2020-08-03

Let's get back to a classic US road song. Before we had the Interstate highway system there was numbered system of patchwork US highways. Even numbers were for east-west roads and the odds were north-south. US route 1 ran from the Florida Keys to Maine while the 101 went from San Diego to Seattle. One of my faves was old route 6 that went from the tip of Cape Cod all the way to San Francisco. Perhaps none was as famous as Route 66, running from Chicago to Los Angeles. It carried countless Midwesterners to California during the depression dust bowl years, spawned a television show, and a classy hit song. Here is jazz singer Diana Krall's version: https://www.youtube.com/watch?v=BgeVx8QoVaQ from the Montreal Jazz Festival accompanied by Paul Keller and Russell Malone. Nat King Cole was the first to make this a hit and here is an old video: https://www.youtube.com/watch?v=dCYApJtsyd0

US COVID-19 STATISTICS - **Infection Rate: 1.4%; CFR: 3.3%** (IR unchanged; CFR unchanged; **note:** the CFR for this current outbreak continues to hover at 2%)

A school superintendent in Arizona faces a tough decision and the funding for his district may be at risk. Dr. Birx notes the US has entered a new phase as deaths have risen. The Washington Post also notes that a COVID-19 vaccine will not be an immediate game changer. Another cautionary tale, this time it is the SF Bay Area that is seeing an uptick in COVID-19 case.

A New Yorker story on how healthcare workers who came to New York City in the Spring have seen the virus come home to them.

The Guardian reports that two cruise ships were hit by COVID-19 just weeks after the industry reopened. There is this opinion piece on the UK effort to contain COVID-19; there is no simple answer.

The New York Times has some observations on college reopenings. Will politics play a role in the approval of a COVID-19 vaccine? Probably, but not if this investigator's view is followed.. Maybe Russia will have the first widely deployable COVID-19 vaccine.

STAT offers the story of a small Texas company that is playing an outsized role in helping get Americans tested for COVID-19. Measuring excess mortality is important to fully understand the pandemic.

Nature has a story on the Chinese vaccine effort. Companies are going to world virus hotspots to test enough patients for licensure. Here is an interesting story on virus naming and whether we should be looking at a new nomenclature in the midst of a pandemic.

I am thankful for a slow day on the preprint front!

MODELING

- A key strategy to prevent a local outbreak during the COVID-19 pandemic is to restrict incoming travel. Once a region has successfully contained the disease, it becomes critical to decide when and how to reopen the borders. Here we explore the impact of border reopening for the example of Newfoundland and Labrador, a Canadian province that has enjoyed no new cases since late April, 2020. We combine a network epidemiology model with machine learning to infer parameters and predict the COVID-19 dynamics upon partial and total airport reopening, with perfect and imperfect quarantine conditions. Our study suggests that upon full reopening,

every other day, a new COVID-19 case would enter the province. Under the current conditions, banning air travel from outside Canada is more efficient in managing the pandemic than fully reopening and quarantining 95% of the incoming population. Our study provides quantitative insights of the efficacy of travel restrictions and can inform political decision making in the controversy of reopening. [**note: island areas are in a position to more effectively manage the pandemic via travel restrictions as this model shows.**] https://www.medrxiv.org/content/10.1101/2020.07.16.20155614v3

NEWLY REGISTERED CLINICAL TRIALS

- Did not check

CLINICAL TRIAL RESULTS

- Nothing new today

DRUG DEVELOPMENT

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019[1,2] and is responsible for the COVID-19 pandemic[3]. Vaccines are an essential countermeasure urgently needed to control the pandemic[4]. Here, we show that the adenovirus-vectored vaccine ChAdOx1 nCoV-19, encoding the spike protein of SARS-CoV-2, is immunogenic in mice, eliciting a robust humoral and cell-mediated response. This response was predominantly Th1, as demonstrated by IgG subclass and cytokine expression profiling. Vaccination with ChAdOx1 nCoV-19 (prime-only and prime-boost regimen) induced a balanced Th1/Th2 humoral and cellular immune response in rhesus macaques. We observed a significantly reduced viral load in bronchoalveolar lavage fluid and lower respiratory tract tissue of vaccinated rhesus macaques challenged with SARS-CoV-2 compared with control animals, and no pneumonia was observed in vaccinated animals. *However, there was no difference in nasal shedding between vaccinated and control animals.* Importantly, no evidence of immune-enhanced disease following viral challenge in vaccinated animals was observed. Safety, immunogenicity and efficacy of ChAdOx1 nCoV-19 against symptomatic PCR-positive COVID-19 disease will now be assessed in randomised controlled human clinical trials. **[note: from Nature, this is an animal study of the Oxford vaccine. I wonder how long vaccinated animals shed virus. This will have implications for mass human immunization as protected individuals might still be hosts for viable virus and infect others. A conundrum worth pondering.]** https://www.nature.com/articles/s41586-020-2608-y

