herpes-Simplex-Virus 1 (HSV-1) as a potential opportunistic virus in COVID-19 patients. Lastly, we observed an unexpectedly high SARS-CoV-2 viral load in an immuno-compromised patient, allowing us to study the SARS-CoV-2 in-vivo life cycle. The development of myeloid dysfunctions and the impairment of lymphoid arm establish a condition of immune paralysis that supports secondary bacteria and virus infection and can progress to immune silence in patients facing death. [**note: this is from a largely Italian group in Verona and further identifies properties of the immune system that lead to poorer outcomes.**] https://www.medrxiv.org/content/10.1101/2020.08.10.20170894v1

DRUG DEVELOPMENT

- We incorporated a membrane-anchored form of the SARS-CoV-2 Spike receptor binding domain (RBD) in place of the neuraminidase (NA) coding sequence in an influenza virus also possessing a mutation that reduces the affinity of hemagglutinin for its sialic acid receptor. The resulting ΔNA(RBD)-Flu virus can be generated by reverse genetics and grown to high titers in cell culture. A single-dose intranasal inoculation of mice with ΔNA(RBD)-Flu elicits serum neutralizing antibody titers against SAR-CoV-2 comparable to those observed in humans following natural infection (~1:250). Furthermore, ΔNA(RBD)-Flu itself causes no apparent disease in mice. It might be possible to produce a vaccine similar to ΔNA(RBD)-Flu at scale by leveraging existing platforms for production of influenza vaccines. [**note: cool outside the box thinking. Spike protein gene is put into influenza virus in place of the neuraminidase coding sequence. Lots of new vaccine approaches!**] https://www.biorxiv.org/content/10.1101/2020.08.12.248823v1
- In an effort to identify therapeutic intervention strategies for the treatment of COVID-19, we have investigated a selection of FDA-approved small molecules and biologics that are commonly used to treat other human diseases. A screen of 19 small molecules and 3 biologics was conducted in cell culture and the impact of treatment on viral titer was quantified by plaque assay. The screen identified 4 FDA-approved small molecules, Maraviroc, FTY720 (Fingolimod), Atorvastatin and Nitazoxanide that were able to inhibit SARS-CoV-2 infection. Confocal microscopy with over expressed S protein demonstrated that Maraviroc reduced the extent of S-protein mediated cell fusion as observed by fewer multinucleate cells in drug-treated cells. Mathematical modeling of drug-dependent viral multiplication dynamics revealed that prolonged drug treatment will exert an exponential decrease in viral load in a

multicellular/tissue environment. Taken together, the data demonstrate that Maraviroc, Fingolimod, Atorvastatin and Nitazoxanide inhibit SARS-CoV-2 in cell culture. [**note: I don't know when this paper was written but all these compounds are listed as being in clinical trials.]** https://www.biorxiv.org/content/10.1101/2020.08.12.246389v1

- The SARS-CoV-2 coronavirus (CoV) causes COVID-19, a current global pandemic. SARS-CoV-2 belongs to an order of Nidovirales with very large RNA genomes. It is proposed that the fidelity of CoV genome replication is aided by an RNA nuclease complex, formed of non-structural proteins 14 and 10 (nsp14-nsp10), an attractive target for antiviral inhibition. Here, we confirm that the SARS-CoV-2 nsp14-nsp10 complex is an RNase. Detailed functional characterisation reveals nsp14-nsp10 is a highly versatile nuclease capable of digesting a wide variety of RNA structures, including those with a blocked 3'-terminus. We propose that the role of nsp14-nsp10 in maintaining replication fidelity goes beyond classical proofreading and purges the nascent replicating RNA strand of a range of potentially replication terminating aberrations. Using the developed assays, we identify a series of drug and drug-like molecules that potently inhibit nsp14-nsp10, including the known Sars-Cov-2 major protease (Mpro) inhibitor ebselen and the HIV integrase inhibitor raltegravir, revealing the potential for bifunctional inhibitors in the treatment of COVID-19. [**note: this is both some new biochemistry but more critically a bifunctional approach to drug development. Ebselen is in clinical trials but not raltegravir. Since a dual drug therapy is used in a lot of viral diseases it might be good to get this combination in trials.**] https://www.biorxiv.org/content/10.1101/2020.08.13.248211v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Large trimeric Spikes decorate SARS-CoV-2 and bind host cells via receptor binding domains (RBDs). We report a conformation in which the trimer is locked into a compact well-ordered form. This differs from previous structures where the RBD can flip up to recognise the receptor. In the locked form regions associated with fusion transitions are stabilised and the RBD harbours curved lipids. The acyl chains bind a hydrophobic pocket in one RBD whilst the polar headgroups attach to an adjacent RBD of the trimer. By functional analogy with enteroviral pocket factors loss of the lipid would destabilise the locked form facilitating receptor attachment, conversion to the postfusion state and virus infection. The nature of lipids available at the site of infection might affect the antigenicity/pathogenicity of released virus. These results reveal a potentially druggable pocket and suggest that the natural prefusion state occludes neutralising RBD epitopes, achieving conformational shielding from antibodies. [**note: some interesting lipid biochemistry in play here and as an old time lipid guy, I'm always intrigued by this stuff.]** https://www.biorxiv.org/content/10.1101/2020.08.13.249177v1
- Gaining detailed insights into the role of host immune responses in viral clearance is critical for understanding COVID-19 pathogenesis and future treatment strategies. While studies analyzing humoral immune responses against SARS-CoV-2 were available rather early during the pandemic, cellular immunity came into focus of investigations just recently. For the present work, we have adapted a protocol, designed for the detection of rare neoantigen-specific Memory T cells in cancer patients for studying cellular immune responses against SARS-CoV-2. Both, CD4+ and CD8+ T cells were detected after 6 days of in vitro expansion using overlapping peptide libraries representing the whole viral protein. The assay readout was an Intracellular cytokine staining and flow cytometric analysis detecting four functional markers simultaneously

(CD154, TNF, IL-2, IFN-γ). We were able to detect SARS-CoV-2-specific T cells in 9 of 9 COVID-19 patients with mild symptoms. All patients had reactive T cells against at least one of 12 analyzed viral antigens and all patients had Spike-specific T cells. While some antigens were detected by CD4+ and CD8+ T cells, Membrane protein was mainly recognized by CD4+ T cells. Strikingly, we were not able to detect SARS-CoV-2-specific T cells in 9 unexposed healthy individuals. We are presenting a highly specific protocol for the detection of SARS-CoV-2-reactive T cells. Our data confirmed the important role of cellular immune responses in understanding SARS-CoV-2 clearance. We showed that Spike is the most immunogenic antigen. We have introduced Membrane protein as interesting target for studying humoral immune responses in convalescent COVID-19 patients. [**note: this is from Germany and looks at cellular immunity.**] https://www.medrxiv.org/content/10.1101/2020.08.10.20150060v1

