2020-08-24

The video is choppy and awful. The sound is passably decent. The singing and guitar playing are sublime. Neil Young and Norah Jones on the stage at the same time for a school benefit concert, what could be better. I was a senior in college when the album with Crazy Horse came out and wore out the grooves on it. A bunch of great songs and to my mind the greatest is 'Down by the River,' here is the benefit performance: https://www.youtube.com/watch?v=RDEfIxWEID0 here is Neil Young and Crazy Horse at Farm Aid (1994): https://www.youtube.com/watch?v=TiX8Rz5C3LY Young was one of the co-founders of Farm Aid in the mid 1980s.

US COVID-19 STATISTICS - **Infection Rate: 1.7%; CFR: 3.1%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

Apparently President Trump's chief of staff, Mark Meadows, does not know how clinical trials work. Subjects need to be enrolled, data needs to be gathered, and case reports need to be analyzed and quantified. Unless Mr. Meadows knows of some magic way to find a shortcut, the time to do the trial and analysis is easy to quantify. Let's look at the Pfizer m-RNA vaccine. This past Friday, Pfizer said they had enrolled 11,000 of the 30,000 patients needed. Two injections are required, the second one is 21 days from the initial shot. The vaccine is being tested globally, currently enrolling in the US, Brazil, and Argentina with additional enrollment in Germany, Turkey, and South Africa. Assuming enrollment is completed early September, the two-shot vaccination regimen will be completed by the end of that month at the latest. I do not know what the evaluation time frame to judge how protective the vaccine is. According to the trial description, confirmed COVID-19 cases are measured beginning seven days after the second vaccination. Pfizer say the expected end date for the trial is November 11. This means the last enrolled patient would be observed for about six weeks If I have done my math correctly. The linked press release states Pfizer may be in a position to seek regulatory review as early as this October and perhaps they will have compelling but incomplete data if that is the goal. This assumes everything goes right with the trial. One further note, Pfizer indicate 100 million doses would be ready by the end of the year. With a two-dose regimen, this would be enough to immunize 50m. We do not know whether all of these 50m would be available to the US.

The Guardian has an article on the latest Emergency Use Authorization announcement from the Administration yesterday evening. Alert readers will note I linked to a study on convalescent serum that showed no mortality benefit in a Rhode Island study. Clinical trials are underway in numerous locations that will provide the definitive answer. The claim for 35% reduction in morality was made with no documentation about how this number was arrived at.

The Washington Post also weighs in on the EUA plasma matter. It may be of help, but is it just an incremental treatment? Will it slow down monoclonal antibody trials? Here is an article that presents a compelling reason to put a halt to the football nonsense gripping some colleges. The director of sports cardiology at Ohio State University (a school that is not playing this fall) shows that there is an alarmingly high rate of myocarditis in college athletes who recovered from COVID-19. Great news for Univ of North Carolina, football players and some other athletic sport are cleared to return to practice on Monday. Of course, normal students cannot return to their classrooms but priorities are priorities! A Virginia family of five had a not so pleasant experience with COVID-19. The parents have mild cases and the two teenage sons end up hospitalized ending up on ECMO. The daughter remained healthy. The boys are home recovering. Anytime you hear that young people are not affected, send them this story

The New York Times also has an article on the FDA EUA for plasma treatment. The downward trend in COVID-19 cases indicates that restrictions may be working. Cases in the US still are much higher than they need to be.

STAT weighs in on the FDA EUA for plasma. Here is their Q&A. There is also a paper discussing whether COVID-19 has become less lethal in the US. I publish infection and mortality numbers daily based on the Washington Post tracking values. For the 'current wave', the CFR has remained at or slightly above 2%. As my readers are painfully aware, we continue to rely on numbers based on 'documented' cases of infection and do not know the real value. As the authors of this piece note, extrapolated mortality rates are coalescing around 0.65% which is the high range of the 'guess' I made back in mid-March where I thought it would level out between 0.3-0.6%.

JAMA have the results of a phase 3 trial of remdesivir. Among patients with moderate COVID-19, those randomized to a 10-day course of remdesivir did not have a statistically significant difference in clinical status compared with standard care at 11 days after initiation of treatment. Patients randomized to a 5-day course of remdesivir had a statistically significant difference in clinical status compared with standard care, but the difference was of uncertain clinical importance. Here is an accompanying editorial. Remdesivir still has not been shown to be anything more than a drug with modest activity against COVID-19. Perhaps that will change with more data. Here is a patient page on prone positioning for acute respiratory distress syndrome (ARDS).

Nature have an interview with the head of EcoHealth Alliance whose research in China was been shut down. NIH has reinstated the grant with conditions. I'll let readers judge for themselves whether this is a good outcome.

MODELING

- Controlling the spread of COVID-19 -- even after a licensed vaccine is available -- requires the effective use of non-pharmaceutical interventions, e.g., physical distancing, limits on group sizes, mask wearing, etc.. To date, such interventions have neither been uniformly nor systematically implemented in most countries. For example, even when under strict stay-at-home orders, numerous jurisdictions granted exceptions and/or were in close proximity to locations with entirely different regulations in place. Here, we investigate the impact of such geographic inconsistencies in epidemic control policies by coupling search and mobility data to a simple mathematical model of SARS-COV2 transmission. Our results show that while stay-at-home orders decrease contacts in most areas of the United States of America (US), some specific activities and venues often see an increase in attendance. Indeed, over the month of March 2020, between 10 and 30% of churches in the US saw increases in attendance; even as the total number of visits to churches declined nationally. This heterogeneity, where certain venues see substantial increases in attendance while others close, suggests that closure can cause individuals to find an open venue, even if that requires longer-distance travel. And, indeed, the average distance travelled to churches in the US rose by 13% over the same period. Strikingly, our mathematical model reveals that, across a broad range of model parameters, partial measures can often be worse than no measures at all. In the most severe cases, individuals not complying with policies by traveling to neighboring jurisdictions can create epidemics when the outbreak would otherwise have been controlled. *Taken together, our data*

analysis and modelling results highlight the potential unintended consequences of inconsistent epidemic control policies and stress the importance of balancing the societal needs of a population with the risk of an outbreak growing into a large epidemic. [**note: this is a useful model looing at inconsistent pandemic control policies.**]
https://www.medrxiv.org/content/10.1101/2020.08.21.20179473v1

- Previous work has indicated that contact tracing and isolation of index case and quarantine of potential secondary cases can, in concert with physical distancing measures, be an effective strategy for reducing transmission of SARS-CoV-2. Currently, contacts traced manually through the NHS Test and Trace scheme in the UK are asked to self-isolate for 14 days from the day they were exposed to the index case, which represents the upper bound for the incubation period. However, following previous work on screening strategies for air travellers it may be possible that this quarantine period could be reduced if combined with PCR testing. Adapting the simulation model for contact tracing, we find that quarantine periods of at least 10 days combined with a PCR test on day 9 may largely emulate the results from a 14-day quarantine period in terms of the averted transmission potential from secondary cases (72% (95%UI: 3%, 100%) vs 75% (4%, 100%), respectively). These results assume the delays from testing index cases' and tracing their contacts are minimised (no longer than 4.5 days on average). If secondary cases are traced and quarantined 1 day earlier on average, shorter quarantine periods of 8 days with a test on day 7 (76% (7%, 100%)) approach parity with the 14 day quarantine period with a 1 day longer delay to the index cases' test. However, the risk of false-negative PCR tests early in a traced case's infectious period likely prevents the use of testing to reduce quarantine periods further than this, and testing immediately upon tracing, with release if negative, will avert just 17% of transmission potential on average. In conclusion, the use of PCR testing is an effective strategy for reducing quarantine periods for secondary cases, while still reducing transmission of SARS-CoV-2, especially if delays in the test and trace system can be reduced, and may improve quarantine compliance rates. [**note: from the UK, testing strategies can help with track and trace quarantine.**]
https://www.medrxiv.org/content/10.1101/2020.08.21.20177808v1
- Background School closures are a well-established non-pharmaceutical intervention in the event of infectious disease outbreaks, and have been implemented in many countries across the world, including the UK, to slow down the spread of SARS-CoV-2. As governments begin to relax restrictions on public life there is a need to understand the potential impact that reopening schools may have on transmission. Methods We used data provided by the UK Department for Education to construct a network of English schools, connected through pairs of pupils resident at the same address. We used the network to evaluate the potential for transmission between schools, and for long range propagation across the network, under different reopening scenarios. Results Amongst the options evaluated we found that reopening only Reception, Year 1 and Year 6 (4-6 and 10-11 year olds) resulted in the lowest risk of transmission between schools, with outbreaks within a single school unlikely to result in outbreaks in adjacent schools in the network. The additional reopening of Years 10 and 12 (14-15 and 16-17 year olds) resulted in an increase in the risk of transmission between schools comparable to reopening all primary school years (4-11 year olds). However, the majority of schools presented low risk of initiating widespread transmission through the school system. Reopening all secondary school years (11-18 year olds) resulted in large potential outbreak clusters putting up to 50% of

households connected to schools at risk of infection if sustained transmission within schools was possible. Conclusions Reopening secondary school years is likely to have a greater impact on community transmission than reopening primary schools in England. Keeping transmission within schools limited is essential for reducing the risk of large outbreaks amongst school-aged children and their household members. [**note: modeling school reopening in the UK.**] https://www.medrxiv.org/content/10.1101/2020.08.21.20167965v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a Phase 2, randomized, double-blind, placebo-controlled, parallel-group, multicenter, dose escalation and proof of concept study to evaluate the safety and efficacy of razuprotafib subcutaneously administered three times daily (TID) in hospitalized subjects with moderate to severe COVID-19. Part 1 of the study is a 2-step dose escalation period conducted in approximately 60 subjects. Part 2 is a safety and efficacy period evaluating razuprotafib doses selected from Part 1 and will be conducted in approximately 120 subjects. Subjects will receive razuprotafib or placebo TID for 7 days or until discharge from the hospital (or death) and will be evaluated for safety and efficacy through Day 28. The effects of razuprotafib on biomarkers of coagulation, inflammation and vascular leakage will also be evaluated. [**note: the trial sponsor is Aerpio Therapeutics**] NCT04511650
- For decades, the antiviral action of Carrageenans has been described in numerous studies with different viruses that infect humans: herpes viruses types 1 and 2, human immunodeficiency virus, human papillomavirus, H1N1 influenza virus, dengue virus, rhinovirus, hepatitis A virus, and enteroviruses. Studies on the dynamics of COVID-19 disease show an intense and rapid pharyngeal multiplication in the first 3-5 days of the onset of symptoms, prior to the onset of pulmonary disease. Finally, this molecule has shown a viricidal effect against SARS-Cov2 in vitro. All this underscores the potential value of a therapy that inhibits the virus in the rhinopharynx. [**note: I linked to a paper showing viricidal activity of this compound and bingo, here is an Argentinian trial looking at a nasal delivery system.**] NCT04521322

CLINICAL TRIAL RESULTS

- The role of renin-angiotensin-aldosterone system (RAAS) inhibitors, notably angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs), in the COVID-19 pandemic has not been fully evaluated. With an increasing number of COVID-19 cases worldwide, it is imperative to better understand the impact of RAAS inhibitors in hypertensive COVID patients. PubMed, Embase and the pre-print database Medrxiv were searched, and studies with data on patients on ACEi/ARB with COVID-19 were included. Random effects models were used to estimate the pooled mean difference with 95% confidence interval using Open Meta[Analyst] software. A total of 28,872 patients were included in this meta-analysis. The use of any RAAS inhibition for any conditions showed a trend to lower risk of death/critical events (OR 0.671, CI 0.435 to 1.034, $p = 0.071$). Within the hypertensive cohort, however, there was a significant lower association with deaths (OR 0.664, CI 0.458 to 0.964, $p = 0.031$) or the combination of death/critical outcomes (OR 0.670, CI 0.495 to 0.908, $p = 0.010$). There was no significant association of critical/death outcomes within ACEi vs non-ACEi (OR 1.008, CI 0.822 to 1.235, $p = 0.941$) and ARB vs non-ARB (OR 0.946, CI 0.735 to 1.218, $p = 0.668$). This is the largest meta-analysis including critical events and mortality data on patients prescribed ACEi/ARB and

found evidence of beneficial effects of chronic ACEi/ARB use especially in hypertensive cohort with COVID-19. As such, we would strongly encourage patients to continue with RAAS inhibitor pharmacotherapy during the COVID-19 pandemic. {**note: this is a large meta-analysis showing possible protection from ACEi/ARB class of drugs. There are a few clinical trials going on with ARBs but no results have been published to date. I saw one news article tout this finding as a general preventative in older patients. That is wildly optimistic at this point.**]
https://link.springer.com/article/10.1007/s11883-020-00880-6

- COVID-19 continues to cause a pandemic, having infected more than 20 million people globally. Successful elimination of the SARS-CoV-2 virus will require an effective vaccine. However, the immune correlates of infection are currently poorly understood. While neutralizing antibodies are believed to be essential for protection against infection, the contribution of the neutralizing antibody response to resolution of SARS-CoV-2 infection has not yet been defined. In this study the antibody responses to the SARS-CoV-2 spike protein and nucleocapsid proteins were investigated in a UK patient cohort, using optimised immunoassays and a retrovirus-based pseudotype entry assay. It was discovered that in severe COVID-19 infections an early antibody response to both antigens was associated with improved prognosis of infection. While not all SARS-CoV-2-reactive sera were found to possess neutralizing antibodies, neutralizing potency of sera was found to be greater in patients who went on to resolve infection, compared with those that died from COVID-19. Furthermore, viral genetic variation in spike protein was found to influence the production of neutralizing antibodies. *Infection with the recently described spike protein variant 614G produced higher levels of neutralizing antibodies when compared to viruses possessing the 614D variant. These findings support the assertion that vaccines targeting generation of neutralizing antibodies may be useful at limiting SARS-CoV-2 infection. Assessment of the antibody responses to SARS-CoV-2 at time of diagnosis will be a useful addition to the diagnostic toolkit, enabling stratification of clinical intervention for severe COVID-19 disease*. [**note: from the UK a study showing early antibody response leads to better outcomes. Patient size was 227 which is on the small side. The finding about the new SARS-CoV-2 614G variant producing higher levels of neutralizing antibodies.**]
https://www.medrxiv.org/content/10.1101/2020.08.22.20176834v1
- BACKGROUND Given that an individual's age and gender are strongly predictive of COVID-19 outcomes, do such factors imply anything about preferable therapeutic options? METHODS An analysis of electronic health records for a large (68,466-case), international COVID-19 cohort, in five-year age strata, revealed age-dependent sex differences. In particular, we surveyed the effects of systemic hormone administration in women. The primary outcome for estradiol therapy was death. Odds Ratios (ORs) and Kaplan-Meier survival curves were analyzed for 37,086 COVID-19 women in two age groups: pre- (15-49 years) and post-menopausal (>50 years). RESULTS The incidence of SARS-CoV-2 infection is higher in women than men (about +15%) and, in contrast, the fatality rate is higher in men (about +50%). Interestingly, the relationships between these quantities are also linked to age. Pre-adolescent girls had the same risk of infection and fatality rate as boys. Adult premenopausal women had a significantly higher risk of infection than men in the same five-year age stratum (about 16,000 vs. 12,000 cases). This ratio changed again in postmenopausal women, with infection susceptibility converging with men. While fatality rates increased continuously with age for both sexes, at 50 years there was a steeper increase for men. Thus far, these types of intricacies have been largely neglected.