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Coronavirus possesses the largest RNA genome among all the RNA viruses. Its genome encodes about 29 proteins. Most of the viral proteins are non-structural proteins (NSP) except envelop (E), membrane (M), nucleocapsid (N) and Spike (S) proteins that constitute the viral nucleocapsid, envelop and surface. We have recently cloned all the 29 SARS-CoV-2 genes into vectors for their expressions in mammalian cells except NSP11 that has only 14 amino acids (aa). We are able to express all the 28 cloned SARS-CoV-2 genes in human cells to characterize their subcellular distributions. The proteins of SARS-CoV-2 are mostly cytoplasmic but some are both cytoplasmic and nuclear. Those punctate staining proteins were further investigated by

immunofluorescent assay (IFA) using specific antibodies or by co-transfection with an organelle marker-expressing plasmid. As a result, we found that NSP15, ORF6, M and ORF7a are related to Golgi apparatus, and that ORF7b, ORF8 and ORF10 colocalize with endoplasmic reticulum (ER). Interestingly, ORF3a distributes in cell membrane, early endosome, endosome, late endosome and lysosome, which suggests that ORF3a might help the infected virus to usurp endosome and lysosome for viral use. Furthermore, we revealed that NSP13 colocalized with SC35, a protein standing for splicing compartments in the nucleus. Our studies for the first time visualized the subcellular locations of SARS-CoV-2 proteins and might provide novel insights into the viral proteins' biological functions. [**note: this is a fascinating paper that looks at intracellular distribution of cloned SARS-CoV-2 proteins. We are learning more and more about this virus.**] https://www.biorxiv.org/content/10.1101/2020.08.02.233023v1

DIAGNOSTIC DEVELOPMENT

- Serology testing for COVID-19 is highly attractive because of the relatively short diagnosis time and the ability to test for an active immune response against the SARS-CoV-2. In many types of serology tests, the sensitivity and the specificity are directly influenced by the quality of the antigens manufactured. Protein purification of these recombinantly expressed viral antigens [e.g., spike and its receptor binding domain (RBD)] is an important step in the manufacturing process. Simple and high-capacity protein purification schemes for spike, RBD, and CR3022 mAb, recombinantly expressed in CHO and HEK293 cells, are reported in this article. The schemes consist of an affinity chromatography step and a desalting step. Purified proteins were validated in ELISA-based serological tests. *Interestingly, extracellular matrix proteins [most notably heparan sulfate proteoglycan (HSPG)] were co-purified from spike-expressing CHO culture with a long cultivation time. HSPG-spike interaction could play a functional role in the pathology and the pathogenesis of SARS-CoV-2 and other coronaviruses*. [**note: good paper from the UK on purification of components for serological testing. The finding of the co-purification of HSPG is an interesting finding.**] https://www.biorxiv.org/content/10.1101/2020.07.31.231282v1

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2020-08-04

What could be better than a nice summer afternoon in Barcelona and music filling the square? Good listening and dancing music from the Sant Abreu Jazz Band under the direction of Joan Chamorro. I don't know where he finds all these great young vocalists and here is Eva Fernandez singing Georgia on my Mind: https://www.youtube.com/watch?v=rRWZxFXXBCE The Tuesday Twofer also brings this Ray Charles Version: https://www.youtube.com/watch?v=qIp9TwSEgFg from the Montreux Jazz Festival.

US COVID-19 STATISTICS - **Infection Rate: 1.4%; CFR: 3.2%** (IR unchanged; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

The Washington Post discusses the vaccine distribution problems that may be forthcoming. Four former FDA commissioners on blood plasma treatment of COVID-19. Cases of COVID-19 are climbing in Midwest states previously unhit. One comedienne finds humor in the COVID-19 pandemic.