- Serodiagnosis of SARS-CoV-2 infection is impeded by immunological cross-reactivity to the human coronaviruses (HCoV) SARS-CoV-2, SARS-CoV-1, MERS-CoV, OC43, 229E, HKU1, and NL63. Here we report the identification of humoral immune responses to SARS-CoV-2 and other HCoV peptides that can be used to detect asymptomatic, mild and, severe SARS-CoV-2 infections, and may enable the discovery of biomarkers for immunity following infection or vaccination. [**note: this is a very useful finding from a Columbia Univ group with some broad applications.**] https://www.biorxiv.org/content/10.1101/2020.08.13.249953v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects cells by binding to the host cell receptor Ace2 and undergoing virus-host membrane fusion. Fusion is triggered by the protease TMPRSS2, which processes the viral Spike (S) protein to reveal the fusion peptide. SARS-CoV-2 has evolved a multibasic site at the S1-S2 boundary, which is thought to be cleaved by furin in order to prime S protein for TMPRSS2 processing. Here we show that CRISPR-Cas9 knockout of furin reduces, but does not prevent, the production of infectious SARS-CoV-2 virus. Comparing S processing in furin knockout cells to multibasic site mutants reveals that while loss of furin substantially reduces S1-S2 cleavage it does not prevent it. SARS-CoV-2 S protein also mediates cell-cell fusion, potentially allowing virus to spread virion-independently. We show that loss of furin in either donor or acceptor cells reduces, but does not prevent, TMPRSS2-dependent cell-cell fusion, unlike mutation of the multibasic site that completely prevents syncytia formation. *Our results show that while furin promotes both SARS-CoV-2 infectivity and cell-cell spread it is not essential, suggesting furin inhibitors will not prevent viral spread*. [**note: this is a bit of a bummer as some drug discovery efforts were targeting this process]** https://www.biorxiv.org/content/10.1101/2020.08.13.243303v1

DIAGNOSTIC DEVELOPMENT

- Nothing new today

>>

2020-08-15

I don't think as many greats have been on one show that I can remember. Here is a television show from 1972 with all the legendary swing singers and players: https://www.youtube.com/watch?v=bNMM8HuX6PM Nothing more needs to be said as the music speaks for itself this Saturday!

US COVID-19 STATISTICS - **Infection Rate: 1.6%; CFR: 3.1%** (IR no change; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

Here is a good article in The Atlantic on the need to radically change the way we test for COVID-19. Some good common sense in this article.

The CDC has updated their COVID-19 guidance implying that if one is recovered do not need to quarantine for three months as long as they do not display any symptoms.

The Washington Post is up to date on how you can sanitize N95 respirators. Just a common pressure cooker does the trick. I looks like Operation Warp Speed is accelerating as HHS are making plans for vaccine distribution. Florida Governor DeSantis plays hard moneyball with one school district that wanted to delay opening by a month. Let's hope the University of Miami football coach is correct about his theory that college football is safe in the COVID-19 era; opening game is in a month!

This is not good. The New York Times is reporting that mAb trials from Regeneron and Lilly are taking longer than expected. Is college becoming a "glorified Skype" experience? Testing for COVID-19 is now on a downward arc; definitely not good news!

Lots of drug/vaccine discovery papers today!

MODELING

- In March 2020 New York City became the USA epicenter for the pandemic. On March 27, 2020 a Malayan tiger (Panthera tigris jacksoni) at the Bronx Zoo in New York City developed a cough and wheezing with subsequent inappetence. Over the next week, an additional Malayan tiger and two Amur tigers (P. t. altaica) in the same building and three lions (Panthera leo krugeri) in a separate building also became ill. The index case was immobilized, and physical examination and bloodwork results were unremarkable. Thoracic radiography and ultrasonography revealed peribronchial cuffing with bronchiectasis, and mild lung consolidation with alveolar-interstitial syndrome, respectively. SARS-CoV-2 RNA was identified by real-time, reverse transcriptase PCR (rRT-PCR) on oropharyngeal and nasal swabs and tracheal wash fluid. Cytologic examination of tracheal wash fluid revealed necrosis, and viral RNA was detected in necrotic cells by in situ hybridization, confirming virus-associated tissue damage. SARS-CoV-2 was isolated from the tracheal wash fluid of the index case, as well as the feces from one Amur tiger and one lion. Fecal viral RNA shedding was confirmed in all seven clinical cases and an asymptomatic Amur tiger. Respiratory signs abated within 1-5 days for most animals, though persisted intermittently for 16 days in the index case. Fecal RNA shedding persisted for as long as 35 days beyond cessation of respiratory signs. This case series describes the clinical presentation, diagnostic evaluation, and management of tigers and lions infected with SARS-CoV-2, and describes the duration of viral RNA fecal shedding in these cases. This report documents the first known natural transmission of SARS-CoV-2 from humans to animals in the USA, and is the first report of SARS-CoV-2 in non-domestic felids. [**note: this paper documents the transmission of SARS-CoV-2 from human to non-domestic big cats at the Bronx zoo. Interesting stuff and we don't know if reverse transmission happens.**]
  https://www.biorxiv.org/content/10.1101/2020.08.14.250928v1

- The COVID-19 global crisis is facilitated by high virus transmission rates and high percentages of asymptomatic and presymptomatic infected individuals. Containing the pandemic hinged on combinations of social distancing and face mask use. Here we examine the efficacy of these measures, using an agent-based modeling approach that evaluates face masks and social distancing in realistic confined spaces scenarios. *We find face masks are more effective than social distancing. Importantly, combining face masks with even moderate social distancing provides optimal protection. The finding that widespread usage of face masks limits COVID-19 outbreaks can inform policies to reopening of social functions*. [**note: this one is mandatory reading for all!!!! Masks WORK!**]
  https://www.medrxiv.org/content/10.1101/2020.08.12.20173047v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a two-center, randomized, placebo-controlled pilot study of anti-SARS-CoV-2 equine immunoglobulin fragments F(ab')2 (INOSARS) to evaluate safety and preliminary efficacy in the treatment of hospitalized COVID-19 patients. Clinical improvement at 28 days from the start of treatment will be evaluated. {**note: this is a trial from Mexico. Equine iG fragments have been tried against Ebola in the past by Chinese researchers. It makes sense to give it a shot here.**]
  NCT04514302
- The therapeutic hypothesis for the use of losmapimod in COVID-19 disease is that increased mortality and severe disease is caused by p38 mitogen-activated protein kinase (MAPK)-mediated exaggerated acute inflammatory response resulting from SARS-CoV-2 infection. The study Sponsor hypothesize's that the early initiation of p38α/β inhibitor therapy in patients hospitalized with moderate COVID-19 who are at increased risk of a poor prognosis based on older age and elevated systemic inflammation will reduce clinical deterioration including progression to respiratory failure and death. To address this hypothesis, Fulcrum Therapeutics is conducting a Phase 3, multicenter, randomized, double-blind, placebo-controlled study that will evaluate the safety and efficacy of losmapimod versus placebo in subjects 50 and older who are hospitalized with moderate COVID-19 disease. [**note: more information is at the above link. GSK developed this drug and apparently gave up on it. Perhaps that is worth noting.**]
  NCT04511819

CLINICAL TRIAL RESULTS

- The COVID-19 outbreak shows a huge variation in prevalence and mortality on geographical level but also within populations. The ACE2 gene, identified as the SARS-CoV2 receptor, has been shown to facilitate the viral invasion and people with higher ACE2 expression generally are more severely affected. As there is a lot of variability in ACE2 expression between individuals we hypothesized that differential DNA methylation profiles could be (one of) the confounding factors explaining this variability. Here we show that epigenetic profiling of host tissue, especially in the ACE2 promoter region and its homologue ACE1, may be important risk factors for COVID-19. Our results propose that variable methylation can explain (part of) the differential susceptibility, symptom severity and death rate for COVID-19. Our findings are a promising starting point to further evaluate the potential of ACE1/2 methylation and other candidates as a predictor for clinical outcome upon SARS-CoV2 infection. [**note: DNA methylation has always been an interesting finding and I don't think we fully understand the full importance. Maybe**

there is a linkage with severe COVID-19.]
https://www.biorxiv.org/content/10.1101/2020.08.14.251538v1