Because the hormone 17β-estradiol has a positive effect on expression of the human ACE2 protein--which plays an essential role for SARS-CoV-2 cellular entry--propensity score matching was performed for the women's sub-cohort, comparing users versus non-users of estradiol. *This retrospective study of hormone therapy in female COVID-19 patients shows that the fatality risk for women >50 yrs receiving estradiol therapy (user group) is reduced by more than 50%; the OR was 0.33, 95 % CI [0.18, 0.62] and the Hazard Ratio was 0.29, 95% CI [0.11,0.76]. For younger, pre-menopausal women (15-49 yrs), the risk of COVID-19 fatality is the same irrespective of estradiol treatment, probably because of higher endogenous estradiol levels. CONCLUSIONS As of this writing, still no effective drug treatment is available for COVID-19; since estradiol shows such a strong improvement regarding fatality in COVID-19, we suggest prospective studies on the potentially more broadly protective roles of this naturally occurring hormone*. [**note: here is a large cohort study from Italy on the effect of estradiol on mortality in women and is an interesting finding.**] https://www.medrxiv.org/content/10.1101/2020.08.21.20179671v1

- A central paradigm of immunity is that interferon (IFN) mediated antiviral responses precede the pro-inflammatory ones, optimizing host protection and minimizing collateral damage. Here, we report that for COVID-19 this does not apply. By investigating temporal IFN and inflammatory cytokine patterns in 32 COVID-19 patients hospitalized for pneumonia and longitudinally followed for the development of respiratory failure and death, we reveal that IFN-λ and type I IFN production is both diminished and delayed, induced only in a fraction of patients as they become critically ill. On the contrary, pro-inflammatory cytokines such as TNF, IL-6 and IL-8 are produced before IFNs, in all patients, and persist for a prolonged time. By comparison, in 16 flu patients hospitalized for pneumonia with similar clinicopathological characteristics to COVID-19 and 24 milder non-hospitalized flu patients IFN-λ and type I IFN are robustly induced, earlier, at higher levels and independently of disease severity, while pro-inflammatory cytokines are only acutely and transiently produced. *Notably, higher IFN-λ levels in COVID-19 patients correlate with lower viral load in bronchial aspirates and faster viral clearance, and a higher IFN-λ:type I IFN ratio with improved outcome of critically ill patients. Moreover, altered cytokine patterns in COVID-19 patients correlate with longer hospitalization time and higher incidence of critical disease and mortality compared to flu. These data point to an untuned antiviral response in COVID-19 contributing to persistent viral presence, hyperinflammation and respiratory failure*. [**note: from Greece, more information on the immune system response.**]
  https://www.medrxiv.org/content/10.1101/2020.08.21.20179291v1

DRUG DEVELOPMENT

- Nothing new

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 is a novel coronavirus that emerged in 2019 and is now classified in the genus Coronavirus with closely related SARS-CoV. SARS-CoV-2 is highly pathogenic in humans and is classified as a biosafety level (BSL)-3 pathogen, which makes manipulating it relatively difficult due to its infectious nature. To circumvent the need for BSL-3 laboratories, an alternative assay was developed that avoids live virus and instead uses a recombinant VSV expressing luciferase and possesses the full length or truncated spike proteins of SARS-CoV-2. Furthermore, to

measure SARS-CoV-2 neutralizing antibodies under BSL2 conditions, a chemiluminescence reduction neutralization test (CRNT) for SARS-CoV-2 was developed. The neutralization values of the serum samples collected from hospitalized patients with COVID-19 or SARS-CoV-2 PCR-negative donors against the pseudotyped virus infection evaluated by the CRNT were compared with antibody titers determined from an immunofluorescence assay (IFA). The CRNT, which used whole blood collected from hospitalized patients with COVID-19, was also examined. As a result, the inhibition of pseudotyped virus infection was specifically observed in both serum and whole blood and was also correlated with the results of the IFA. In conclusion, the CRNT for COVID-19 is a convenient assay system that can be performed in a BSL-2 laboratory with high specificity and sensitivity for evaluating the occurrence of neutralizing antibodies against SARS-CoV-2. [**note: these Japanese researchers cloned the Spike protein into vesicular stomatitis virus that can be used as a pseudo-virus for evaluating neutralizing antibodies. This is also the vector that Merck are using for one of their vaccine candidates.**]
https://www.biorxiv.org/content/10.1101/2020.08.21.262295v1

DIAGNOSTIC DEVELOPMENT

- Nothing new

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

2020-08-25

I don't think I have had any Handel since the Messiah excepts around Easter time. Let us remedy this oversight with the 'Water Music' played on period instruments by the Akademie für alte Musik Belin. This is a live performance from 2016: https://www.youtube.com/watch?v=EVAB2z1RPu4 The first performance took place on the River Thames in London. I cannot find any record about how many times it has been played in/on the original setting.

US COVID-19 STATISTICS - **Infection Rate: 1.7%; CFR: 3.0%** (IR unchanged; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post has an op-ed criticizing the FDA EUA decision for convalescent plasma along with a news article. China seems to be using their COVID-19 vaccine way beyond clinical trial numbers. Is it considered still experimental? In Manaus Brazil the COVID-19 epidemic appears to have abated though the reason for this is unclear. Is it herd or relative immunity? IIRC, there was a paper back in late March or Early April that predicted something similar for the Lombardy region of Italy. West Germany conducted an indoor concert experiment to see how such gatherings can be conducted safely. EPA have given an emergency use authorization for a long lived viricide that can be used on environmental surfaces. The product is an electrostatic applied quaternary ammonium polymer that is non-toxic to humans.

The New York Times also weighs in on the FDA's misunderstanding of basic statistics about the use of convalescent plasma. Here is a reinfection case from Hong Kong but the man did not experience any clinical symptoms. It's not clear what this means. More on oleandrin, "the miracle cure" for COVID-19.

The Guardian covers the new outbreak of COVID-19 in Spain. Van Morrison is a great singer but has no understanding of infectious disease.

STAT discuss four scenarios for developing immunity to SARS-CoV-2. FDA Commissioner Stephen Hahn is having "…a terrible, horrible, no good, very bad day." (apologies to Judith Viorst and Ray Cruz for appropriating the title of their wonderful children's book) There is this interesting piece on personal risk assessment and polarization.

I would point your attention to an intriguing paper down in the Virus Biochemistry section on a novel SARS-CoV-2 mutation that appears to raise neutralizing antibodies but has lost the ability to cause lethal infection in an animal model. Could this be an attenuated version of the virus that could outcompete the lethal strain? There are also a number of good drug discovery papers today.

MODELING

- The coronavirus disease 2019 (COVID-19) outbreak is reasonably contained in China. In this paper, we evaluated the effectiveness of different containment strategies in halting the pandemic spread in both short- and long-term. We combined a networked metapopulation SEIR model featuring undocumented infections, actual mobility data and Bayesian inference to simulate the counterfactual outbreak scenarios removing each one or a combination of the following three policies in place: i) city lockdowns, ii) intercity travel bans, and iii) testing, detection, and quarantine. Our estimates revealed that 11.4% [95% credible interval (CI): 9.7-13.0%] of the infected cases were unidentified before January 23, 2020. The rate grew to 92.5% [95% credible interval (CI): 85.9-94.5%] in early March, thanks to the boost in coronavirus testing capacity. We show that increasing the detection rate of infections from 11.4% to 92.5% alone would explain 75% of the reduction in infections from a no-policy baseline by March 15, 2020. The most pronounced policy implication is that city lockdowns appeared to be the more effective intervention in the short-run but effective testing is essential in containing the COVID-19 spread in the long run. By March 15, restoring within-city personal contact to its 2019 level would lead to a 678% growth in infections with all the other interventions remaining unaffected. Removing intercity travel restrictions and effective detection measures would lead to 3% and 477% growth, respectively. Extending the time horizon to July 15, the counterfactual increase in infections would become 581%, 3% and 30000% had the three classes of interventions been lifted individually. [**note: modeling various policies in China.**]
https://www.medrxiv.org/content/10.1101/2020.08.22.20179697v1
- Cloth face coverings and surgical masks have become commonplace across the United States in response to the SARS-CoV-2 epidemic. While evidence suggests masks help curb the spread of respiratory pathogens, research is limited. Face masks have quickly become a topic of public debate as government mandates have started requiring their use. Here we investigate the association between self-reported mask wearing, social distancing and community SARS-CoV-2 transmission in the United States, as well as the effect of statewide mandates on mask uptake. Methods: Serial cross-sectional surveys were administered June 3 through July 31, 2020 via web platform. Surveys queried individuals' likelihood to wear a face mask to the grocery store or with family and friends. Responses (N=378,207) were aggregated by week and state and combined with measures of the instantaneous reproductive number (Rt), social distancing proxies, respondent demographics and other potential sources of confounding. We fit multivariate logistic regression models to estimate the association between mask wearing and community transmission control (Rt <1) for each state and week. Multiple sensitivity analyses were considered to corroborate findings across mask wearing definitions, Rt estimators and

data sources. Additionally, mask wearing in 12 states was evaluated two weeks before and after statewide mandates. Results: We find an upward trend in mask usage across the U.S., although uptake varies by geography and demographic groups. A multivariate logistic model controlling for social distancing and other variables found a 10% increase in mask wearing was associated with a 3.53 (95% CI: 2.03, 6.43) odds of transmission control (Rt <1). We also find that communities with high mask wearing and social distancing have the highest predicted probability of a controlled epidemic. These positive associations were maintained across sensitivity analyses. Segmented regression analysis of mask wearing found no statistical change following mandates, however the positive trend of increased mask wearing over time was preserved. Conclusion: Widespread utilization of face masks combined with social distancing increases the odds of SARS-CoV-2 transmission control. Mask wearing rose separately from government mask mandates, suggesting supplemental public health interventions are needed to maximize mask adoption and disrupt the spread of SARS-CoV-2, especially as social distancing measures are relaxed. [**note: mask wearing works to control SARS-CoV-2 infections. Unless there is some dramatic new evidence, the will be the last mask paper posted in the newsletter.**] https://www.medrxiv.org/content/10.1101/2020.08.23.20078964v1

- We propose a novel Timed Intervention SPEIQRD extended SEIR model for predicting the evolution of the Covid 19 Pandemic in the USA. The model can, by parameter, assignment, be reduced to the model of Hong et al, which appeared in February, 2020. Novel aspects of the proposed model include 1. Formulation of a "Protected" population P, which can be viewed as a "Sheltered in Place", unexposed population which, starting at time t = \Tau_P, builds up and stores a reservoir of unexposed Population; 2. This "Protection Intervention" provides the basis for a second, "Timed Release" Intervention: on receiving a "reopening signal" at time t = \tau_R$, this second intervention initiates a release of the stored population P back into the general population S. 3. Selection of model parameters to "optimize" the approximation of the model up to the present, and then projecting simulation of the model based on the value thus obtained 100,200, 365, and 730 days into the future. This model shows excellent qualitative results and quantitative results that are good, considering the chosen nationwide scope. These results compare favorably to University of Washington projections, as published Daily on the Worldometers.info website. The qualitative and quantitative behavior of all 7}state variables S,P,E,I,Q,R,D will be illustrated and discussed, in this and possible future further Intervention cycles\footnote{"How long, I wondered, will this thing, last?} Lyric from "A Foggy Day" by George and Ira Gershwin} [**note: any author who references a Gershwin song in an abstract is guaranteed mention in my newsletter!!!! This is an interesting model paper to read.**] https://www.medrxiv.org/content/10.1101/2020.08.23.20180174v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check

CLINICAL TRIAL RESULTS

- Nothing new

DRUG DEVELOPMENT

- Influenza virus and coronavirus, belonging to enveloped RNA viruses, are major causes of human respiratory diseases. The aim of this study was to investigate the broad spectrum antiviral activity of a naturally existing sulfated polysaccharide, lambda-carrageenan (λ-CGN), purified from marine red algae. Cell culture-based assays revealed that the macromolecule efficiently inhibited both influenza A and B viruses, as well as currently circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with EC50 values ranging from 0.3-1.4 μg/ml. No toxicity to host cells was observed at concentrations up to 300 μg/ml. Plaque titration and western blot analysis verified that λ-CGN reduced expression of viral proteins in cell lysates and suppressed progeny virus production in culture supernatants in a dose-dependent manner. This polyanionic compound exerts antiviral activity by targeting viral attachment to cell surface receptors and preventing entry. Moreover, intranasal administration to mice during influenza A viral challenge not only alleviated infection-mediated reductions in body weight but also protected 60% of mice from virus-induced mortality. Thus, λ-CGN could be a promising antiviral agent for preventing infection by several respiratory viruses. [**note: another carrageenan paper!!! Time to start cultivating red algae!! You can even buy this stuff right HERE & this one is non-GMO and Kosher certified. It's perfect for your DIY clinical trial. Be the first one on your block to cure COVID-19!**]
  https://www.biorxiv.org/content/10.1101/2020.08.23.255364v1
- Given the aggressive spread of COVID-19-related deaths, there is an urgent public health need to support the development of vaccine candidates to rapidly improve the available control measures against SARS-CoV-2. To meet this need, we are leveraging our existing vaccine platform to target SARS-CoV-2. Here, we generated cellular heat shock chaperone protein, glycoprotein 96 (gp96), to deliver SARS-CoV-2 protein S (spike) to the immune system and to induce cell-mediated immune responses. We showed that our vaccine platform effectively stimulates a robust cellular immune response against protein S. Moreover, we confirmed that gp96-Ig, secreted from allogeneic cells expressing full-length protein S, generates powerful, protein S polyepitope-specific CD4+ and CD8+ T cell responses in both lung interstitium and airways. These findings were further strengthened by the observation that protein-S -specific CD8+ T cells were induced in human leukocyte antigen (HLA)-A2-02-01 transgenic mice thus providing encouraging translational data that the vaccine is likely to work in humans, in the context of SARS-CoV-2 antigen presentation. [**note: another vaccine prototype]**
  https://www.biorxiv.org/content/10.1101/2020.08.24.265090v1
- We screened steroid compounds to obtain a drug expected to block host inflammatory responses and MERS-CoV replication. Ciclesonide, an inhaled corticosteroid, suppressed replication of MERS-CoV and other coronaviruses, including SARS-CoV-2, the cause of COVID-19, in cultured cells. The effective concentration (EC_{90}) of ciclesonide for SARS-CoV-2 in differentiated human bronchial tracheal epithelial cells was 0.55 μM. Ciclesonide inhibited formation of double membrane vesicles, which anchor the viral replication-transcription complex in cells. Eight consecutive passages of 43 SARS-CoV-2 isolates in the presence of ciclesonide generated 15 resistant mutants harboring single amino acid substitutions in non-structural protein 3 (nsp3) or nsp4. Of note, ciclesonide still suppressed replication of all these mutants by 90% or more, suggesting that these mutants cannot completely overcome ciclesonide blockade. These observations indicate that the suppressive effect of ciclesonide on viral replication is specific to coronaviruses, highlighting it as a candidate drug for the treatment

of COVID-19 patients. [**note: give everybody an inhaler!!!**]
https://www.biorxiv.org/content/10.1101/2020.08.22.258459v1

- Effective clinical intervention strategies for COVID-19 are urgently needed. Although several clinical trials have evaluated the use of convalescent plasma containing virus-neutralizing antibodies, the effectiveness has not been proven. We show that hamsters treated with a high dose of human convalescent plasma or a monoclonal antibody were protected against weight loss showing reduced pneumonia and pulmonary virus replication compared to control animals. *However, a ten-fold lower dose of convalescent plasma showed no protective effect. Thus, variable and relatively low levels of virus neutralizing antibodies in convalescent plasma may limit their use for effective antiviral therapy, favouring concentrated, purified (monoclonal) antibodies*. [**note: from Erasmus Univ in Holland who were one of the first groups to identify an mAb. Note the conclusion!!! Is FDA's decision to move forward with plasma hurting mAb clinical trial recruitment?**] https://www.biorxiv.org/content/10.1101/2020.08.24.264630v1
- In the light of the recent accumulated knowledge on SARS-CoV-2 and its mode of human cells invasion, the binding of viral spike glycoprotein to human Angiotensin Converting Enzyme 2 (hACE2) receptor plays a central role in cell entry. We designed a series of peptides mimicking the N-terminal helix of hACE2 protein which contains most of the contacting residues at the binding site and have a high helical folding propensity in aqueous solution. Our best peptide mimics bind to the virus spike protein with high affinity and are able to block SARS-CoV-2 human pulmonary cell infection with an inhibitory concentration (IC50) in the nanomolar range. These first in class blocking peptide mimics represent powerful tools that might be used in prophylactic and therapeutic approaches to fight the coronavirus disease 2019 (COVID-19). [**note: from Paris, a ACE2 peptide mimic that blocks SARS-CoV-2 pulmonary cell infection. There are other research groups that are doing similar work.**]
https://www.biorxiv.org/content/10.1101/2020.08.24.264077v1
- The outbreak of COVID-19 has severely impacted global health and the economy. Cost-effective, highly efficacious therapeutics are urgently needed. Here, we used camelid immunization and proteomics to identify a large repertoire of highly potent neutralizing nanobodies (Nbs) to the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD). We discovered multiple elite Nbs with picomolar to femtomolar affinities that inhibit viral infection at sub-ng/ml concentration, more potent than some of the best human neutralizing antibodies. We determined a crystal structure of such an elite neutralizing Nb in complex with RBD. Structural proteomics and integrative modeling revealed multiple distinct and non-overlapping epitopes and indicated an array of potential neutralization mechanisms. Structural characterization facilitated the bioengineering of novel multivalent Nb constructs into multi-epitope cocktails that achieved ultrahigh neutralization potency (IC50s as low as 0.058 ng/ml) and may prevent mutational escape. These thermostable Nbs can be rapidly produced in bulk from microbes and resist lyophilization, and aerosolization. These promising agents are readily translated into efficient, cost-effective, and convenient therapeutics to help end this once-in-a-century health crisis. [**note: more good nanobody work. Will any of this ever make it into the clinic?**]
https://www.biorxiv.org/content/10.1101/2020.08.24.264333v2
- SARS-CoV2 infection leads to cardiac injury and dysfunction in 20-30% of hospitalized patients and higher rates of mortality in patients with pre-existing cardiovascular disease. Inflammatory factors released as part of the 'cytokine storm' are thought to play a critical role in cardiac

dysfunction in severe COVID-19 patients. Here we use human cardiac organoids combined with high sensitivity phosphoproteomics and single nuclei RNA sequencing to identify inflammatory targets inducing cardiac dysfunction. This state-of-the-art pipeline allowed rapid deconvolution of mechanisms and identification of putative therapeutics. We identify a novel interferon-γ driven BRD4 (bromodomain protein 4)-fibrosis/iNOS axis as a key intracellular mediator of inflammation-induced cardiac dysfunction. This axis is therapeutically targetable using BRD4 inhibitors, which promoted full recovery of function in human cardiac organoids and prevented severe inflammation and death in a cytokine-storm mouse model. The BRD inhibitor INCB054329 was the most efficacious, and is a prime candidate for drug repurposing to attenuate cardiac dysfunction and improve COVID-19 mortality in humans. [**note: good work from Australia on a novel drug target.]**
https://www.biorxiv.org/content/10.1101/2020.08.23.258574v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 infection induces a T cell response that most likely contributes to virus control in COVID-19 patients, but may also induce immunopathology. Until now, the cytotoxic T cell response has not been very well characterized in COVID-19 patients. Here, we analyzed the differentiation and cytotoxic profile of T cells in 30 cases of mild COVID-19 during acute infection. SARS-CoV-2 infection induced a cytotoxic response of CD8+ T cells, but not CD4+ T cells, characterized by the simultaneous production of granzyme A and B, as well as perforin within different effector CD8+ T cell subsets. PD-1 expressing CD8+ T cells also produced cytotoxic molecules during acute infection indicating that they were not functionally exhausted. *However, in COVID-19 patients over the age of 80 years the cytotoxic T cell potential was diminished, especially in effector memory and terminally differentiated effector CD8+ cells, showing that elderly patients have impaired cellular immunity against SARS-CoV-2.* Our data provides valuable information about T cell responses in COVID-19 patients that may also have important implications for vaccine development. [**note: more good information on immune response but not for those in the elderly category. The big question is whether a vaccine will be useful in this patient population and which one it would be.**]
https://www.biorxiv.org/content/10.1101/2020.08.21.262329v1
- SARS-CoV-2 contains a PRRA polybasic cleavage motif considered critical for efficient infection and transmission in humans. We previously reported that virus variants with spike protein S1/S2 junction deletions spanning this motif are attenuated. Here we characterize a further cell-adapted SARS-CoV-2 variant, Ca-DelMut. Ca-DelMut replicates more efficiently than wild type or parental virus in cells, but causes no apparent disease in hamsters, despite replicating in respiratory tissues. Unlike wild type virus, Ca-DelMut does not induce proinflammatory cytokines in hamster infections, but still triggers a strong neutralizing antibody response. Ca-DelMut-immunized hamsters challenged with wild type SARS-CoV-2 are fully protected, demonstrating sterilizing immunity. [**note: this is really interesting. Here is a virus variant that replicates more efficiently than the wild type but causes no disease in hamsters. It does trigger a strong neutralizing antibody response. The authors note that this provokes a different type of immune response than the wild type and that it might be an ideal strain for production of an inactivated vaccine in addition to holding promise as a live attenuated**

vaccine – paging Dr. Albert Sabin!]
https://www.biorxiv.org/content/10.1101/2020.08.24.264192v1

- The D614G mutation of the Spike protein is thought to be relevant for SARS-CoV-2 infection. Here we report biological and epidemiological aspects of this mutation. Using pseudotyped lentivectors, we were able to confirm that the G614 variant of the Spike protein is markedly more infectious than the ancestral D614 variant. We demonstrate by molecular modelling that the replacement of aspartate by glycine in position 614 facilitates the transition towards an open state of the Spike protein. To understand whether the increased infectivity of the D614 variant explains its epidemiological success, we analysed the evolution of 27,086 high-quality SARS-CoV-2 genome sequences from GISAID. We observed striking coevolution of D614G with the P323L mutation in the viral polymerase. Importantly, exclusive presence of G614 or L323 did not become epidemiologically relevant. In contrast, the combination of the two mutations gave rise to a viral G/L variant that has all but replaced the initial D/P variant. There was no significant correlation between reported COVID mortality in different countries and the prevalence of the Wuhan versus G/L variant. However, when comparing the speed of emergence and the ultimate predominance in individual countries, the G/L variant displays major epidemiological supremacy. Our results suggest that the P323L mutation, located in the interface domain of the RNA-dependent RNA polymerase (RdRp), is a necessary alteration that led to the epidemiological success of the present variant of SARS-CoV-2. [**note: more on the D614G viral mutation.**] https://www.medrxiv.org/content/10.1101/2020.08.23.20180281v1
- The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) affects millions of people and killed hundred-thousands of individuals. While acute and intermediate interactions between SARS-CoV-2 and the immune system have been studied extensively, long-term impacts on the cellular immune system remained to be analyzed. Here, we comprehensively characterized immunological changes in peripheral blood mononuclear cells in 49 COVID-19 convalescent individuals (CI) in comparison to 27 matched SARS-CoV-2 unexposed individuals (UI). Despite recovery from the disease for more than 2 months, CI showed significant decreases in frequencies of invariant NKT and NKT-like cells compared to UI. Concomitant with the decrease in NKT-like cells, an increase in the percentage of Annexin V and 7-AAD double positive NKT-like cells was detected, suggesting that the reduction in NKT-like cells results from cell death months after recovery. Significant increases in regulatory T cell frequencies, TIM-3 expression on CD4 and CD8 T cells, as well as PD-L1 expression on B cells were also observed in CI, while the cytotoxic potential of T cells and NKT-like cells, defined by GzmB expression, was significantly diminished. However, both CD4 and CD8 T cells of CI showed increased Ki67 expression and were fully capable to proliferate and produce effector cytokines upon TCR stimulation. Collectively, we provide the first comprehensive characterization of immune signatures in patients recovering from SARS-CoV-2 infection, suggesting that the cellular immune system of COVID-19 patients is still under a sustained influence even months after the recovery from disease. [**note: from China and Germany, long term impact on the immune system in recovered patients. It will be important to gather more data on this to see how prevalent this is and what symptoms if any these patients exhibit. Someone needs to set up a registry that can track long-term outcomes with respect to a variety of impacts.**]
https://www.medrxiv.org/content/10.1101/2020.08.21.20179358v1

- The breadth of the humoral immune response following SARS-CoV-2 infection was indicated to be important for recovery from COVID-19. Recent studies have provided valuable insights regarding the dynamics of the antibody response in symptomatic COVID-19 patients. However, the information regarding the dynamics of the serological and cellular memory in COVID-19 recovered patients in scarce. It is imperative to determine the persistence of humoral memory in COVID-19 recovered patients as it will help to evaluate the susceptibility of recovered patients to re-infection. Here, we describe the dynamics of both the SARS-CoV-2 specific serological and B cell response in COVID-19 recovered patients. We found that symptomatic SARS-CoV-2 patients mount a robust antibody response following infection however, the serological memory decays in recovered patients over the period of 6 months. On the other hand, the B cell response as observed in the SARS-CoV-2 specific memory B cell compartment, was found to be stable over time. Moreover, the frequency of SARS-CoV-2 specific B cell plasmablasts was found to be associated with the SARS-CoV-2 specific antibody levels. *These data, suggests that the differentiation of short-lived plasmablasts to become long-lived plasma cells is impaired and the main contributor of antibody production are the short-lived plasmablasts. Overall, our data provides insights regarding the humoral memory persistence in recovered COVID-19 patients. Notwithstanding the insights from this study, it is still to be determined if the persistence of SARS-CoV-2 memory B cells can be considered as a correlate of protection in the absence of serological memory.* [**note: important data from Irael on long term stability of B cell memory.**] https://www.medrxiv.org/content/10.1101/2020.08.23.20179796v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-08-26

As long as the Sant Andreu Jazz Band drops new videos, I will feature them!! Remember that except for the occasional soloist, these are all kids 20 years old and under! Here is 'He's My Guy' which has been covered by some impressive musicians: https://www.youtube.com/watch?v=8X0gMTRo7_c Here is Sarah Vaughn and Clifford Brown: https://www.youtube.com/watch?v=SL4k3CJKJOM and of course Peggy Lee: https://www.youtube.com/watch?v=9KfQk0I_oBM

US COVID-19 STATISTICS - **Infection Rate: 1.7%; CFR: 3.0%** (IR unchanged; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post covers the reopening of Purdue University. If anyone can pull off a reopening at a large land-grant school, it's Mitch Daniels. Faculty are still divided on in class teaching. Other schools are not faring as well, with 500 cases at Alabama. Here is an interesting story on the genetics of the Boston superspreading event. It's good to see college football keeping their eye on the ball announcing football playoffs will go on! That is what these student-athletes are paid to do (oops, I forgot, they really are not paid, they just get a scholarship). The cruise line industry may not be back.

In a New York Times op-ed, the author of 'Friday Night Lights' argues that college football players should strike to get what is due them. Money quote: "It is a system of serfdom unlike any not just in sports but in corporate America." Germany offers a solution to opening schools, can the US learn from this?

It appears the US may have officially given up on Track and Trace as CDC changes the guidelines regarding testing.

The Guardian has a story on a Japanese supercomputer's mask simulation. Non-woven polypropylene masks came out on top. Where do we get them? Google suggests THIS SITE but this just compares various surgical masks. The search will continue!

STAT have a cautionary story on long haulers who may have a hard time proving they were infected with SARS-CoV-2. Maybe the US messaging on COVID-19 risks is wrong.

Nature explain why the US has a coronavirus data crisis. Underfunding of public health is never a good idea! Here is a story on COVID-19 vaccine pre-orders.

The BMJ offers a point/counterpoint on whether doctors should recommend treatments and vaccines when full data are not publicly available. It's a tough call!

Medscape have a story on an observational study of famotidine showing some benefit. We still do not have data on the large controlled trial which to my knowledge has been completed.