- There has been speculation that non-steroidal anti-inflammatory drugs (NSAIDs) may negatively affect coronavirus disease 2019 (COVID-19) outcomes, yet clinical evidence is limited. Objective: To assess the association between NSAID use and deaths from COVID-19 using OpenSAFELY, a secure analytical platform. Design: Two cohort studies (1st March-14th June 2020). Setting: Working on behalf of NHS England, we used routine clinical data from >17 million patients in England linked to death data from the Office for National Statistics. Participants: Study 1: General population (people with an NSAID prescription in the last three years). Study 2: people with rheumatoid arthritis/osteoarthritis. Exposures: Current NSAID prescription within the 4 months before 1st March 2020. Main Outcome and Measure: We used Cox regression to estimate hazard ratios (HRs) for COVID-19 related death in people currently prescribed NSAIDs, compared with those not currently prescribed NSAIDs, adjusting for age, sex, comorbidities and other medications. Results: In Study 1, we included 535,519 current NSAID users and 1,924,095 non-users in the general population. The crude HR for current use was 1.25 (95% CI, 1.07-1.46), versus non-use. We observed no evidence of difference in risk of COVID-19 related death associated with current use (HR, 0.95, 95% CI, 0.80-1.13) in the fully adjusted model. In Study 2, we included 1,711,052 people with rheumatoid arthritis/osteoarthritis, of whom 175,631 (10%) were current NSAID users. The crude HR for current use was 0.43 (95% CI, 0.36-0.52), versus non-use. In the fully adjusted model, we observed a lower risk of COVID-19 related death (HR, 0.78, 95% CI, 0.65-0.94) associated with current use of NSAID versus non-use. Conclusion and Relevance: We found no evidence of a harmful effect of NSAIDs on COVID-19 related deaths. Risks from COVID-19 do not need to influence decisions about therapeutic use of NSAIDs. [**note: wow is this a large study! One good thing about the UK NHS is that you can do these types of study quickly and on a lot of subjects. Anyone think that this can be done here in the US? You don't have to toss out your NSAIDS as there appears to be no link to COVID-19 related mortality. Of course there are other issues with NSAIDS that we are all aware of.**]
https://www.medrxiv.org/content/10.1101/2020.08.12.20171405v1
- An interleukin-6 inhibition strategy could be effective in selected COVID-19 patients. The objective is to present our experience of tocilizumab use in patients with severe COVID-19. Methods: Observational retrospective cohort study. Hospitalized patients were evaluated by our multidisciplinary team for eventual use of tocilizumab. Patients with progressive ventilatory impairment and evidence of a hyperinflammatory state despite usual treatment received tocilizumab 8 mg/kg intravenous (maximum dose 800 mg), in addition to standard treatment. The use and time of use of mechanical ventilation (MV), the change of the Alveolar-arterial (A-a) gradient, of the ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) and of inflammation laboratory parameters after 72 hours of tocilizumab use was evaluated. Results: 29 patients received tocilizumab. 93.1% were men, 37.9% were obese, and 34.5% had hypertension. Of the 20 patients who were not on MV when receiving tocilizumab, 11 required non-invasive MV, for an average of five days, and one of them required intubation. A-a gradient, PaO2/FiO2, and inflammation parameters improved significantly. A better lymphocyte count, which improved significantly after tocilizumab use, was significantly associated with less use of MV. Five patients presented positive culture samples after tocilizumab, three being of clinical significance. A lower lymphocyte count was associated with

having a positive culture. No other significant adverse events were seen. Conclusion: Our study suggests the utility and shows the safety of tocilizumab use in COVID-19 patients who have respiratory failure and evidence of hyperinflammation. Lymphocyte improvement was a predictor of good response. [**note: this is a small Chilean study of tocilizumab. They did see an improved lymphocyte count but larger studies elsewhere don't show that this is a useful drug.b]** https://www.medrxiv.org/content/10.1101/2020.08.12.20173104v1

- Acute lung inflammatory edema is a major pathological finding in autopsies explaining O2 diffusion failure and hypoxemia. Only dexamethasone has been shown to reduce mortality in severe cases, further supporting a role for inflammation in disease severity. SARS-CoV-2 enters cells employing angiotensin converting enzyme 2 (ACE2) as a receptor, which is highly expressed in lung alveolar cells. ACE2 is one of the components of the cellular machinery that inactivates the potent inflammatory agent bradykinin, and SARS-CoV-2 infection could interfere with the catalytic activity of ACE2, leading to accumulation of bradykinin. In this open-label, randomized clinical trial, we tested two pharmacological inhibitors of the kinin-kallikrein system that are currently approved for the treatment of hereditary angioedema, icatibant and inhibitor of C1 esterase/kallikrein, in a group of 30 patients with severe COVID-19. Neither icatibant nor inhibitor of C1 esterase/kallikrein resulted in significant changes in disease mortality and time to clinical improvement. However, both compounds promoted significant improvement of lung computed tomography scores and increased blood eosinophils, which has been reported as an indicator of disease recovery. In this small cohort, we found evidence for a beneficial role of pharmacological inhibition of the kinin-kallikrein system in two markers that indicate improved disease recovery. [**note: from Brazil, a small number of patients in a proof of concept study. Icatibant is in clinical trials and there are others who are looking at this pathway.**] https://www.medrxiv.org/content/10.1101/2020.08.11.20167353v1

DRUG DEVELOPMENT

- Background: SARS-coronavirus 2 (SARS-CoV-2) is currently causing a worldwide pandemic. Potential drugs identified for the treatment of SARS-CoV-2 infection include chloroquine (CQ), its derivative hydroxychloroquine (HCQ), and the anesthetic propofol. Their mechanism of action in SARS-CoV-2 infection is poorly understood. Recently, anesthetics, both general and local, were shown to disrupt ordered lipid domains. These same lipid domains recruit the SARS-CoV-2 surface receptor angiotensin converting enzyme 2 (ACE2) to an endocytic entry point and their disruption by cholesterol depletion decreases ACE2 recruitment and viral entry. Methods: Viral entry was determined using a SARS-CoV-2 pseudovirus (SARS2-PV) and a luciferase reporter gene expressed by the virus after treatment of the cells with 50 micromolar propofol, tetracaine, HCQ, and erythromycin. HCQ disruption of monosialotetrahexosylganglioside1 (GM1) lipid rafts, phosphatidylinositol 4,5-bisphosphate (PIP2) domains, and ACE2 receptor at nanoscale distances was monitored by direct stochastic reconstruction microscopy (dSTORM). Cells were fixed, permeabilized, and then labeled with either fluorescent cholera toxin B (CTxB) or antibody and then fixed again prior to imaging. Cluster analysis of dSTORM images was used to determine size and number and cross pair correlation was used to determine trafficking of endogenously expressed ACE2 in and out of lipid domains. Results: Propofol, tetracaine, and HCQ inhibit SARS2-PV viral entry. HCQ directly perturbs both GM1 lipid rafts and PIP2 domains. GM1 rafts increased in size and number similar to anesthetic disruption of lipid rafts; PIP2

domains decreased in size and number. HCQ blocked both GM1 and PIP2 domains ability to attract and cluster ACE2. Conclusions: We conclude HCQ is an anesthetic-like compound that disrupts GM1 lipid rafts similar to propofol and other local or general anesthetics. Furthermore, we conclude disruption of GM1 raft function, and not the concentration of GM1 raft molecules, governs the antiviral properties of HCQ. HCQ disruption of the membrane appears to also disrupt the production of host defense peptide, hence an antimicrobial such as erythromycin could be an important combined treatment. Nonetheless erythromycin has anti-SARS-CoV-2 activity and may combine with HCQ to reduce infection. [**note: at last someone has come up with a putative mechanism for HCQ. Maybe everyone was missing the co-administration of erythromycin rather than focusing on azithromycin as the magic additive. As a former lipid biochemist, I put little faith in this paper as there a lot of compounds that will do exactly the same thing. I think mellitin is a good drug for treating COVID-19 as it can perturb membranes in an analogous manner! (disclosure: part of my PhD work was on this peptide)**] https://www.biorxiv.org/content/10.1101/2020.08.13.250217v1