Derek Lowe on the use of convalescent plasma & on preparing for vaccine results. Amidst all the political comments to the second article, is a pretty good one: *Has anyone done the math and has hazarded to make a guess how many covid19 cases will appear within 3 months of the placebo group? Let me make a guess. I imagine that there will be about 60 cases in the first three months for the 15,000 who get the placebo. Now, how many in the vaccine arm? 10? 15? Even 20 (out of 15,000 in the first three months and the vaccine should meet FDA specifications (approval depending also on side effect profile, of course). Of course, if everyone who enters the trials tends to be "cautious" by nature, there may be few cases even in the placebo arm, and therefore less of a difference between vaccine and placebo. I don't imagine that these studies are attracting many 28 year old pub-crawlers or Daytona beach partiers. What if only 15 people in the placebo group get Covid (and maybe 6 in the vaccine arm). Such small numbers are less impressive. This means that it might take 5 or 6 months to generate meaningful differences.* Interesting issue to ponder given the small numbers in the trial.

MODELING

- SARS-CoV-2 has caused a severe, ongoing outbreak of COVID-19 in Massachusetts with 111,070 confirmed cases and 8,433 deaths as of August 1, 2020. To investigate the introduction, spread, and epidemiology of COVID-19 in the Boston area, we sequenced and analyzed 772 complete SARS-CoV-2 genomes from the region, including nearly all confirmed cases within the first week of the epidemic and hundreds of cases from major outbreaks at a conference, a nursing facility, and among homeless shelter guests and staff. The data reveal over 80 introductions into the Boston area, predominantly from elsewhere in the United States and Europe. We studied two superspreading events covered by the data, events that led to very different outcomes because of the timing and populations involved. One produced rapid spread in a vulnerable population

but little onward transmission, while the other was a major contributor to sustained community transmission, including outbreaks in homeless populations, and was exported to several other domestic and international sites. The same two events differed significantly in the number of new mutations seen, raising the possibility that SARS-CoV-2 superspreading might encompass disparate transmission dynamics. Our results highlight the failure of measures to prevent importation into MA early in the outbreak, underscore the role of superspreading in amplifying an outbreak in a major urban area, and lay a foundation for contact tracing informed by genetic data. [**note: this is the phylogenetic analysis of the Boston superspreading event. Good work!**] https://www.medrxiv.org/content/10.1101/2020.08.23.20178236v1

- Risk-cost-benefit analysis requires the enumeration of decision alternatives, their associated outcomes, and the quantification of uncertainty. Public and private decision-making surrounding the COVID-19 pandemic must contend with uncertainty about the probability of infection during activities involving groups of people, in order to decide whether that activity is worth undertaking. We propose a deterministic linear model of SARS-CoV-2 infection probability that can produce estimates of relative risk for diverse activities, so long as those activities meet a list of assumptions, including that they do not last longer than one day. We show how the model can be used to inform decisions facing governments and industry, such as opening stadiums or flying on airplanes. We prove that the model is a good approximation of a more refined model in which we assume infections come from a series of independent risks. The linearity assumption makes interpreting and using the model straightforward, and we argue that it does so without significantly diminishing the reliability of the model. [**note: this is an interesting paper to read regarding estimates of relative risk. The math is not all that difficult to follow and I found it an interesting read.**] https://www.medrxiv.org/content/10.1101/2020.08.23.20180349v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow, but check the following out:
- BACKGROUND: A recently published randomized trial (Boulware et al., 2020, NCT04308668) of hydroxychloroquine (HCQ) for post-exposure prophylaxis found a reduction in Covid-19 of 17%. In the context of ambitious powering to detect a 50% reduction, this non-statistically significant finding could translate to a reduction of 22,000/130,828 cases (CDC 8/12/20) among US health care workers (HCW), impacting trajectory and resource utilization models that drive decisions on lockdowns and social distancing. Data found only in the appendix of Boulware et al. suggested greater differences in the effect of HCQ among sub-groups. There were reductions (36%) in younger (<35 years) and increases (110%) in older (>50 years) subjects. Our preliminary analysis revealed a significant negative correlation (slope -0.211, CI -0.328-0.094, p=0.016) between treatment lag and disease reduction, reaching 49% when initiated within one day (RR 0.51, CI 0.176-1.46, p=0.249). There were also differences in disease reduction by HCQ by type of exposure (HCW - 8% vs. household contacts - 31%; RR 0.691, CI 0.398-1.2). The definitions of exposure severity did not discriminate between the numbers or duration (> 10 minutes) of exposures. Differences between exposure types may result from younger HCW and higher risks in less trained household contacts with little access to advanced PPE. The ex-protocol use of zinc and ascorbic acid were likely confounders, as was the possibly active folate placebo. Exploratory reanalysis of the raw dataset may inform an age- and stage- nuanced approach to COVID-19

using HCQ testable by prospective studies and may provide insight into the various proposed mechanisms of HCQ. OBJECTIVES: To conduct an exploratory re-analysis of the de-identified raw dataset from a randomized study of the use of HCQ for post-exposure prophylaxis of COVID-19 with view to further defining: a) The time dependent effect of HCQ, b) The age dependent effect of HCQ; c) The sub-stratification of time- and age-dependent effects by exposure type and risk level, as well as by the use of zinc and ascorbic acid. d) The design of future clinical trials to test the hypotheses generated by this study. METHODS: Should granularity of data (by age, time-lag, level and type of exposure) be greater than that originally reported, Fisher Exact test will be used to compare the incidence of COVID-19 in HCQ- and control groups, for each sub-group stratification. Since the degree of loss of data granularity due to de-identification is yet unknown, exploratory analyses involving other demographic characteristics cannot be planned. Where sufficient data granularity exists, univariate regression analyses will be conducted to examine the effect of age- and time lag on any effect of HCQ. The possibility will be explored of conducting multivariate Cox regression analyses with propensity score matching to examine observational data relating to the use of zinc and ascorbic acid. This analysis will be expanded should a dataset from a similarly designed study (Mitja et al., 2020, NCT04304053), with directionally similar results, become available. This protocol was devised using the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) incorporating the WHO Trial Registration Data Set. Study Status: Protocol version 1.1, (August 19 2020) Protocol registered at: OSF Registries August 19 2020 Registration doi: https://doi.org/10.17605/OSF.IO/9RPYT [**note: I don't know what to make of this. The Boulware paper was published in the NEJM showing no effect of HCQ. Is this an attempt to slice and ice data or come up with a new statistical approach? There still appear to be folks out there that want HCQ to work. A lot of studies HCQ studies have been stopped but there are still some ongoing and if there was a major benefit a DSMB would have certainlyu stopped the trial. There is also the big Duke HERO registry.**] https://www.medrxiv.org/content/10.1101/2020.08.19.20178376v1

CLINICAL TRIAL RESULTS

- Hydroxychloroquine has not been associated with improved survival among hospitalized COVID-19 patients in the majority of observational studies and similarly was not identified as an effective prophylaxis following exposure in a prospective randomized trial. We aimed to explore the role of hydroxychloroquine therapy in mildly symptomatic patients diagnosed in the outpatient setting. Methods: We examined the association between outpatient hydroxychloroquine exposure and the subsequent progression of disease among mildly symptomatic non-hospitalized patients with documented SARS-CoV-2 infection. The primary outcome assessed was requirement of hospitalization. Data was obtained from a retrospective review of electronic health records within a New Jersey USA multi-hospital network. We compared outcomes in patients who received hydroxychloroquine with those who did not applying a multivariable logistic model with propensity matching. Results: Among 1274 outpatients with documented SARS-CoV-2 infection 7.6% were prescribed hydroxychloroquine. In a 1067 patient propensity matched cohort, 21.6% with outpatient exposure to hydroxychloroquine were hospitalized, and 31.4% without exposure were hospitalized. In the primary multivariable logistic regression analysis with propensity matching there was an association between exposure to hydroxychloroquine and a decreased rate of hospitalization

from COVID-19 (OR 0.53; 95% CI, 0.29, 0.95). Sensitivity analyses revealed similar associations. QTc prolongation events occurred in 2% of patients prescribed hydroxychloroquine with no reported arrhythmia events among those with data available. Conclusions: In this retrospective observational study of SARS-CoV-2 infected non-hospitalized patients hydroxychloroquine exposure was associated with a decreased rate of subsequent hospitalization. Additional exploration of hydroxychloroquine in this mildly symptomatic outpatient population is warranted. [**note: if at first you don't succeed, find a statistical method that proves something works. This is a poorly done paper trying to show HCQ prevents hospitalization in some subset of patients. I will be surprised if this is ever published in a mainstream journal but it won't prevent the HCQ addicts from saying "I told you it worked." the abstract obscures the fact that only 97 patients in the study were given HCQ. I'll leave it to the rest of you to find all the holes in this paper of which there are many.]**
https://www.medrxiv.org/content/10.1101/2020.08.20.20178772v1

- Background: Thrombotic microangiopathy (TMA) has been repeatedly described in COVID-19 and may contribute to SARS-CoV-2 associated hypercoagulability. The underlying mechanisms remain elusive. We hypothesized that endothelial damage may lead to substantially increased concentrations of Von Willebrand Factor (VWF) with subsequent relative deficiency of ADAMTS13. Methods: A prospective controlled trial was performed on 75 patients with COVID-19 of mild to critical severity and 10 healthy controls. VWF antigen (VWF:Ag), ADAMTS13 and VWF multimer formation were analyzed in a German hemostaseologic laboratory. Results: VWF:Ag was 4.8 times higher in COVID-19 patients compared to healthy controls ($p<0.0001$), whereas ADAMTS13 activities were not significantly different ($p=0.24$). The ADAMTS13/VWF:Ag ratio was significantly lower in COVID-19 than in the control group (24.4±20.5 vs. 79.7±33.2, $p<0.0001$). Fourteen patients (18.7%) undercut a critical ratio of 10 as described in thrombotic thrombocytopenic purpura (TTP). Gel analysis of multimers resembled the TTP constellation with loss of the largest multimers in 75% and a smeary triplet pattern in 39% of the patients. The ADAMTS13/VWF:Ag ratio decreased continuously from mild to critical disease (ANOVA $p=0.026$). Moreover, it differed significantly between surviving patients and those who died from COVID-19 ($p=0.001$) yielding an AUC of 0.232 in ROC curve analysis. Conclusion: *COVID-19 is associated with a substantial increase in VWF levels, which can exceed the ADAMTS13 processing capacity resulting in the formation of large VWF multimers identical to TTP. The ADAMTS13/VWF:Ag ratio is an independent predictor of severity of disease and mortality. These findings render further support to perform studies on the use of plasma exchange in COVID-19 and to include VWF and ADAMTS13 in the diagnostic workup*. [**note: more information on the COVID-19 associated hyper coagulability.**]
https://www.medrxiv.org/content/10.1101/2020.08.23.20177824v1
- Background: COVID-19 is associated with hypercoagulability and an increased incidence of thrombosis. We evaluated the clinical outcomes of adults hospitalized with COVID-19 who either continued therapeutic anticoagulants previously prescribed or who were newly started on anticoagulants during hospitalization. Methods: We performed an observational study of adult inpatients with COVID-19 at 10 hospitals affiliated with Northwestern Medicine in the Chicagoland area from March 9 to June 26, 2020. We evaluated clinical outcomes of subjects with COVID-19 who were continued on their outpatient therapeutic anticoagulation during hospitalization and those who were newly started on these medications compared to those who

were on prophylactic doses of these medications based on the World Health Organization (WHO) Ordinal Scale for Clinical Improvement. The primary outcome was overall death while secondary outcomes were critical illness (WHO score >5), need for mechanical ventilation, and death among those subjects who first had critical illness adjusted for age, sex, race, body mass index (BMI), Charlson score, glucose on admission, and use of antiplatelet agents. Results: 1,716 subjects with COVID-19 were included in the analysis. 171 subjects (10.0%) were continued on their outpatient therapeutic anticoagulation and 201(11.7%) were started on new therapeutic anticoagulation during hospitalization. In subjects continued on home therapeutic anticoagulation, there were no differences in overall death, critical illness, mechanical ventilation, or death among subjects with critical illness compared to subjects on prophylactic anticoagulation. Subjects receiving new therapeutic anticoagulation for COVID-19 were more likely to die (OR 5.93; 95% CI 3.71-9.47), have critical illness (OR 14.51; 95% CI 7.43-28.31), need mechanical ventilation (OR 11.22; 95% CI 6.67-18.86), and die after first having critical illness. (OR 5.51; 95% CI 2.80 -10.87). Conclusions: *Continuation of outpatient prescribed anticoagulant was not associated with improved clinical outcomes. Therapeutic anticoagulation for COVID-19 in absence of other indications was associated with worse clinical outcomes*. [**note: from Northwestern Univ medical school. Patients already on anticoagulants do not have improved health outcomes. Therapeutic anticoagulation seems not to be beneficial.]**
https://www.medrxiv.org/content/10.1101/2020.08.22.20179911v1

DRUG DEVELOPMENT

- Introduction Emerging infectious diseases, especially the coronavirus disease identified in 2019 (COVID-19), can be complicated by a severe exacerbation in the Th17 cell-mediated IL-17 proinflammatory immune storm. This enhanced immune response plays a major role in mortality and morbidity, including neurological symptoms. We hypothesized that countering the cytokine storm with thiamine may have therapeutic efficacy in lowering the Th17 cell proinflammatory response. We used an in vitro study and corroborated those results in disease controls (DC). We developed an effective dose range and model for key pharmacokinetic measures with the potential of targeting the cytokine storm and neurological symptoms of COVID-19. Study Participants and Methods We investigated the effect of a three-week 200 mg dose of thiamine in lowering the Th17 response in sixteen DC (proinflammatory origin due to heavy alcohol drinking) patients; and eight healthy control/volunteers (HV) as a pilot clinical-translational investigation. To further investigate, we performed an in vitro study evaluating the effectiveness of thiamine treatment in lowering the Th17 proinflammatory response in a mouse macrophage cell line (RAW264.7) treated with ethanol. In this in vitro study, 100 mg/day equivalent (0.01 ug/ml) thiamine was used. Based on recent publications, we compared the results of the IL-17 response from our clinical and in vitro study to those found in other proinflammatory disease conditions (metabolic conditions, septic shock, viral infections and COVID-19), including symptoms, and dose ranges of effective and safe administration of thiamine. We developed a dose range and pharmacokinetic profile for thiamine as a novel intervention strategy in COVID-19 to alleviate the effects of the cytokine storm and neurological symptoms. Results The DC group showed significantly elevated proinflammatory cytokines compared to HV. Three-week of 200 mg daily thiamine treatment significantly lowered the baseline IL-17 levels while increased IL-22 levels (anti-inflammatory response). This was

validated by an in vitro macrophage response using a lower thiamine dose equivalent (100 mg), which resulted in attenuation of IL-17 and elevation of IL-22 at the mRNA level compared to the ethanol-only treated group. In humans, a range of 79-474 mg daily of thiamine was estimated to be effective and safe as an intervention for the COVID-19 cytokine storm. A literature review showed that several neurological symptoms of COVID-19 (which exist in 45.5% of the severe cases) occur in other viral infections and neuroinflammatory states that may also respond to thiamine treatment. Discussion The Th17 mediated IL-17 proinflammatory response can potentially be attenuated by thiamine. Thiamine, a very safe drug even at very high doses, could be repurposed for treating the cytokine/immune storm of COVID-19 and the subsequent neurological symptoms observed in COVID-19 patients. Further studies using thiamine as an interventional/prevention strategy in severe COVID-19 patients could identify its precise anti-inflammatory role. [**note: rationale for using thiamine as a interventional treatment in severe COVID-19 patients. Maybe we should be adding this to the OTC things we are already taking.]** https://www.medrxiv.org/content/10.1101/2020.08.23.20177501v1

- Human angiotensin-converting enzyme 2 (ACE2) is the primary receptor of SARS-CoV-2 to enter the host cells and start the infection process. Therefore, it is prudent to design therapeutics based on the critical binding region of ACE2, which is a ~30 aa long helix with a kink in the middle. However, the small peptide in solution may lose its helical conformation and subsequently lose its binding potential to the SARS-CoV-2 RBD, which it utilizes to bind to that helical region. Here we report the design of four stapled peptides based on that helix, which is expected to bind to SARS-CoV-2 with high affinity and prevent the binding of the virus to the ACE2 receptor and disrupt the infection. All stapled peptides showed high helical contents (50 - 94% helicity). On the contrary, the linear control peptide NYBSP-C showed no helicity (19%). We have evaluated the peptides in a pseudovirus based single-cycle assay in HT1080 and human lung cells, A549. Three of the four stapled peptides showed potent antiviral activity in HT1080 (IC50: 1.9 – 4.1 μ) and A549 cells (IC50: 2.2 – 2.8 μ). It is noteworthy that the stapled peptide, NYBSP-3, which showed the least helical content, also had the lowest antiviral activity in both cell lines. The linear peptides NYBSP-C and SBP1, reported recently to bind SARS-CoV-2 with KD of ~47nM affinity, showed no antiviral activity. Most significantly, none of the stapled peptides show any appreciable cytotoxicity at the highest dose tested. We determined the proteolytic stability of one of the most active stapled peptides, NYBSP-4, in human plasma, which showed a half-life (T1/2) of >289 min. [**note: another approach to using peptide mimics of the ACE2 receptor. This one preserves the helical biding site.**] https://www.biorxiv.org/content/10.1101/2020.08.25.266437v1
- Here, we present the identification of 200 approved drugs, appropriate for repurposing against COVID-19. We constructed a SARS-CoV-2-induced protein (SIP) network, based on disease signatures defined by COVID-19 multi-omic datasets(Bojkova et al., 2020; Gordon et al., 2020), and cross-examined these pathways against approved drugs. This analysis identified 200 drugs predicted to target SARS-CoV-2-induced pathways, 40 of which are already in COVID-19 clinical trials(Clinicaltrials.gov, 2020) testifying to the validity of the approach. Using artificial neural network analysis we classified these 200 drugs into 9 distinct pathways, within two overarching mechanisms of action (MoAs): viral replication (130) and immune response (70). A subset of drugs implicated in viral replication were tested in cellular assays and two (proguanil and sulfasalazine) were shown to inhibit replication. This unbiased and validated analysis opens new

avenues for the rapid repurposing of approved drugs into clinical trials. [**note: another drug discovery paper based on SARS-CoV-2 pathways. At least they offer a suggested mechanism of action, "This analysis thereby highlights the possibility that targeting NO production through multiple pathways may be the reason for the efficacy of Sulfasalazine and Proguanil in reducing viral replication" Will these compounds ever enter the clinic?**]
https://www.biorxiv.org/content/10.1101/2020.08.24.265496v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- Nothing today

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2020-08-27

With everything that swirling around these days I needed to find something uplifting to listen to today (don't even ask me to post one of IceT's provocative videos; while I admire his work, it won't make the cut for a family friendly newsletter). Vivaldi composed a lot of stuff, but I go back to The Four Seasons for enjoyment. Here is the great German violinist, Anne-Sophie Mutter as the featured soloist: https://www.youtube.com/watch?v=9eEap53WxKY

US COVID-19 STATISTICS - **Infection Rate: 1.8%; CFR: 3.0%** (IR up 0.1%; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post has an article on the change of the CDC testing guidelines that was mentioned in yesterday's newsletter. Basic statistics course for FDA Commissioner Hahn who misspoke regarding the utility of convalescent plasma. Gaza could be in real trouble with COVID-19 because of population density and lack of health care. Maybe sleep away camp was a good idea this past summer.

STAT have an article on the politization of the FDA.

The Lancet offer an editorial on air travel in the time of COVID-19.

Nature have a paper on sex differences in immune responses that underlie COVID-19 disease outcomes. Hint: it sucks to be an older male.

Medscape discuss whether the FDA EUA on convalescent plasma will delay clinical trial recruitment. This is of concern and I don't think we know the answer. With schools reopening, asymptomatic SARS-CoV-2 infections in kids becomes a concern. I hope none of my loyal readers have to confront this, but here are some issues regarding home healthcare for COVID-19.

From Kaiser Health News, the mystery of why patients take much longer to regain consciousness after coming off a ventilator. Let us not forget some of the immigrant healthcare providers who helped out vulnerable communities only to perish from COVID-19.

MODELING

- No news is good news.

NEWLY REGISTERED CLINICAL TRIALS

- The aim of the clinical study is to determine the safety, reactogenicity and immunogenicity parameters of the EpiVacCorona vaccine in volunteers aged 18-60 years. [**note: this appears to be a second Russian COVID-19 vaccine. Not much detail other than some synthetic peptides are linked to a carrier protein and it is administered with an adjuvant.**] NCT04527575
- The severe acute respiratory syndrome coronavirus 2 (SARS-CoC-2), the virus responsible for coronavirus disease 2019 (COVID-19), is associated with a high incidence of acute respiratory distress syndrome (ARDS) and death. Aging, obesity, diabetes, hypertension and other risk factors associated with abnormal lipid and carbohydrate metabolism are risk factors for death in COVID-19. Recent studies suggest that COVID-19 progression is dependent on metabolic mechanisms. Moreover, gene expression analyses in cultured human bronchial cells infected with SARS-CoV-2 and lung tissue from patients with COVID-19, indicated a marked shift in cellular metabolism, with excessive intracellular lipid generation. In this cell culture system, fenofibrate (a widely available low-cost generic drug approved by the FDA and multiple other regulatory agencies around the world to treat dyslipemias) at concentrations that can be achieved clinically, markedly inhibited SARS-CoV-2 viral replication. Fenofibrate also has immunomodulatory effects that may be beneficial in the setting of COVID-19. The aim of this trial is to assess the clinical impact of fenofibrate (145 mg/d of Tricor or dose-equivalent preparations for 10 days, with dose adjustment in chronic kidney disease ([CKD]) to improve clinical outcomes in patients with COVID-19. [**note: this is a Univ of Pennsylvania trial. Another hail Mary trial?**] NCT04517396
- The primary purpose of this research is to determine whether Valproate alone, and in combination with Quetiapine, lowers confusion and agitation in persons with severe Corona Virus Disease (COVID)19 pneumonia during weaning from the breathing machine (ventilator). Though Valproate and Quetiapine are often given to persons with severe confusion with agitation, the purpose of this small research study is specifically for: a) persons infected with COVID 2019 on a ventilator whose agitation is not responding to the usual medications (like dexmedetomidine), and b) to reduce the time persons are treated with dexmedetomidine, which requires continuous close monitoring in an ICU. [**note: trial is at Univ of Miami and for delirium accompanying severe COVID-19**] NCT04513314
- This is a prospective, multicenter, randomized, open-label study to investigate the efficacy and safety of eltrombopag plus recombinant human thrombopoietin (rhTPO) versus eltrombopag as treatment for corticosteroid-resistant or relapsed immune thrombocytopenia (ITP) during the COVID-19 pandemic. [**note: this is a Chinese trial of these two drugs.**] NCT04516837

CLINICAL TRIAL RESULTS

- Sadly, nothing new.

DRUG DEVELOPMENT

- Globally accessible preventive and therapeutic molecules against SARS-CoV-2 are urgently needed. DARPin molecules are an emerging class of novel therapeutics based on naturally occurring repeat proteins (about 15 kDa in size) and can be rapidly produced in bacteria in large quantities. Here, we report the identification of 380 DARPin molecules specifically targeting the SARS-CoV-2 spike protein selected from a naive library of 10^12 DARPin molecules. Using extensive biophysical and biochemical characterization, (pseudo)virus neutralization assays and cryo EM analysis, 11 mono-DARPin molecules targeting either the receptor binding domain (RBD), the S1 N-terminal-domain (NTD) or the S2 domain of the SARS-CoV-2 spike protein were chosen. Based on these 11 mono-DARPin molecules, 31 anti-SARS-CoV-2 multi-DARPin molecules were constructed which can broadly be grouped into 2 types; multi-paratopic RBD-neutralizing DARPin molecules and multi-mode DARPin molecules targeting simultaneously RBD, NTD and the S2 domain. Each of these multi-DARPin molecules acts by binding with 3 DARPin modules to the SARS-CoV-2 spike protein, leading to potent inhibition of SARS-CoV-2 infection down to 1 ng/ml (12 pM) and potentially providing protection against viral escape mutations. Additionally, 2 DARPin modules binding serum albumin, conferring an expected half-life of about 3 weeks in humans, were included in the multi-DARPin molecules. The protective efficacy of one multi-DARPin molecule was studied in a Golden Syrian hamster SARS-CoV-2 infection model, resulting in a significant reduction in viral load and pathogenesis. In conclusion, the multi-DARPin molecules reported here display very high antiviral potency, high-production yield, and a long systemic half-life, and thereby have the potential for single-dose use for prevention and treatment of COVID-19. [**note: this is a technology new to me and it looks pretty cool. The nice thing is these molecules can be easily produced in E. coli, a well used recombinant production system. The company is Swiss based Molecular Partners and the academic researchers are Dutch. As with all innovative stuff that COVID-19 related the big question is will these ever hit the clinic? I hope so!**] https://www.biorxiv.org/content/10.1101/2020.08.25.256339v1
- Coronaviruses (CoVs) are important human pathogens for which no specific treatment is available. Here, we provide evidence that pharmacological reprogramming of ER stress pathways can be exploited to suppress CoV replication. We found that the ER stress inducer thapsigargin efficiently inhibits coronavirus (HCoV-229E, MERS-CoV, SARS-CoV-2) replication in different cell types, (partially) restores the virus-induced translational shut-down, and counteracts the CoV-mediated downregulation of IRE1α and the ER chaperone BiP. Proteome-wide data sets revealed specific pathways, protein networks and components that likely mediate the thapsigargin-induced antiviral state, including HERPUD1, an essential factor of ER quality control, and ER-associated protein degradation complexes. The data show that thapsigargin hits a central mechanism required for CoV replication, suggesting that thapsigargin (or derivatives thereof) may be developed into broad-spectrum anti-CoV drugs. [**note: this is a plant sesquiterpene lactone with an interesting chemical structure. It's been looked at as a potential treatment for some solid tumors but I don't know how much progress has been made. References at the link above.**]
  https://www.biorxiv.org/content/10.1101/2020.08.26.266304v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 has resulted in a global pandemic and shutdown economies around the world. Sequence analysis indicates that the novel coronavirus (CoV) has an insertion of a furin cleavage

site (PRRAR) in its spike protein. Absent in other group 2B CoVs, the insertion may be a key factor in the replication and virulence of SARS-CoV-2. To explore this question, we generated a SARS-CoV-2 mutant lacking the furin cleavage site (ΔPRRA) in the spike protein. This mutant virus replicated with faster kinetics and improved fitness in Vero E6 cells The mutant virus also had reduced spike protein processing as compared to wild-type SARS-CoV-2. In contrast, the ΔPRRA had reduced replication in Calu3 cells, a human respiratory cell line, and had attenuated disease in a hamster pathogenesis model. Despite the reduced disease, the ΔPRRA mutant offered robust protection from SARS-CoV-2 rechallenge. Importantly, plaque reduction neutralization tests (PRNT50) with COVID-19 patient sera and monoclonal antibodies against the receptor-binding domain found a shift, with the mutant virus resulting in consistently reduced PRNT50 titers. Together, these results demonstrate a critical role for the furin cleavage site insertion in SARS-CoV-2 replication and pathogenesis. In addition, these findings illustrate the importance of this insertion in evaluating neutralization and other downstream SARS-CoV-2 assays. [**note: it is useful to read the Wikipedia entry on furin to see why this is important. It is interesting that the mutant virus that was prepared with an altered furin cleavage site behaves quite differently.**] https://www.biorxiv.org/content/10.1101/2020.08.26.268854v1

- Early in the current pandemic, the D614G mutation arose in the Spike protein of SARS-CoV-2 and quickly became the dominant variant globally. Mounting evidence suggests D614G enhances viral entry. Here we use a direct competition assay with single-cycle viruses to show that D614G outcompetes the wildtype. We developed a cell line with inducible ACE2 expression to confirm that D614G more efficiently enters cells with ACE2 levels spanning the different primary cells targeted by SARS-CoV-2. Using a new assay for crosslinking and directly extracting Spike trimers from the pseudovirus surface, we found an increase in trimerization efficiency and viral incorporation of D614G protomers. Our findings suggest that D614G increases infection of cells expressing a wide range of ACE2, and informs the mechanism underlying enhanced entry. The tools developed here can be broadly applied to study other Spike variants and SARS-CoV-2 entry, to inform functional studies of viral evolution and vaccine development. [**note: more research on the D614G mutation.**]
  https://www.biorxiv.org/content/10.1101/2020.08.25.267500v1
- There is growing evidence pointing to the protective role of T cells against COVID-19. Vaccines eliciting targeted T cell responses have the potential to provide robust, long-lasting immunity. However, their design requires knowledge of the SARS-CoV-2-specific epitopes that can elicit a T cell response and confer protection across a wide population. Here, we provide a unified description of emerging data of SARS-CoV-2 T cell epitopes compiled from results of 8 independent studies of convalescent COVID-19 patients. We describe features of these epitopes relevant for vaccine design, while indicating knowledge gaps that can, in part, be augmented using prior immunological data from SARS-CoV. The landscape of SARS-CoV-2 T cell epitopes that we describe can help guide SARS-CoV-2 vaccine development as well as future immunological studies. A web-based platform has also been developed to complement these efforts. [**note: from Hong Kong, a description of epitopes targeted by T cells in convalescent COVID-19 patients.**] https://www.biorxiv.org/content/10.1101/2020.08.26.267724v1

DIAGNOSTIC DEVELOPMENT

- We identify a mutation in the N gene of SARS-CoV-2 that adversely affects annealing of a commonly used RT-PCR primer; epidemiologic evidence suggests the virus retains pathogenicity and competence for spread. This reinforces the importance of using multiple targets for robust SARS-CoV-2 detection. [**note: this highlights the fact that diagnostic manufacturers need to be vigilant in looking for polymorphisms that can impact the quality of their test.**] https://www.biorxiv.org/content/10.1101/2020.08.25.265074v1

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2020-08-28

Beethoven's 'Hammerkalvier' Sonata may be one of the more difficult solo pieces in the repertoire. A number of young pianists attack this piece with force but little subtlety. Notes come cascading out at the sacrifice of a musical line. Not so with Vladimir Ashkenazy. Here is a fine live performance from 1980: https://www.youtube.com/watch?v=FjJPNVJJMN4

US COVID-19 STATISTICS - **Infection Rate: 1.8%; CFR: 3.0%** (IR unchanged%; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

The New York Times offers and update on vaccine development. It echoes my thinking "…what if the first coronavirus vaccines aren't the best…?" Virus clusters in the US. New Jersey's school reopening plan is running into trouble. YIKES!!! Not only do schools have to worry about COVID-19 but there may be lurking Legionnaire's bacteria in the water supply & a nice op-ed on risk assessment that school kids should be aware of.