- The COVID-19 pandemic is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) which enters the body principally through the nasal and larynx mucosa and progress to the lungs through the respiratory tract. SARS-CoV-2 replicates efficiently in respiratory epithelial cells motivating the development of alternative and rapidly scalable vaccine inducing mucosal protective and long-lasting immunity. We have previously developed an immunologically optimized multi-neoepitopes-based peptide vaccine platform which has already demonstrated tolerance and efficacy in hundreds of lung cancer patients. Here, we present a multi-target CD8 T cell peptide COVID-19 vaccine design targeting several structural (S, M, N) and non-structural (NSPs) SARS-CoV-2 proteins with selected epitopes in conserved regions of the SARS-CoV-2 genome. We observed that a single subcutaneous injection of a serie of epitopes induces a robust immunogenicity in-vivo as measured by IFNγ ELIspot. Upon tetramer characterization we found that this serie of epitopes induces a strong proportion of virus-specific CD8 T cells expressing CD103, CD44, CXCR3 and CD49a, the specific phenotype of tissue-resident memory T lymphocytes (Trm). Finally, we observed broad cellular responses, as characterized by IFNγ production, upon restimulation with structural and non-structural protein-derived epitopes using blood T cells isolated from convalescent asymptomatic, moderate and severe COVID-19 patients. These data provide insights for further development of a second generation of COVID-19 vaccine focused on inducing lasting Th1-biased memory CD8 T cell sentinels protection using immunodominant epitopes naturally observed after SARS-CoV-2 infection resolution. [**note: we are moving on to 2nd generation COVID-19 vaccines with this paper from a French company! It is based on memory T-cell induction technology containing a combination of 12 CD8 T cell synthetic peptides from 11 SARS-CoV-2 structural and non-structural proteins. It will be interesting to see if this technology progresses to clinical trials. This is a very interesting paper and worth reading!**] https://www.biorxiv.org/content/10.1101/2020.08.14.240093v1
- The current COVID-19 pandemic caused by SARS-CoV-2 has resulted in millions of confirmed cases and thousands of deaths globally. Extensive efforts and progress have been made to develop effective and safe vaccines against COVID-19. A primary target of these vaccines is the SARS-CoV-2 spike (S) protein, and many studies utilized structural vaccinology techniques to either stabilize the protein or fix the receptor-binding domain at certain states. In this study, we extended an evolutionary protein design algorithm, EvoDesign, to create thousands of stable S

protein variants without perturbing the surface conformation and B cell epitopes of the S protein. We then evaluated the mutated S protein candidates based on predicted MHC-II T cell promiscuous epitopes as well as the epitopes' similarity to human peptides. The presented strategy aims to improve the S protein's immunogenicity and antigenicity by inducing stronger CD4 T cell response while maintaining the protein's native structure and function. The top EvoDesign S protein candidate (Design-10705) recovered 31 out of 32 MHC-II T cell promiscuous epitopes in the native S protein, in which two epitopes were present in all seven human coronaviruses. This newly designed S protein also introduced nine new MHC-II T cell promiscuous epitopes and showed high structural similarity to its native conformation. The proposed structural vaccinology method provides an avenue to rationally design the antigen's structure with increased immunogenicity, which could be applied to the rational design of new COVID-19 vaccine candidates. [**note: here is another design approach for vaccine, this time focusing on CD4 T cell response.**]
https://www.biorxiv.org/content/10.1101/2020.08.14.251496v1

- Angiotensin-converting enzyme 2 (ACE2) is the entry receptor for SARS-CoV-2, and recombinant ACE2 decoys are being evaluated as new antiviral therapies. We designed and tested an ACE2-Fc fusion protein, which has the benefits of a long pharmacological half-life and the potential to facilitate immune clearance of the virus. Out of the concern that the intrinsic catalytic activity of ACE2 may unintentionally alter the balance of its hormonal substrates and cause adverse cardiovascular effects in treatment, we performed a mutagenesis screening for inactivating the enzyme. Three mutants, R273A, H378A and E402A, completely lost their enzymatic activity for either surrogate or physiological substrates. All of them remained capable of binding SARS-CoV-2 and could suppress the transduction of a pseudotyped virus in cell culture. This study established new ACE2-Fc candidates as antiviral treatment for SARS-CoV-2 without potentially harmful side effects from ACE2 catalytic actions toward its vasoactive substrates. [**note: cool work from Northwestern in creating a decoy version of ACE2 that does not have enzymatic activity but can still bind SARS-CoV-2. I believe there is a trial with recombinant ACE2 but I've not seen any results.]** https://www.biorxiv.org/content/10.1101/2020.08.13.248351v1
- Methylene blue is an FDA and EMA approved drug with an excellent safety profile. It displays broad-spectrum virucidal activity in the presence of UV light and has been shown to be effective in inactivating various viruses in blood products prior to transfusions. In addition, its use has been validated for methemoglobinemia and malaria treatment. Here we show the virucidal activity of methylene blue at low micromolar concentrations and in the absence of UV activation against SARS-CoV2. [**note: the drug hits keep coming today. The malaria dose is apparently about 5mg/kg and long term treatment may result in fully reversible bluish coloration of urine, sclera, and skin. Too bad it doesn't turn the skin green as I could go out at Halloween as the Incredible Hulk!**] https://www.biorxiv.org/content/10.1101/2020.08.14.251090v1
- Antiviral therapeutics against SARS-CoV-2 are needed to treat the pandemic disease COVID-19. Pharmacological targeting of a host factor required for viral replication can suppress viral spread with a low probability of viral mutation leading to resistance. Here, we used a genome-wide loss of function CRISPR/Cas9 screen in human lung epithelial cells to identify potential host therapeutic targets. Validation of our screening hits revealed that the kinase SRPK1, together with the closely related SRPK2, were jointly essential for SARS-CoV-2 replication; inhibition of SRPK1/2 with small molecules led to a dramatic decrease (more than 100,000-fold) in SARS-CoV-

2 virus production in immortalized and primary human lung cells. Subsequent biochemical studies revealed that SPRK1/2 phosphorylate the viral nucleocapsid (N) protein at sites highly conserved across human coronaviruses and, due to this conservation, even a distantly related coronavirus was highly sensitive to an SPRK1/2 inhibitor. Together, these data suggest that SRPK1/2-targeted therapies may be an efficacious strategy to prevent or treat COVID-19 and other coronavirus-mediated diseases. [**note: yet another target for drug design.**] https://www.biorxiv.org/content/10.1101/2020.08.14.251207v1

- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the etiological agent for coronavirus disease 2019 (COVID-19), has emerged as an ongoing global pandemic. Presently, there are no clinically approved vaccines nor drugs for COVID-19. Hence, there is an urgent need to accelerate the development of effective antivirals. Here in, we discovered Clioquinol (5-chloro-7-iodo-8-quinolinol (CLQ)), a FDA approved drug and two of its analogues (7-bromo-5-chloro-8-hydroxyquinoline (CLBQ14); and 5, 7-Dichloro-8-hydroxyquinoline (CLCQ)) as potent inhibitors of SARS-CoV-2 infection induced cytopathic effect in vitro. In addition, all three compounds showed potent anti-exopeptidase activity against recombinant human angiotensin converting enzyme 2 (rhACE2) and inhibited the binding of rhACE2 with SARS-CoV-2 Spike (RBD) protein. CLQ displayed the highest potency in the low micromolar range, with its antiviral activity showing strong correlation with inhibition of rhACE2 and rhACE2-RBD interaction. Altogether, our findings provide a new mode of action and molecular target for CLQ and validates this pharmacophore as a promising lead series for clinical development of potential therapeutics for COVID-19. [**note: is there any drug that does not inhibit SARS-CoV-2 *in vitro*?**] https://www.biorxiv.org/content/10.1101/2020.08.14.250480v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The genome of the SARS-CoV-2 coronavirus contains 29 proteins, of which 15 are nonstructural. Nsp10 and Nsp16 form a complex responsible for the capping of mRNA at the 5' terminus. In the methylation reaction the S-adenosyl-L-methionine serves as the donor of the methyl group that is transferred to Cap-0 at the first transcribed nucleotide to create Cap-1. The presence of Cap-1 makes viral RNAs mimic the host transcripts and prevents their degradation. To investigate the 2'- O methyltransferase activity of SARS-CoV-2 Nsp10/16, we applied fixed-target serial synchrotron crystallography (SSX) which allows for physiological temperature data collection from thousands of crystals, significantly reducing the x-ray dose while maintaining a biologically relevant temperature. We determined crystal structures of Nsp10/16 that revealed the states before and after the methylation reaction, for the first time illustrating coronavirus Nsp10/16 complexes with the m7GpppAm2'-O Cap-1, where 2'OH of ribose is methylated. We compare these structures with structures of Nsp10/16 at 297 K and 100 K collected from a single crystal. This data provide important mechanistic insight and can be used to design small molecules that inhibit viral RNA maturation making SARS-CoV-2 sensitive to host innate response. [**note: here is a paper on a different kind of methylation from what was mentioned above regarding DNA methylation. This appears to be some sort of defense mechanism for the viral mRNA.**] https://www.biorxiv.org/content/10.1101/2020.08.14.251421v1
- Binding of the viral spike protein (SARS-2-S) to cell surface receptor angiotensin-converting enzyme 2 (ACE2) mediates host cell infection. In the present study, we demonstrate that in addition to ACE2, the S1 subunit of SARS-2-S binds to HDL and that SARS-CoV-2 hijacks the SR-

B1-mediated HDL uptake pathway to facilitate its entry. SR-B1 facilitates SARS-CoV-2 entry into permissive cells by augmenting virus attachment. MAb (monoclonal antibody)-mediated blocking of SARS-2-S-HDL binding and SR-B1 antagonists strongly inhibit HDL-enhanced SARS-CoV-2 infection. Notably, SR-B1 is co-expressed with ACE2 in human pulmonary and extrapulmonary tissues. These findings revealed a novel mechanism for SARS-CoV-2 entry and could provide a new target to treat SARS-CoV-2 infection. [**note: another paper from China showing that there may be an alternate entry way into cells via the HDL uptake pathway. Clever virus.**] https://www.biorxiv.org/content/10.1101/2020.08.13.248872v1

- The recently emerged SARS-CoV-2 virus is currently causing a global pandemic and cases continue to rise. The majority of infected individuals experience mildly symptomatic coronavirus disease 2019 (COVID-19), but it is unknown whether this can induce persistent immune memory that might contribute to herd immunity. Thus, we performed a longitudinal assessment of individuals recovered from mildly symptomatic COVID-19 to determine if they develop and sustain immunological memory against the virus. We found that recovered individuals developed SARS-CoV-2-specific IgG antibody and neutralizing plasma, as well as virus-specific memory B and T cells that not only persisted, but in some cases increased numerically over three months following symptom onset. Furthermore, *the SARS-CoV-2-specific memory lymphocytes exhibited characteristics associated with potent antiviral immunity: memory T cells secreted IFN-γ and expanded upon antigen re-encounter, while memory B cells expressed receptors capable of neutralizing virus when expressed as antibodies. These findings demonstrate that mild COVID-19 elicits memory lymphocytes that persist and display functional hallmarks associated with antiviral protective immunity*. [**note: from the Univ of Washington some really good news!!! Function protective immunity is present in those who experience mild symptomatic COVID-19. Now we just need to figure out how to stop severe COVID-19 and things will be almost all good.**]
  https://www.medrxiv.org/content/10.1101/2020.08.11.20171843v1
- The development of vaccines against SARS-CoV-2 would be greatly facilitated by the identification of immunological correlates of protection in humans. However, to date, studies on protective immunity have only been performed in animal models and correlates of protection have not been established in humans. Here, we describe an outbreak of SARS-CoV-2 on a fishing vessel associated with a high attack rate. Predeparture serological and viral RT-PCR testing along with repeat testing after return to shore was available for 120 of the 122 persons on board over a median follow-up of 32.5 days (range 18.8 to 50.5 days). A total of 104 individuals had an RT-PCR positive viral test with Ct <35 or seroconverted during the follow-up period, yielding an attack rate on board of 85.2% (104/122 individuals). Metagenomic sequencing of 39 viral genomes suggested the outbreak originated largely from a single viral clade. Only three crewmembers tested seropositive prior to the boat's departure in initial serological screening and also had neutralizing and spike-reactive antibodies in follow-up assays. None of these crewmembers with neutralizing antibody titers showed evidence of bona fide viral infection or experienced any symptoms during the viral outbreak. Therefore, the presence of neutralizing antibodies from prior infection was significantly associated with protection against re-infection (Fisher's exact test, p=0.002). [**note: more good news from Washington, this time showing that neutralizing antibodies are indeed protective.**]
  https://www.medrxiv.org/content/10.1101/2020.08.13.20173161v1

- CD8+ T cells are critical for the elimination and long-lasting protection of many viral infections, but their role in the current SARS-CoV-2 pandemic is unclear. Emerging data indicates that SARS-CoV-2-specific CD8+ T cells are detectable in the majority of individuals recovering from SARS-CoV-2 infection. However, optimal virus-specific epitopes, the role of pre-existing heterologous immunity as well as their kinetics and differentiation program during disease control have not been defined in detail. Here, we show that both pre-existing and newly induced SARS-CoV-2-specific CD8+ T-cell responses are potentially important determinants of immune protection in mild SARS-CoV-2 infection. In particular*, our results can be summarized as follows: First, immunodominant SARS-CoV-2-specific CD8+ T-cell epitopes are targeted in the majority of individuals with convalescent SARS-CoV-2 infection. Second, MHC class I tetramer analyses revealed the emergence of phenotypically diverse and functionally competent pre-existing and newly induced SARS-CoV-2-specific memory CD8+ T cells that showed similar characteristics compared to influenza-specific CD8+ T cells. Third, SARS-CoV-2-specific CD8+ T-cell responses are more robustly detectable than antibodies against the SARS-CoV-2-spike protein. This was confirmed in a longitudinal analysis of acute-resolving infection that demonstrated rapid induction of the SARS-CoV-2-specific CD8+ T cells within a week followed by a prolonged contraction phase that outlasted the waning humoral immune response indicating that CD8+ T-cell responses might serve as a more precise correlate of antiviral immunity than antibody measurements after convalescence. Collectively, these data provide new insights into the fine specificity, heterogeneity, and dynamics of SARS-CoV-2-specific memory CD8+ T cells, potentially informing the rational development of a protective vaccine against SARS-CoV-2*. [**note: from Germany, some good work on the role of CD8 T cells**]
  https://www.biorxiv.org/content/10.1101/2020.08.13.249433v1