This Washington Post article on physical distancing is worth pondering. I don't know about you, but I certainly don't carry a tape measure with me when I'm out and about. Density of people, mask wearing compliance and air circulation are what I look for. Here is the BMJ paper the article references. Vaccine trials are halfway enrolled but are lacking in diversity.

Derek Lowe on the new Abbott quick laminar flow test for COVID-19. It has a very nice description of how it works!

STAT have a piece on recovered COVID-19 individuals and fear of reinfection. President Trump pledges a COVID-19 vaccine will be ready by the end of the year. Here is one doctor's first person account with severe COVID-19.

The Lancet have a pathophysiology study of COVID-19 associated acute respiratory distress syndrome (ARDS) from Italy. Here is a study on ARDS from the UK along with a commentary on the two papers.

JAMA have an interesting opinion piece on what health insurers ought to do with the surprising profits from this year brought about by sharp declines in elective care during the pandemic.

MODELING

- Transmission of severe acute respiratory coronavirus-2 (SARS-CoV-2) between livestock and humans is a potential public health concern. We demonstrate the susceptibility of rabbits to SARS-CoV-2, which excrete infectious virus from the nose and throat upon experimental inoculation. Therefore, investigations on the presence of SARS-CoV-2 in farmed rabbits should be considered. [**note: looks like rabbits can be a host for SARS-CoV-2. Wild rabbits pretty much**

avoid humans so I wonder if this is a concern in our neighborhood which has numerous rabbits.] https://www.biorxiv.org/content/10.1101/2020.08.27.263988v1

NEWLY REGISTERED CLINICAL TRIALS

- RT-CoV-2 is a Phase I, open-label, dose escalation multicenter clinical trial to assess safety and immunogenicity of the candidate Coronavirus disease (COVID-19) vaccine GRAd-COV2 in Italian healthy volunteers aged 18-55 years and 65-85 years inclusive. GRAd-COV2 is based on a novel replication defective Gorilla Adenovirus and encodes for SARS-COV-2 Spike protein. [**note: this is another vaccine into clinical trials using an adenovirus platform, this time a defective gorilla sourced one. It is from an Italian company. Looks like every country wants to have their own COVID-19 vaccine.]** NCT04528641
- A phase 2, placebo-controlled study of the safety and efficacy of STI-5656 (Abivertinib Maleate) in subjects hospitalized due to COVID-19 [**note: this is a tyrosine kinase inhibitor that has been studied for non-small cell lung cancer. Sponsor is Sorrento Therpeutics.**] NCT04528667
- To evaluate the safety and efficacy of antroquinonol treatment of mild to moderate pneumonia due to COVID-19, as measured by the proportion of patients alive and free of respiratory failure. [**note: sponsor is Golden Biotech, a Taiwan company. The compound is a fungal product of an expensive mushroom found in Taiwan (see the link above).**] NCT04523181
- This pilot study is being performed to assess the efficacy and safety of inhaled ensifentrine delivered via pMDI compared with a matching placebo in conjunction with standard of care treatments on recovery in patients hospitalized due to COVID-19 infection [**note: sponsor is Verona Pharma and more info on the drug and company are at the above link. Compound is a dual inhibitor of phosphodiesterases 3 & 4**] NCT04527471
- Clinical research focused to evaluate the effect as coadyuvant of a combination of L. plantarum and P. acidilactici in adults positive for SARS-CoV-2 with mild clinical COVID-19 symptoms. Main objective is to evaluate how this combination of probiotics reduce the risk to progress to moderate or severe COVID and associated advantages such as reduce the risk of death. Adittionnally this RCT is launching to explore the benefits of this combination of strains to modulate fecal microbiome and explore how this correlate with clinical improvement. [**note: here is a trial for you probiotic fans! Sponsor is AB Biotics, SA. The company has a cool looking website!**] NCT04517422

CLINICAL TRIAL RESULTS

- Again, nothing of note.

DRUG DEVELOPMENT

- The FDA has granted Remdesivir (RDV, GS-5734) an emergency use authorization on the basis of an acceleration of clinical recovery in hospitalized patients with COVID-19. Unfortunately, the drug must be administered intravenously, restricting its use to those with relatively advanced disease. RDV is also unstable in plasma and has a complex activation pathway which may contribute to its highly variable antiviral efficacy in SARS-CoV-2 infected cells. A potent orally bioavailable antiviral for early treatment of SARS-CoV-2 infection is needed. We focused on making simple orally bioavailable lipid analogs of Remdesivir nucleoside (RVn, GS-441524) that are processed to RVn-monophosphate, the precursor of the active RVn-triphosphate, by a single

step intracellular cleavage. *In addition to likely improved oral bioavailability and simpler metabolic activation, two of the three new lipid prodrugs of RVn had anti-SARS-CoV-2 activity 9 to 24 times greater than that of RDV in Vero E6 cells*. [**note: from UCSD, an interesting new synthetic chemistry approach to improving remdesivir by turning it into a lipid pro-drug.**] https://www.biorxiv.org/content/10.1101/2020.08.26.269159v1

- SARS-CoV-2 is a single stranded RNA (ssRNA) virus and contains GU-rich sequences distributed abundantly in the genome. In COVID-19, the infection and immune hyperactivation causes accumulation of inflammatory immune cells, blood clots, and protein aggregates in lung fluid, increased lung alveolar wall thickness, and upregulation of serum cytokine levels. A serum protein called serum amyloid P (SAP) has a calming effect on the innate immune system and shows efficacy as a therapeutic for fibrosis in animal models and clinical trials. In this report, we show that aspiration of the GU-rich ssRNA oligonucleotide ORN06 into mouse lungs induces all of the above COVID-19-like symptoms. Men tend to have more severe COVID-19 symptoms than women, and in the aspirated ORN06 model, male mice tended to have more severe symptoms than female mice. Intraperitoneal injections of SAP starting from day 1 post ORN06 aspiration attenuated the ORN06-induced increase in the number of inflammatory cells and formation of clot-like aggregates in the mouse lung fluid, reduced ORN06-increased alveolar wall thickness and accumulation of exudates in the alveolar airspace, and attenuated an ORN06-induced upregulation of the inflammatory cytokines IL-1β, IL-6, IL-12p70, IL-23, and IL-27 in serum. Together, these results suggest that aspiration of ORN06 is a simple model for both COVID-19 as well as cytokine storm in general, and that SAP is a potential therapeutic for diseases with COVID-19-like symptoms as well as diseases that generate a cytokine storm. [**note: I learn something new every day. I had not hears of SAP before reading this paper. I also did not know anything about the oligonucleotide used to mimic inflammatory response.**] https://www.biorxiv.org/content/10.1101/2020.08.26.269183v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The molecular basis for the severity and rapid spread of the COVID-19 disease caused by SARS-CoV-2 is largely unknown. ORF8 is a rapidly evolving accessory protein that has been proposed to interfere with immune responses. The crystal structure of SARS-CoV-2 ORF8 was determined at 2.04 Angstrom resolution by x-ray crystallography. The structure reveals a ~60 residue core similar to SARS-CoV ORF7a with the addition of two dimerization interfaces unique to SARS-CoV-2 ORF8. A covalent disulfide-linked dimer is formed through an N-terminal sequence specific to SARS-CoV-2, while a separate non-covalent interface is formed by another SARS-CoV-2-specific sequence, 73YIDI76. Together the presence of these interfaces shows how SARS-CoV-2 ORF8 can form unique large-scale assemblies not possible for SARS-CoV, potentially mediating unique immune suppression and evasion activities. [**note: from Univ of California, the crystal structure of the ORF8 protein and comparison with the original SARS protein of the same function showing differences.**] https://www.biorxiv.org/content/10.1101/2020.08.27.270637v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease (COVID-19) started at the end of 2019 in Wuhan, China has spread rapidly and became a pandemic. Since there is no therapy available that is proven as fully protective against COVID 19, a vaccine to protect against deadly COVID 19 is urgently needed. Nucleocapsid protein (N protein), is one of the most abundant proteins in coronaviruses and is a potential target for both

vaccine development and point of care diagnostics. The variable mass of N protein (45 to 60 kDa), suggests the presence of post-translational modifications (PTMs), and it is critical to clearly define these PTMs to gain the structural understanding necessary for further vaccine research. There have been several reports suggesting that the N protein is phosphorylated but lacks glycosylation. Our comprehensive glycomics and glycoproteomics experiments confirm that the N protein is highly O glycosylated and also contains significant levels of N glycosylation. We were able to confirm the presence of O glycans on seven sites with substantial glycan occupancy, in addition to less abundant O glycans on four sites. We also detected N glycans on two out of five potential N glycosylation sites. Moreover, we were able to confirm one phosphorylation site. Recent studies have indicated that the N protein can serve as an important diagnostic marker for coronavirus disease and a major immunogen by priming protective immune responses. Thus, detailed structural characterization of the N protein may provide useful insights for understanding the roles of glycosylation on viral pathogenesis and also in vaccine design and development. [**note: from Univ of Georgia, a study of the glycosylation of the nucleocapsid (N) protein**] https://www.biorxiv.org/content/10.1101/2020.08.26.269043v1

- The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) becomes a tremendous threat to global health. Although vaccines against the virus are under development, the antigen epitopes on the virus and their immunogenicity are poorly understood. Here, we simulated the three-dimensional structures of SARS-CoV-2 proteins with high performance computer, predicted the B cell epitopes on spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins of SARS-CoV-2 using structure-based approaches, and then validated the epitope immunogenicity by immunizing mice. Almost all 33 predicted epitopes effectively induced antibody production, six of which were immunodominant epitopes in patients identified via the binding of epitopes with the sera from domestic and imported COVID-19 patients, and 23 were conserved within SARS-CoV-2, SARS-CoV and bat coronavirus RaTG13. We also found that the immunodominant epitopes of domestic SARS-CoV-2 were different from that of the imported, which may be caused by the mutations on S (G614D) and N proteins. Importantly, we validated that eight epitopes on S protein elicited neutralizing antibodies that blocked the cell entry of both D614 and G614 pseudo-virus of SARS-CoV-2, three and nine epitopes induced D614 or G614 neutralizing antibodies, respectively. *Our present study shed light on the immunodominance, neutralization, and conserved epitopes on SARS-CoV-2 which are potently used for the diagnosis, virus classification and the vaccine design tackling inefficiency, virus mutation and different species of coronaviruses*. [**note: here is some clever work from China, deciphering antigen epitopes via computer and then confirming the results in an animal model. This can be useful in looking across coronavirus species.**] https://www.biorxiv.org/content/10.1101/2020.08.27.267716v1

DIAGNOSTIC DEVELOPMENT

- Nothing new

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2020-08-29

One of my top ten faves in the entire music world is the Bach Partita #2 in D minor. Many newsletters ago I had a performance of the final movement from this piece. Today we have the whole thing played by a young Itzhak Perlman and every note is just right on. Enjoy: https://www.youtube.com/watch?v=qtyTaE7LvVs

I TOLD YOU IT WOULD WORK!!! The Washington Post reports that Univ of Arizona stopped a COVID-19 outbreak before it started by screening the sewage from the dormitory. More schools can do this and it would be good for engineering and biochem students to take part in this research! Virus cases are rising in Europe though not with the same levels of mortality. Notre Dame resumes in class teaching next week as cases recede following the two week closure; no word on whether any of the football practices were affected. Moderna gets some bad news, no the vaccine trials are not being questioned, the company failed to disclose the federal support they received in vaccine patents.

Well, this was a short-lived job – eleven days. The New York Times reports that two public affairs experts at FDA were shown the door after the botched White House announcement about convalescent plasma. One was a political appointee who was installed by the White House. The other was long time PR exec Wayne Pines who had an outside contract with FDA.

My Yahoo News feed continues to pump out miracle COVID-19 cures!!

Medscape discuss a recent meta-analysis of four studies showing the *potential* protection of statins. Here is the original paper. I note that this is an observational study and that there are some clinical trials with statins registered. Correlation does not imply causation.

An alert reader sends me this link regarding another HCQ observational trial. They counter the UK RECOVERY trial results that showed HCQ not effective by noting "…the dose of HCQ used in that trial was almost double of that administered in our real-life conditions. A reduced mortality was also observed by other observational studies using low or intermediate doses of HCQ…" [there is also a report from Belgium on this as well] This is a peculiar statement to make unless one is a proponent of homeopathic remedies. Does anyone know of a drug having a therapeutic effect at a low dose but not a high dose? Leave aside the possibility for adverse drug reactions that can occur at high doses. I am not aware of this phenomenon. How much more effort need be focused on chasing this effect or non-effect? My reading shows the UK RECOVERY trial as well designed and definitive. Unless someone can come up with a scientific rationale for low dose use of HCQ, it will just be chalked up as another among the many anomalies of observational research.

MODELING

- Nothing new

NEWLY REGISTERED CLINICAL TRIALS

- I checked yesterday and that should be it for another day or two as new trials are rare

CLINICAL TRIAL RESULTS

- Nothing in the preprint literature

DRUG DEVELOPMENT

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the etiological agent of Coronavirus Disease 2019 (COVID-19), a pandemic that has claimed over 700,000 human lives. The only SARS-CoV-2 antiviral, for emergency use, is remdesivir, targeting the viral polymerase complex. PF-00835231 is a pre-clinical lead compound with an alternate target, the main SARS-CoV-2 protease 3CLpro (Mpro). Here, we perform a comparative analysis of PF-00835231 and remdesivir in A549+ACE2 cells, using isolates of two major SARS-CoV-2 clades. PF-00835231 is antiviral for both clades, and, in this assay, statistically more potent than remdesivir. A time-of-drug-addition approach delineates the timing of early SARS-CoV-2 life cycle steps and validates PF-00835231s time of action. Both PF-00835231 and remdesivir potently inhibit SARS-CoV-2 in human polarized airway epithelial cultures. Thus, our study provides in vitro evidence for the potential of PF-00835231 as an effective antiviral for SARS-CoV-2, addresses concerns from non-human in vitro models, and supports further studies with this compound. [**note: at last we see the 'secret' Pfizer COVID-19 drug candidate!! This is the *in vitro* data and shows it is as good or perhaps better than remdesivir. The paper discussing the drug discovery work is HERE where you can find the drug's chemical structure. According to the paper the drug was developed back in 2003 for the earlier SARS-CoV epidemic. As with remdesivir, it is an IV drug]** https://www.biorxiv.org/content/10.1101/2020.08.28.272880v1
- A consensus virtual screening protocol has been applied to ca. 2000 approved drugs to seek inhibitors of the main protease (Mpro) of SARS-CoV-2, the virus responsible for COVID-19. 42 drugs emerged as top candidates, and after visual analyses of the predicted structures of their complexes with Mpro, 17 were chosen for evaluation in a kinetic assay for Mpro inhibition. Remarkably 14 of the compounds at 100 µM concentration were found to reduce the enzymatic activity and 5 provided IC50 values below 40 µM: manidipine (4.8 µM), boceprevir (5.4 µM), lercanidipine (16.2 µM), bedaquiline (18.7 µM), and efonidipine (38.5 µM). Structural analyses reveal a common cloverleaf pattern for the binding of the active compounds to the P1, P1', and P2 pockets of Mpro. Further study of the most active compounds in the context of COVID-19 therapy is warranted, while all of the active compounds may provide a foundation for lead optimization to deliver valuable chemotherapeutics to combat the pandemic. [**note: and the drug hits just keep on coming. These Yale investigators look for inhibitors of the Mpro enzyme using a kinetic assay, the old tried and true method that I learned about years ago when I took B.R. Baker's course in medicinal chemistry at UCSB. This is certainly a strange group of drugs, two calcium channel blockers, a Hep C antiviral that was withdrawn when superior drugs were developed, an anti-hypertensive, and an antimicrobial used for TB treatment. Anyone want to make a bet whether these ever get into the clinic?**] https://www.biorxiv.org/content/10.1101/2020.08.28.271957v1
- Until now, no approved effective vaccine and antiviral therapeutic are available for treatment or prevention of SARS coronavirus 2 (SCoV2) virus infection. In this study, we established a SCoV2 Spike glycoprotein (SP), including a SP mutant D614G, pseudotyped HIV based vector system and tested their ability to infect ACE2-expressing cells. This study revealed that a C-terminal 17 amino acid deletion in SCoV-2 SP significantly increases the incorporation of SP into the pseudotyped viruses and enhanced its infectivity, which may be helpful in the design of SCoV2 SP based vaccine strategies. Moreover, based on this system, we have demonstrated that an aqueous extract from the Chinese herb Prunella vulgaris (CHPV) and a compound, suramin, displayed potent inhibitory effects on both wild type and mutant (G614) SCoV2 SP pseudotyped

virus (SCoV2 SP-PVs)-mediated infection. The 50% inhibitory concentration (IC50) for CHPV and suramin on SCoV2 SP-PVs are 30, and 40 ug/ml, respectively. To define the mechanisms of their actions, we demonstrated that both CHPV and suramin are able to directly interrupt SCoV2 SP binding to its receptor ACE2 and block the viral entry step. Importantly, our results also showed that CHPV or suramin can efficiently reduce levels of cytopathic effect caused by SARS-CoV-2 virus infection in Vero cells. Furthermore, our results demonstrated that the combination of CHPV/suramin with an antiSARS CoV2 neutralizing antibody mediated more potent blocking effect against SCoV2 SP-PVs. Overall, this study provides evidence that CHPV and suramin has anti-SARSCoV2 activity and may be developed as a novel antiviral approach against SARSCoV-2 infection. [**note: these Canadian scientists develop a pseudovirus assay and find two compounds that block viral entry. Check out the link to Prunella vulgaris, aka "heal all' the flowers are quite attractive and the plant is edible! All you gardeners, get this stuff growing and cure COVID-19; another great DIY project. Suramin is an interesting drug but cannot be taken orally and it has a half life even longer than HCQ (41-78 days)**]
https://www.biorxiv.org/content/10.1101/2020.08.28.270306v1

- Plasmablast responses and derived IgG monoclonal antibodies (MAbs) have been analysed in three COVID-19 patients. An average of 13.7% and 13.0% of plasmablast-derived IgG MAbs were reactive with virus spike glycoprotein or nucleocapsid, respectively. Of thirty-two antibodies specific for the spike glycoprotein, ten recognised the receptor-binding domain (RBD), thirteen were specific for non-RBD epitopes on the S1 subunit, and nine recognised the S2 subunit. A subset of anti-spike antibodies (10 of 32) cross-reacted with other betacoronaviruses tested, five targeted the non-RBD S1, and five targeted the S2 subunit. Of the plasmablast-derived MAbs reacting with nucleocapsid, over half of them (19 of 35) cross-reacted with other betacoronaviruses tested. The cross-reactive plasmablast-derived antibodies harboured extensive somatic mutations, indicative of an expansion of memory B cells upon SARS-CoV-2 infection. We identified 14 of 32 anti-spike MAbs that neutralised SARS-CoV-2 in independent assays at less than or equal to 133 nM (20 lower case Greek mug/ml) (five of 10 anti-RBD, three of 13 anti-non-RBD S1 subunit, six of nine anti-S2 subunit). Six of 10 anti-RBD MAbs showed evidence of blockade of ACE2 binding to RBD, and five of six of these were neutralising. Non-competing pairs of neutralising antibodies were identified, which offer potential templates for the development of prophylactic and therapeutic agents against SARS-CoV-2. [**note: more monoclonal antibody work from Taiwan.]**
https://www.biorxiv.org/content/10.1101/2020.08.28.267526v1
- Development of a safe and effective SARS-CoV-2 vaccine is a public health priority. We designed subunit vaccine candidates using self-assembling ferritin nanoparticles displaying one of two multimerized SARS-CoV-2 spikes: full-length ectodomain (S-Fer) or a C-terminal 70 amino acid deletion (SΔC-Fer). Ferritin is an attractive nanoparticle platform for production of vaccines and ferritin-based vaccines have been investigated in humans in two separate clinical trials. We confirmed proper folding and antigenicity of spike on the surface of ferritin by cryo-EM and binding to conformation-specific monoclonal antibodies. After a single immunization of mice with either of the two spike ferritin particles, a lentiviral SARS-CoV-2 pseudovirus assay revealed mean neutralizing antibody titers at least 2-fold greater than those in convalescent plasma from COVID-19 patients. Additionally, a single dose of SΔC-Fer elicited significantly higher neutralizing responses as compared to immunization with the spike receptor binding domain (RBD)

monomer or spike ectodomain trimer alone. After a second dose, mice immunized with SΔC-Fer exhibited higher neutralizing titers than all other groups. Taken together, these results demonstrate that multivalent presentation of SARS-CoV-2 spike on ferritin can notably enhance elicitation of neutralizing antibodies, thus constituting a viable strategy for single-dose vaccination against COVID-19. [**note: sound the trumpets and bang the drums, another vaccine prototype to consider. This is from Stanford and uses self-assembling ferritin nanoparticles. The ferritin for this vaccine comes from our old friend *Heilcobacter pylori* and has been previously used in other vaccine prototypes. This is not a new approach as the group at Walter Reed published on this several months ago, but that one has not yet started clinical trials.**] https://www.biorxiv.org/content/10.1101/2020.08.28.272518v1

- Effective and safe vaccines against SARS-CoV-2 are highly desirable to prevent casualties and societal cost caused by Covid-19 pandemic. The receptor binding domain (RBD) of the surface-exposed spike protein of SARS-CoV-2 represents a suitable target for the induction of neutralizing antibodies upon vaccination. Small protein antigens typically induce weak immune response while particles measuring tens of nanometers are efficiently presented to B cell follicles and subsequently to follicular germinal center B cells in draining lymph nodes, where B cell proliferation and affinity maturation occurs. Here we prepared and analyzed the response to several DNA vaccines based on genetic fusions of RBD to four different scaffolding domains, namely to the foldon peptide, ferritin, lumazine synthase and β-annulus peptide, presenting from 6 to 60 copies of the RBD on each particle. Scaffolding strongly augmented the immune response with production of neutralizing antibodies and T cell response including cytotoxic lymphocytes in mice upon immunization with DNA plasmids. The most potent response was observed for the 24-residue β-annulus peptide scaffold that forms large soluble assemblies, that has the advantage of low immunogenicity in comparison to larger scaffolds. Our results support the advancement of this vaccine platform towards clinical trials. [**note: and yet another scaffold vaccine approach and I think this is the first paper with a majority of researchers from Slovenia! We are all in this together. This approach is quite interesting in that multiple copies of antigen can be displayed. One is still left to wonder what the best COVID-19 vaccine is.**] https://www.biorxiv.org/content/10.1101/2020.08.28.244269v1
- The adenosine analogue remdesivir has emerged as a front-line antiviral treatment for SARS-CoV-2, with preliminary evidence that it reduces the duration and severity of illness. Prior clinical studies have identified adverse events, and remdesivir has been shown to inhibit mitochondrial RNA polymerase in biochemical experiments, yet little is known about the specific genetic pathways involved in cellular remdesivir metabolism and cytotoxicity. Through genome-wide CRISPR-Cas9 screening and RNA sequencing, we show that remdesivir treatment leads to a repression of mitochondrial respiratory activity, and we identify five genes whose loss significantly reduces remdesivir cytotoxicity. In particular, we show that loss of the mitochondrial nucleoside transporter SLC29A3 mitigates remdesivir toxicity without a commensurate decrease in SARS-CoV-2 antiviral potency and that the mitochondrial adenylate kinase AK2 is a remdesivir kinase required for remdesivir efficacy and toxicity. This work elucidates the cellular mechanisms of remdesivir metabolism and provides a candidate gene target to reduce remdesivir cytotoxicity. [**note: interesting approach to looking at potential drug cytotoxicity using CRISPR for remdesivir.**] https://www.biorxiv.org/content/10.1101/2020.08.27.270819v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The spike (S) protein of Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) binds to a host cell receptor which facilitates viral entry. A polybasic motif detected at the cleavage site of the S protein has been shown to broaden the cell tropism and transmissibility of the virus. Here we examine the properties of SARS-CoV-2 variants with mutations at the S protein cleavage site that undergo inefficient proteolytic cleavage. Virus variants with S gene mutations generated smaller plaques and exhibited a more limited range of cell tropism compared to the wild-type strain. These alterations were shown to result from their inability to utilize the entry pathway involving direct fusion mediated by the host type II transmembrane serine protease, TMPRSS2. Notably, viruses with S gene mutations emerged rapidly and became the dominant SARS-CoV-2 variants in TMPRSS2-deficient cells including Vero cells. Our study demonstrated that the S protein polybasic cleavage motif is a critical factor underlying SARS-CoV-2 entry and cell tropism. As such, researchers should be alert to the possibility of de novo S gene mutations emerging in tissue-culture propagated virus strains. [**note: interesting article on viral mutations *in vitro***] https://www.biorxiv.org/content/10.1101/2020.08.28.271163v1
- We assessed the infectivity, replication dynamics and cytopathogenicity of the first Swedish isolate of SARS-CoV-2 in six different cell lines of human origin and compared their growth characteristics. High replication kinetics in absence of cytopathic-effect observed in many cell lines provided important clues on SARS-CoV-2 pathogenesis. [**note: this is a useful paper from the Karolinska Institute showing the variability results using the Swedish isolated virus and different cell culture systems.**] https://www.biorxiv.org/content/10.1101/2020.08.28.271684v1

DIAGNOSTIC DEVELOPMENT

- Nothing new.

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2020-08-30

As my readers know, I am always impressed by good youth performances. For this reflection Sunday here is an exceptional performance that was on my YouTube feed yesterday. It is the Dvorak Symphony #9 From the New World: https://www.youtube.com/watch?v=O_tPb4JFgmw The orchestra is Gimnazija Kranj Symphony Orchestra under the baton of maestro Nejc Bečan. It is a school in Slovenia and this is the extent of my knowledge. There are some other YouTube clips of performances worth watching like this one of the Barry Manilow hit: https://www.youtube.com/watch?v=ULCAJrC1Ia4 or this one of the Bert Bacharach hit: https://www.youtube.com/watch?v=D-EE9xCG9oY Stuff like this makes me realize that the kids are alright and things will get better!! Optimism is far better than pessimism. BTW, how do the learn such good American accents in Slovenia?

The New Yorker has a long form story of a man's quest for a cheap COVID-19 diagnostic test.

Keeping on the testing meme, The New York Times leads with this headline, 'Your Coronavirus Test is Positive. Maybe it Shouldn't Be.' UK schools are opening next week; will it be a successful reopening?

According to the Washington Post, things are not going well with the opening of the University of Alabama. Lots of COVID-19 cases with a two week total of over 1000. No word on how many of Nick Saban's football players are infected. The Post also has a take on the current COVID-19 testing situation. If any school can open safely it should be a military academy. Is strict discipline the key to controlling COVID-19? If you are a college student or the parent of one, this article is for you! Nine ways to reduce your risk when living close to your school. Speaking of numbers, I'm reminded of the great Julia Stiles movie, 'Ten Things I Hate About You', a great retelling of 'The Taming of The Shrew.' I'll have to stream it later on today. Aren't you glad you subscribe to this newsletter?

My Yahoo News feed reports 87 coronavirus cases linked to a wedding in Maine. I hope my Maine readers did not attend this event.