DIAGNOSTIC DEVELOPMENT

- Background Access to rapid diagnosis is key to the control and management of SARS-CoV-2. Reverse Transcriptase- Polymerase Chain Reaction (RT-PCR) testing usually requires a centralised laboratory and significant infrastructure. We describe the development and diagnostic accuracy assessment of a novel, rapid point-of-care RT-PCR test, the DnaNudge platform CovidNudge test, which requires no laboratory handling or sample pre-processing. Methods Nasopharyngeal swabs are inserted directly into a cartridge which contains all reagents and components required for RT-PCR reactions, including multiple technical replicates of seven SARS-CoV-2 gene targets (rdrp1, rdrp2, e-gene, n-gene, n1, n2 and n3) and human ribonuclease P (RNaseP) as a positive control. Between April and May 2020, swab samples were tested in parallel using the CovidNudge direct-to-cartridge platform and standard laboratory RT-PCR using swabs in viral transport medium. Samples were collected from three groups: self-referred healthcare workers with suspected COVID-19 (Group 1, n=280/386; 73%); patients attending the emergency department with suspected COVID-19 (Group 2, n=15/386; 4%) and hospital inpatient admissions with or without suspected COVID-19 (Group 3, n=91/386; 23%). Results Of 386 paired samples tested across all groups, 67 tested positive on the CovidNudge platform and 71 with standard laboratory RT-PCR. The sensitivity of the test varied by group (Group 1 93% [84-98%], Group 2 100% [48-100%] and Group 3 100% [29-100%], giving an average sensitivity of 94.4% (95% confidence interval 86-98%) and an overall specificity of 100% (95%CI 99-100%; Group 1 100% [98-100%]; Group 2 100% [69-100%] and Group 3 100% [96-100%]). Point of care

testing performance was comparable during a period of high (25%) and low (3%) background prevalence. Amplification of the viral nucleocapsid (n1, n2, n3) targets were most sensitive for detection of SARS-CoV2, with the assay able to detect 1x104 viral particles in a single swab. Conclusions The CovidNudge platform offers a sensitive, specific and rapid point of care test for the presence of SARS-CoV-2 without laboratory handling or sample pre-processing. The implementation of such a device could be used to enable rapid decisions for clinical care and testing programs. [**note: this novel new POC PCR test comes from the UK. Good approach to the problem.]** https://www.medrxiv.org/content/10.1101/2020.08.13.20174193v1

- The on-going coronavirus disease 2019 (COVID-19) pandemic has mobilized a global effort to develop vaccines and therapeutics that inhibit viral entry by inducing or transferring antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike glycoprotein (CoV2-S). Phase I/II vaccine clinical trials, monoclonal antibodies, and convalescent sera have all shown promise. However, these efforts often require extensive screening with the live virus under onerous high biocontainment conditions (BSL-3). Virus neutralization assays (VNAs) remain the gold standard for evaluating the anti-viral potency of antibodies and entry inhibitors. The proliferation of pseudotyped virus systems that can be used in BSL-2 compatible VNAs is a positive development. *Yet, there is marked variability between VNAs and how the findings are presented, making inter-group comparisons difficult. To address these limitations, we developed a standardized VNA using VSVdeltaG based CoV-2-S pseudotyped particles (CoV2pp) that can be robustly produced at scale. We used our CoV2pp to interrogate the role of exogenous and endogenous proteases in CoV-2-S mediated entry and standardized our VNA based on that understanding. Our CoV2pp VNA showed a strong positive correlation with CoV2-S ELISA and live virus neutralizations in a validated set of patient sera.* Our system was subsequently validated by three independent groups as an out-of-the-box VNA. More than 120 patient sera were screened, and we report descriptive statistics for absolute (abs) IC50, IC80, and IC90 values from all positive patient sera. Lastly, we used our CoV2pp in a screen to identify ultrapermissive 293T clones that stably express ACE2 or ACE2+TMPRSS2. When used in combination with our CoV2pp, we can now produce CoV2pp sufficient for 150,000 standardized VNA/week. [**note: more good work from the Mt. Sinai group. Here is a standardized viral neutralization assay that allows for cross cohort comparisons of COVID-19 sera.**]
  https://www.medrxiv.org/content/10.1101/2020.08.13.20157222v1

>>>>>>>>>>>>>>>>>>>>

2020-08-16

For reflection Sunday I am in a gospel mood. 'Just a Closer Walk with Thee' has been around since 1941. It has been covered by a huge number of artists from a variety of musical styles. Here is a version done by Wynton Marsalis and his band at Lincoln Center with guests Eric Clapton and Taj Mahal: https://www.youtube.com/watch?v=q5krFNUMQHI Here is my favorite Barcelona group headed by Joan Chamorro with Magali Datzira on the vocal line: https://www.youtube.com/watch?v=bylCFXdW0c0 This one is a recording, but who does it better than Patsy Cline and Willie Nelson: https://www.youtube.com/watch?v=OOKaircCiGI ? and finally the pure voice of Joan Baez: https://www.youtube.com/watch?v=9mR1P4nc-bk

US COVID-19 STATISTICS - **Infection Rate: 1.6%; CFR: 3.1%** (IR no change; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

Some good news on the diagnostic front, a quick saliva-based test developed by Yale researchers received a FDA Emergency Use Authorization! I linked the preprint last week.

The Washington Post update for today notes that infections among children under 17 have risen since March and New York continues to see hospitalizations drop.

The Guardian covers the recent outbreak in New Zealand.

STAT on the FDA EUA of the Yale saliva test.

The Lancet has the results of a large observational study in New Jersey on the use of tocilizumab. Tocilizumab exposure among patients with severe SARS-CoV-2 infection requiring ICU support was associated with a reduction in hospital-related mortality. An accompanying editorial discusses the conundrum of IL-6 blockade in COVID-19 as the first controlled trial showed no effect.

JAMA have a report on the phase 2 trial of the Chinese inactivated SARS-CoV-2 vaccine along with an accompanying editorial.

A BMJ editorial discusses the need to break the chain of household transmission of COVID-19.

MODELING

- A vaccine, when available, will likely become our best tool to control the current COVID-19 pandemic. Even in the most optimistic scenarios, vaccine shortages will likely occur. Using an age-stratified mathematical model, we determined optimal vaccine allocation for four different metrics (deaths, symptomatic infections, and maximum non-ICU and ICU hospitalizations) under a wide variety of assumptions. We find that a vaccine with effectiveness ≥50% would be enough to substantially mitigate the ongoing pandemic provided that a high percentage of the population is optimally vaccinated. *When minimizing deaths, we find that for low vaccine effectiveness, it is optimal to allocate vaccine to high-risk (older) age-groups first. In contrast, for higher vaccine effectiveness, there is a switch to allocate vaccine to high-transmission (younger) age-groups first for high vaccination coverage. While there are other societal and ethical considerations, this work can provide an evidence-based rationale for vaccine prioritization*. [**note: this paper poses a very good question for optimizing use of the COVID-19 vaccine, "who should be vaccinated first?"**]
  https://www.medrxiv.org/content/10.1101/2020.08.14.20175257v1
- On May 1, a 4-phase reopening plan began. If implemented without interruptions, all types of public interactions were planned to resume by July 15. We investigated whether adjunctive prevention strategies would allow less restrictive physical distancing to avoid second epidemic waves and secure safe school reopening. Methods We developed a mathematical model, stratifying the population by age (0-19 years, 20-49 years, 50-69 years, and 70+ years), infection status (susceptible, exposed, asymptomatic, pre-symptomatic, symptomatic, recovered) and treatment status (undiagnosed, diagnosed, hospitalized) to project SARS-CoV-2 transmission during and after the reopening period. The model was parameterized with demographic and contact data from King County, WA and calibrated to confirmed cases, deaths (overall and by