The Guardian covers some countries outside the US: New Zealand, France, Germany, & Brazil.

Kaiser Health News reminds us that this is an especially good year to get a flu shot. This UCLA teaching hospital deserves credit for working to insure vulnerable patients are part of an AstraZeneca COVID-19 vaccine trial.

The Lancet has a commentary on the need of an efficacious COVID-19 vaccine. The points in this article are all well taken! This economist wants to pay people to take the COVID-19 vaccine. Hey, we all need a little more pocket money.

MODELING

- Large events and gatherings, particularly those taking place indoors, have been linked to multi-transmission events that have accelerated the pandemic spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To provide real-time, geo-localized risk information, we developed an interactive online dashboard that estimates the risk that at least one individual with SARS-CoV-2 is present in gatherings of different sizes in the United States. The website combines documented case reports at the county level with ascertainment bias information obtained via population-wide serological surveys to estimate real time circulating, per-capita infection rates. These rates are updated daily as a means to visualize the risk associated with gatherings, including county maps and state-level plots. The website provides data-driven information to help individuals and policy-makers make prudent decisions (e.g., increasing mask wearing compliance and avoiding larger gatherings) that could help control the spread of SARS-CoV-2, particularly in hard-hit regions. [**note: this is pretty cool; these guys have developed a real-time event risk assessment website that you can use to help figure out what is going on where you live! The link in the paper doesn't work but Google does.]**
  https://www.medrxiv.org/content/10.1101/2020.08.24.20181271v1
- **Background** Accurate estimates of SARS-CoV-2 seroprevalence are crucial for the implementation of effective public health measures, but are currently largely lacking in regions with low infection rates. This is further complicated by inadequate test performance of many widely used serological assays. We therefore aimed to assess SARS-CoV-2 seroprevalence in a region with low COVID-19 burden, especially focusing on neutralizing antibodies that presumably constitute a major component of acquired immunity. **Methods** We invited all individuals who were enrolled in the Rhineland Study, an ongoing community-based prospective cohort study in people aged 30 years and above in the city of Bonn, Germany (N=5427).

Between April 24th and June 30th, 2020, 4771 (88%) of these individuals participated in the serosurvey. Anti-SARS-CoV-2 IgG levels were measured using an ELISA assay, and all positive or borderline results were subsequently examined through both a recombinant immunofluorescent assay and a plaque reduction neutralisation test (PRNT). **Findings** Seroprevalence was 0.97% (95% CI: 0.72-1.30) by ELISA and 0.36% (95% CI: 0.21-0.61) by PRNT, and did not vary with either age or sex. All PRNT+ individuals reported having experienced at least one symptom (odds ratio (OR) of PRNT+ for each additional symptom: 1.12 (95% CI: 1.04-1.21)). Apart from living in a household with a SARS-CoV-2 confirmed or suspected person, a recent history of reduced taste or smell, fever, chills/hot flashes, pain while breathing, pain in arms/legs, as well as muscle pain and weakness were significantly associated with the presence of neutralizing antibodies in those with mild to moderate infection (ORs 3.44 to 9.97, all $p<0.018$). **Interpretation** Our findings indicate a relatively low SARS-CoV-2 seroprevalence in Bonn, Germany (until June 30th, 2020), with neutralizing antibodies detectable in only one third of those with a positive immunoassay result, implying that almost the entire population in this region remains susceptible to SARS-CoV-2 infection. [**note: here is a population serology study of Bonn Germany through the end of June. Low background of infection was found.**] https://www.medrxiv.org/content/10.1101/2020.08.24.20181206v1

- Understanding the outbreak dynamics of the COVID-19 pandemic has important implications for successful containment and mitigation strategies. Recent studies suggest that the population prevalence of SARS-CoV-2 antibodies, a proxy for the number of asymptomatic cases, could be an order of magnitude larger than expected from the number of reported symptomatic cases. Knowing the precise prevalence and contagiousness of asymptomatic transmission is critical to estimate the overall dimension and pandemic potential of COVID-19. However, at this stage, the effect of the asymptomatic population, its size, and its outbreak dynamics remain largely unknown. Here we use reported symptomatic case data in conjunction with antibody seroprevalence studies, a mathematical epidemiology model, and a Bayesian framework to infer the epidemiological characteristics of COVID-19. Our model computes, in real time, the time-varying contact rate of the outbreak, and projects the temporal evolution and credible intervals of the effective reproduction number and the symptomatic, asymptomatic, and recovered populations. Our study quantifies the sensitivity of the outbreak dynamics of COVID-19 to three parameters: the effective reproduction number, the ratio between the symptomatic and asymptomatic populations, and the infectious periods of both groups For nine distinct locations, our model estimates the fraction of the population that has been infected and recovered by Jun 15, 2020 to 24.15% (95% CI: 20.48%-28.14%) for Heinsberg (NRW, Germany), 2.40% (95% CI: 2.09%-2.76%) for Ada County (ID, USA), 46.19% (95% CI: 45.81%-46.60%) for New York City (NY, USA), 11.26% (95% CI: 7.21%-16.03%) for Santa Clara County (CA, USA), 3.09% (95% CI: 2.27%-4.03%) for Denmark, 12.35% (95% CI: 10.03%-15.18%) for Geneva Canton (Switzerland), 5.24% (95% CI: 4.84%-5.70%) for the Netherlands, 1.53% (95% CI: 0.76%-2.62%) for Rio Grande do Sul (Brazil), and 5.32% (95% CI: 4.77%-5.93%) for Belgium. Our method traces the initial outbreak date in Santa Clara County back to January 20, 2020 (95% CI: December 29, 2019 - February 13, 2020). Our results could significantly change our understanding and management of the COVID-19 pandemic: A large asymptomatic population will make isolation, containment, and tracing of individual cases challenging. Instead, managing community transmission through increasing population awareness, promoting physical distancing, and encouraging behavioral changes

could become more relevant. [**note: this is from the Stanford group that conducted the 'infamous' serology study of Santa Clara county several months ago. This is model approach that attempts to look at a large asymptomatic population. It is also the third revision of an early posted paper.**] https://www.medrxiv.org/content/10.1101/2020.05.23.20111419v3

NEWLY REGISTERED CLINICAL TRIALS

- This study is a randomized, double-blind, controlled clinical trial to evaluate the effects of toremifene and/or melatonin in adults with mild COVID-19. [**note: this is an investigator sponsored trial of an estrogen receptor modulator at the Cleveland Clinic**] NCT04531748

CLINICAL TRIAL RESULTS

- To determine the effect of COVID-19 convalescent plasma on mortality, we aggregated patient outcome data from randomized clinical trials, matched control, case series, and case report studies. Fixed-effects analyses demonstrated that hospitalized COVID-19 patients transfused with convalescent plasma exhibited a ~57% reduction in mortality rate (10%) compared to matched-patients receiving standard treatments (22%; OR: 0.43, P < 0.001). These data provide evidence favouring the efficacy of human convalescent plasma as a therapeutic agent in hospitalized COVID-19 patients. [**note: this is from the Mayo Clinic and is an aggregate of data from preprint studies through August 25. You will need to read the paper to get an understanding of which studies were included an excluded as well as the statistical analysis. I continue to believe that we really need a good controlled clinical trial to provide the definitive answer. Take a look at the main data table and you see very low number of patients in some studies.**] https://www.medrxiv.org/content/10.1101/2020.07.29.20162917v2

DRUG DEVELOPMENT

- Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the COVID-19 pandemic, remains viable and therefore potentially infectious on several materials. One strategy to discourage the fomite-mediated spread of COVID-19 is the development of materials whose surface chemistry can spontaneously inactivate SARS-CoV-2. Silicon nitride, a material used in spine fusion surgery, is one such candidate because it has been shown to inactivate several bacterial species and viral strains. This study hypothesized that contact with silicon nitride would inactivate SARS-CoV-2, while mammalian cells would remain unaffected. Materials: SARS-CoV-2 virions (2x10^4 PFU/mL diluted in growth media) were exposed to 5, 10, 15, and 20% (w/v) of an aqueous suspension of sintered silicon nitride particles for durations of 1, 5, and 10 minutes, respectively. Before exposure to the virus, cytotoxicity testing of silicon nitride alone was assessed in Vero cells at 24 and 48 hour post-exposure times. Following each exposure to silicon nitride, the remaining infectious virus was quantitated by plaque assay. Results: Vero cell viability increased at 5% and 10% (w/v) concentrations of silicon nitride at exposure times up to 10 minutes, and there was only minimal impact on cell health and viability up to 20% (w/v). However, the SARS-CoV-2 titers were markedly reduced when exposed to all concentrations of silicon nitride; the reduction in viral titers was between 85% - 99.6%, depending on the dose and duration of exposure. Conclusions: Silicon nitride was non-toxic to the Vero cells while showing strong antiviral activity against SARS-CoV-2. The viricidal effect increased with increasing concentrations of silicon nitride and

longer duration of exposure. Surface treatment strategies based on silicon nitride may offer novel methods to discourage SARS-CoV-2 persistence and infectivity on surfaces and discourage the spread of COVID-19. [**note: this is a study of silicon nitride which might be a useful viricide on environmental surfaces**] https://www.biorxiv.org/content/10.1101/2020.08.29.271015v1

- Characterisation of germinal centre B and T cell responses yields critical insights into vaccine immunogenicity. Non-human primates are a key pre-clinical animal model for human vaccine development, allowing both lymph node and circulating immune responses to be longitudinally sampled for correlates of vaccine efficacy. However, patterns of vaccine antigen drainage via the lymphatics after intramuscular immunisation can be stochastic, driving uneven deposition between lymphoid sites, and between individual lymph nodes within larger clusters. In order to improve the accurate isolation of antigen-exposed lymph nodes during biopsies and necropsies, we developed and validated a method for co-formulating candidate vaccines with tattoo ink, which allows for direct visual identification of vaccine-draining lymph nodes and evaluation of relevant antigen-specific B and T cell responses by flow cytometry. This approach improves the assessment of vaccine-induced immunity in highly relevant non-human primate models. [**note: who would have thought tattoo ink had a biomedical application. This is from a Melbourne research group and uses a coformulation of ink and vaccine to evaluate vaccine induced immunity. Way Cool!**] https://www.biorxiv.org/content/10.1101/2020.08.27.270975v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- While the antibody response to SARS-CoV-2 has been extensively studied in blood, relatively little is known about the mucosal immune response and its relationship to systemic antibody levels. Since SARS-CoV-2 initially replicates in the upper airway, the antibody response in the oral cavity is likely an important parameter that influences the course of infection, but how it correlates to the antibody response in serum is not known. Here, we profile by enzyme linked immunosorbent assays (ELISAs) IgG, IgA and IgM responses to the SARS-CoV-2 spike protein (full length trimer) and its receptor binding domain (RBD) in serum (n=496) and saliva (n=90) of acute and convalescent patients with laboratory-diagnosed COVID-19 ranging from 3-115 days post-symptom onset (PSO), compared to negative controls. Anti-CoV-2 antibody responses were readily detected in serum and saliva, with peak IgG levels attained by 16-30 days PSO. Whereas anti-CoV-2 IgA and IgM antibodies rapidly decayed, IgG antibodies remained relatively stable up to 105 days PSO in both biofluids. In a surrogate neutralization ELISA (snELISA), neutralization activity peaks by 31-45 days PSO and slowly declines, though a clear drop is detected at the last blood draw (105-115 days PSO). Lastly, IgG, IgM and to a lesser extent IgA responses to spike and RBD in the serum positively correlated with matched saliva samples. This study confirms that systemic and mucosal humoral IgG antibodies are maintained in the majority of COVID-19 patients for at least 3 months PSO. Based on their correlation with each other, IgG responses in saliva may serve as a surrogate measure of systemic immunity. [**note: This is from Toronto and compares systemic and humoral IgG response. Antibodies appear to be maintained for three months which was the duration of the study.**] https://www.medrxiv.org/content/10.1101/2020.08.01.20166553v2
- Abstract Objectives Castiglione D'Adda is one of the municipalities more precociously and severely affected by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) epidemic in Lombardy. With our study we aimed to understand the diffusion of the infection by

mass seroprevalence screening. Methods We searched for SARS-CoV-2 IgGs in the entire voluntary population using lateral flow immune-cromatographic tests on capillary blood (rapid tests). We then performed chemioluminescent serological assays (CLIA) and naso-pharyngeal swabs in a randomized representative sample of 562 subjects and in every subject with a positive rapid test. Results Based on CLIA serologies on the representative random sample (509 subjects), we estimated a 23% IgG seroprevalence. We also found a strong correlation between age and prevalence, with the elderly showing the highest probability of a positive serological test. Conclusions In an area of unrestricted viral circulation less than one-fourth of the population tested positive for SARS-CoV-2 IgG. Seroprevalence increased with increasing age, possibly suggesting differences in susceptibility to the infection. [**note: this is a serology study from one of the hard hit regions of Italy and breaks it down by age.**]
https://www.medrxiv.org/content/10.1101/2020.06.24.20138875v2

DIAGNOSTIC DEVELOPMENT

- The COVID-19 pandemic continues to have an unprecedented impact on societies and economies worldwide. Despite rapid advances in diagnostic test development and scale-up, there remains an ongoing need for SARS-CoV-2 tests which are highly sensitive, specific, minimally invasive, cost-effective and scalable for broad testing and surveillance. Here we report development of a highly sensitive single molecule array (Simoa) immunoassay on the automated HD-X platform for the detection of SARS-CoV-2 Nucleocapsid protein (N-protein) in venous and capillary blood (fingerstick). In pre-pandemic and clinical sample sets, the assay has 100% specificity and 97.4% sensitivity for serum / plasma samples. The limit of detection (LoD) estimated by titration of inactivated SARS-CoV-2 virus is 0.2 pg/ml, corresponding to 0.05 Median Tissue Culture Infectious Dose (TCID50) per ml, > 2000 times more sensitive than current EUA approved antigen tests. No cross-reactivity to other common respiratory viruses, including hCoV229E, hCoVOC43, hCoVNL63, Influenza A or Influenza B, was observed. We detected elevated N-protein concentrations in symptomatic, asymptomatic, and pre-symptomatic PCR+ individuals using capillary blood from a finger-stick collection device. *The Simoa SARS-CoV-2 N-protein assay has the potential to detect COVID-19 infection via antigen in blood with similar or better performance characteristics of molecular tests, while also enabling at home and point of care sample collection.* [**note: this work comes from Quanterix Corporation and this is an assay for the N-protein of the virus.**]
https://www.medrxiv.org/content/10.1101/2020.08.14.20175356v2