age) and epidemic peak timing. Adjunctive prevention interventions were simulated assuming different levels of pre-COVID physical interactions (pC_PI) restored. We made several predictions related to adjunctive interventions or increased pC_PI. Results The best model fit estimated ~35% pC_PI under lockdown. Gradually restoring 75% pC_PI for all age groups between May 15-July 15 resulted in ~350 daily deaths by early September 2020. Maintaining less than 45% pC_PI was required with current testing practices to ensure low levels of daily infections and deaths. If widespread community transmission persisted, isolating the elderly does not lower daily death rates significantly. Increased testing, isolation of symptomatic infections, and contact tracing permitted 60% pC_PI without significant increases in daily deaths before September, although this strategy may not be sufficient to eliminate community transmission. This combination strategy also allowed opening of schools with <15 daily deaths. Inpatient antiviral treatment reduces deaths significantly without lowering cases or hospitalizations. Conclusions *We predict that widespread implementation of "test and isolate" policy alone is insufficient to prevent the rapid re-emergence of SARS CoV-2 without moderate physical distancing. However, widespread testing, contact tracing and case isolation would allow relaxation of physical distancing, as well as opening of schools, without a surge in local cases and deaths*. [**note: from Hutchinson Cancer Research Center in Seattle a way forward for school reopening based on modelling in that area.**]
https://www.medrxiv.org/content/10.1101/2020.08.14.20174649v1

- Background: To suppress the COVID-19 outbreak, the Norwegian government closed all schools on March 13, 2020. The kindergartens reopened on April 20, and the schools on April 27 and May 11 of 2020. The effect of these measures is largely unknown since the role of children in the spread of the SARS-CoV-2 virus is still unclear. There are only a few studies of school closures as a separate intervention to other social distancing measures, and little research exists on the effect of school opening during a pandemic. Objective: This study aimed to model the effect of opening kindergartens and the schools in Norway in terms of a change in the reproduction number (R). A secondary objective was to assess if we can use the estimated R after school openings to infer the rates of transmission between children in schools. Methods: We used an individual-based model (IBM) to assess the reopening of kindergartens and schools in two Norwegian cities, Oslo, the Norwegian capital, with a population of approximately 680 000, and Tromsoe, which is the largest city in Northern Norway, with a population of approximately 75 000. The model uses demographic information and detailed data about the schools in both cities. We carried out an ensemble study to obtain robust results in spite of the considerable uncertainty that remains about the transmission of SARS-CoV-2. Results: We found that reopening of Norwegian kindergartens and schools are associated with a change in R of 0.10 (95%CI 0.04-0.16) and 0.14 (95%CI 0.01-0.25) in the two cities under investigation if the in-school transmission rates for the SARS-CoV-2 virus are equal to what Ferguson et al. have previously estimated for influenza pandemics [1]. *Conclusion: We found only a limited effect of reopening schools on the reproduction number, and we expect the same to hold true in other countries where nonpharmaceutical interventions have suppressed the pandemic. Consequently, current R-estimates are insufficiently accurate for determining the transmission rates in schools. For countries that have not opened schools yet, planned interventions, such as the opening of selected schools, can be useful to infer general knowledge about children-to-children transmission of SARS-CoV-2*. [**note: a good summary of the Norwegian experience with school**

reopenings this past spring. Norway has less population density than many US school districts so the experience may not translate.]
https://www.medrxiv.org/content/10.1101/2020.08.14.20174896v1

- The current explosive outbreak of coronavirus (COVID-19) is posing serious threats to public health and economy around the world. To clarify the coupling mechanism between this disease and economy, a new dynamical system is established. It is theoretically proved that the basic reproduction number is a nonlinear combination of parameters regarding disease transmission, intervention and economy effect, which totally determines the stability of the disease-free and endemic equilibria. Further results indicate the existence of interaction and mutual restraint among the transmission, intervention and economy, in which strong coupling of COVID-19 and economy would trigger disease outbreak and form poverty trap, while adaptive isolation of at-risk population could effectively reduce morbidity at the cost of least economic loss. Our findings can offer new insights to improve the intervention strategies against COVID-19. [**note: here is a mode for the academic economists among my readers! It's from China and focuses on economic development and COVID-19.**]
https://www.medrxiv.org/content/10.1101/2020.08.13.20174755v1

NEWLY REGISTERED CLINICAL TRIALS

- Hey, it's Sunday! Did you really think I would spend time pouring over the clinical trials database?

CLINICAL TRIAL RESULTS

- COVID-19 represents a global crisis, yet major knowledge gaps remain about human immunity to SARS-CoV-2. We analyzed immune responses in 76 COVID-19 patients and 69 healthy individuals from Hong Kong and Atlanta. In PBMCs of COVID-19 patients, there was reduced expression of HLA-DR and pro-inflammatory cytokines by myeloid cells, and impaired mTOR-signaling and IFN-α production by plasmacytoid DCs. In contrast, there were enhanced plasma levels of inflammatory mediators, including EN-RAGE, TNFSF14, and oncostatin-M, which correlated with disease severity and increased bacterial products in human plasma. Single-cell transcriptomics revealed no type-I IFN, reduced HLA-DR in myeloid cells of severe patients, and transient expression of IFN-stimulated genes. This was consistent with bulk PBMC transcriptomics, and transient, low plasma IFN-α levels during infection. These results reveal mechanisms and potential therapeutic targets for COVID-19. [**note: this is a good article to read about the immune system going out of control. Interestingly, they find enhanced levels of bacterial DNA and lipopolysaccharides suggesting that some lung involvement here that augments inflammatory cytokines. The ability to tamp down on these inflammations will be key to controlling severe COVID-19 but we still don't have much more than dexamethasone at this point.**] https://science.sciencemag.org/content/early/2020/08/10/science.abc6261
- Severe COVID-19 is characterised by fever, cough, and dyspnoea. Symptoms affecting other organ systems have been reported. The clinical associations of different patterns of symptoms can influence diagnostic and therapeutic decision-making: for example, significant differential therapeutic effects in sub-groups of patients with different severities of respiratory failure have already been reported for the only treatment so far shown to reduce mortality in COVID-19, dexamethasone. We obtained structured clinical data on 68914 patients in the UK (the ISARIC

Coronavirus Clinical Characterisation Consortium, 4C) and used a principled, unsupervised clustering approach to partition the first 33468 cases according to symptoms reported at recruitment. We validated our findings in a second group of 35446 cases recruited to ISARIC-4C, and in separate cohort of community cases. A core symptom set of fever, cough, and dyspnoea co-occurred with additional symptoms in three patterns: fatigue and confusion, diarrhoea and vomiting, or productive cough. Presentations with a single reported symptom of dyspnoea or confusion were common, and a subgroup of patients reported few or no symptoms. Patients presenting with gastrointestinal symptoms were more commonly female, had a longer duration of symptoms before presentation, and had lower 30-day mortality. Patients presenting with confusion, with or without core symptoms, were older and had a higher unadjusted mortality. Symptom clusters were highly consistent in replication analysis using a further 35446 individuals subsequently recruited to ISARIC-4C. Similar patterns were externally verified in 4445 patients from a study of self-reported symptoms of mild disease. The large scale of ISARIC-4C study enabled robust, granular discovery and replication of patient clusters. Clinical interpretation is necessary to determine which of these observations have practical utility. *We propose that four patterns are usefully distinct from the core symptom groups: gastro-intestinal disease, productive cough, confusion, and pauci-symptomatic presentations. Importantly, each is associated with an in-hospital mortality which differs from that of patients with core symptoms. These observations deepen our understanding of COVID-19 and will influence clinical diagnosis, risk prediction, and future mechanistic and clinical studies.* [**note: of course this comes from the UK who can do large observational studies quickly. These are useful criteria for clinical evaluation.**] https://www.medrxiv.org/content/10.1101/2020.08.14.20168088v1

- Objective To compare survival of subjects with COVID-19 treated in hospitals that either did or did not routinely treat patients with hydroxychloroquine or chloroquine. Methods We analysed data of COVID-19 patients treated in 9 hospitals in the Netherlands. Inclusion dates ranged from February 27th 2020, to May 15th, when the Dutch national guidelines no longer supported the use of (hydroxy)chloroquine. Seven hospitals routinely treated subjects with (hydroxy)chloroquine, two hospitals did not. Primary outcome was 21-day all-cause mortality. We performed a survival analysis using log-rank test and Cox-regression with adjustment for age, sex and covariates based on premorbid health, disease severity, and the use of steroids for adult respiratory distress syndrome, including dexamethasone. Results Among 1893 included subjects, 21-day mortality was 23.4% in 1552 subjects treated in hospitals that routinely prescribed (hydroxy)chloroquine, and 17.0% in 341 subjects that were treated in hospitals that did not. In the adjusted Cox-regression models this difference disappeared, with an adjusted hazard ratio of 1.17 (95%CI 0.88-1.55). When stratified by actually received treatment in individual subjects, the use of (hydroxy)chloroquine was associated with an increased 21-day mortality (HR 1.58; 95%CI 1.25-2.01) in the full model. Conclusions After adjustment for confounders, mortality was not significantly different in hospitals that routinely treated patients with (hydroxy)chloroquine, compared with hospitals that did not. We compared outcomes of hospital strategies rather than outcomes of individual patients to reduce the chance of indication bias. This study adds evidence against the use of (hydroxy)chloroquine in patients with COVID-19. [**note: ho hum, another negative HCQ study, this time from The Netherlands.**] https://www.medrxiv.org/content/10.1101/2020.08.14.20173369v1

DRUG DEVELOPMENT

- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the current pandemic, coronavirus disease 2019 (COVID-19), has taken a huge toll on human lives and the global economy. Therefore, effective treatments against this disease are urgently needed. Here, we established a fluorescence resonance energy transfer (FRET)-based high-throughput screening platform to screen compound libraries to identify drugs targeting the SARS-CoV-2 main protease (Mpro), in particular those which are FDA-approved, to be used immediately to treat patients with COVID-19. Mpro has been shown to be one of the most important drug targets among SARS-related coronaviruses as impairment of Mpro blocks processing of viral polyproteins which halts viral replication in host cells. Our findings indicate that the anti-malarial drug tafenoquine (TFQ) induces significant conformational change in SARS-CoV-2 Mpro and diminishes its protease activity. Specifically, TFQ reduces the alpha-helical content of Mpro, which converts it into an inactive form. Moreover, TFQ greatly inhibits SARS-CoV-2 infection in cell culture system. Hence, the current study provides a mechanistic insight into the mode of action of TFQ against SARS-CoV-2 Mpro. Moreover, the low clinical toxicity of TFQ and its strong antiviral activity against SARS-CoV-2 should warrant further testing in clinical trials. [**note: from China here is yet another antimalarial drug that may be useful. At least this one has a plausible mode of action. It's a GSK drug and has a little better half-life than HCQ of only 14 days. Will anyone take this into clinical trials?**]
  https://www.biorxiv.org/content/10.1101/2020.08.14.250258v1
- Entry of SARS-CoV-2 is facilitated by endogenous and exogenous proteases. These proteases proteolytically activate the SARS-CoV-2 spike glycoprotein and are key modulators of virus tropism. We show that SARS-CoV-2 naïve serum exhibits significant inhibition of SARS-CoV-2 entry. We identify alpha-1-antitrypsin (AAT), and to a lesser degree, alpha-2-macroglobulin (A2M) as highly abundant serum protease inhibitors that potently restrict protease-mediated entry of SARS-CoV-2. AAT inhibition of protease-mediated SARS-CoV-2 entry in vitro occurs at concentrations far below what is present in serum and bronchoalveolar tissues, suggesting that AAT effects are physiologically relevant. Moreover, AAT mutations that have been characterized to affect abundance or function are highly prevalent. In addition to the effects that AAT may have on viral entry itself, we argue that the anti-inflammatory and coagulation regulatory activity of AAT have implications for coronavirus disease 2019 (COVID-19) pathogenicity, SARS-CoV-2 tissue restriction, convalescent plasma therapies, and even potentially AAT therapy. [**note: while a nice finding, some Spanish investigators have already registered a clinical trial for AAT.**] https://www.biorxiv.org/content/10.1101/2020.08.14.248880v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- We conducted an extensive serological study to quantify population-level exposure and define correlates of immunity against SARS-CoV-2. We found that relative to mild COVID-19 cases, individuals with severe disease exhibited elevated authentic virus-neutralizing titers and antibody levels against nucleocapsid (N) and the receptor binding domain (RBD) and the S2 region of spike protein. Unlike disease severity, age and sex played lesser roles in serological responses. All cases, including asymptomatic individuals, seroconverted by 2 weeks post-PCR confirmation. RBD- and S2-specific and neutralizing antibody titers remained elevated and stable for at least 2-3 months post-onset, whereas those against N were more variable with rapid declines in many samples. Testing of 5882 self-recruited members of the local community

demonstrated that 1.24% of individuals showed antibody reactivity to RBD. However, 18% (13/73) of these putative seropositive samples failed to neutralize authentic SARS-CoV-2 virus. Each of the neutralizing, but only 1 of the non-neutralizing samples, also displayed potent reactivity to S2. Thus, inclusion of multiple independent assays markedly improved the accuracy of antibody tests in low seroprevalence communities and revealed differences in antibody kinetics depending on the viral antigen. In contrast to other reports, we conclude that immunity is durable for at least several months after SARS-CoV-2 infection. [**note: a community serology study from Arizona.**] https://www.medrxiv.org/content/10.1101/2020.08.14.20174490v1

- It is currently unknown whether acquired immunity to common alpha- and beta-coronaviruses provides cross-protection against SARS-CoV-2. In this study, we found that certain patient sera and intravenous immunoglobulins (IVIG) collected prior to the COVID-19 outbreak were cross-reactive to SARS-CoV-2 full-length Spike, S2 domain, and nucleoprotein. However, their presence did not translate into neutralizing activity against SARS-CoV-2 in vitro. Importantly, we detected serum IgG reactivity to common coronaviruses in the early sera of patients with severe COVID-19 before the appearance of anti-SARS-CoV-2 antibodies. Collectively, the results of our study indicate that pre-existing immunity to common coronaviruses does not confer cross-protection against SARS-CoV-2 in vivo. [**note: this French study point to the lack of cross-immunity to SARS-CoV-2 if exposed to common coronaviruses.**] https://www.medrxiv.org/content/10.1101/2020.08.14.20173393v1

DIAGNOSTIC DEVELOPMENT

- The Yale EUA was already reported on above the fold.

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>