The Atlantic's Ed Yong on how the pandemic defeated America. It didn't have to happen this way. Perhaps the push to open schools in the fall is doomed to failure.

Another cautionary tale about school reopening is in today's The New York Times, specifically on the Israeli experience. Lockdown 2.0 in Melbourne leads to question about what one can and cannot do. There is a fine article on how to think like an epidemiologist; there are some good links in this article. The craft distillers who repurposed to making hand sanitizer have seen that market dry up.

These New Zealand newlyweds had a long trip home after the pandemic left them stranded on their honeymoon in the Falkland Islands.

The Guardian talks to three epidemiologists on herd immunity. Is Gilead studying the wrong drug? This opinion piece likens the pandemic to a wildfire. How do we take away the fuel and stop it?

The National Academies of Scinces, Engineering, and Medicine have two publications of interest to newsletter readers: Rapid Expert Consultation on Staffing Considerations for Crisis Standards of Care for the COVID-19 Pandemic (July 28, 2020) and Genomic Epidemiology Data Infrastructure Needs for SARS-CoV-2.

STAT on COVID-19 apps and wearables. Do they provide any benefit? The surgeon general of California weighs in on the stress related costs of COVID-19 on children.

The Lancet has a commentary on school reopenings. There links to useful research papers in this piece. There is also a good commentary with references on COVID-19 mortality during the first wave in Germany. I found this article on using facemasks during the COVID-19 pandemic to be a little confusing, but maybe that is just that I am not yet fully awake. I am not confused about this commentary on Vitamin D for COVID-19; I am taking 1000 IU of the vitamin daily.

This Kaiser Health News article on what seniors can expect as a new normal in post-COVID-19 vaccine world is a bit of a downer. I am continuing to look towards building a *tiny house* as a retreat from everything. There is also a nice overview of vaccine development efforts.

I'm putting this Indian DIY mask paper above the fold as the technological approach is quite interesting and inexpensive to implement. It adds a third impermeable layer to the traditional cotton mask that many of us wear.

Does anyone know what 'Tik Tok' is? I obviously do not.

Lots of stuff to review today including a somewhat depressing model that projects herd immunity at >70%.

MODELING

- SARS-CoV-2 rapidly spread from a regional outbreak to a global pandemic in just a few months. Global research efforts have focused on developing effective vaccines against SARS-CoV-2 and the disease it causes, COVID-19. However, some of the basic epidemiological parameters, such as the exponential epidemic growth rate and the basic reproductive number, R0, across

geographic areas are still not well quantified. Here, we developed and fit a mathematical model to case and death count data collected from the United States and eight European countries during the early epidemic period before broad control measures were implemented. Results show that the early epidemic grew exponentially at rates between 0.19-0.29/day (epidemic doubling times between 2.4-3.6 days). We discuss the current estimates of the mean serial interval, and argue that existing evidence suggests that the interval is between 6-8 days in the absence of active isolation efforts. *Using parameters consistent with this range, we estimated the median R0 value to be 5.8 (confidence interval: 4.7-7.3) in the United States and between 3.6 and 6.1 in the eight European countries. This translates to herd immunity thresholds needed to stop transmission to be between 73% and 84%. We further analyze how vaccination schedules depends on R0, the duration of vaccine-induced immunity to SARS-CoV-2, and show that individual-level heterogeneity in vaccine induced immunity can significantly affect vaccination schedules.* [**note: this is a bummer if true. I was estimating 60% for herd immunity and happy to see other papers modeling heterogeneity approaches that lowered this value.**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20166298v1

- In the present work, we outline a set of coarse-grain analytical models that can be used by decision-makers to bound the potential impact of the COVID-19 pandemic on specific communities with known or estimated social contact structure and to assess the effects of various non-pharmaceutical interventions on slowing the progression of disease spread. This work provides a multi-dimensional view of the problem by examining steady-state and dynamic disease spread using a network-based approach. In addition, Bayesian-based estimation procedures are used to provide a realistic assessment of the severity of outbreaks based on estimates of the average and instantaneous basic reproduction number R_0. [**note: this is a math heavy paper from a MITRE Corporation group. I wish they would have been clearer on the conclusions as I could not figure out how to implement the model (but it has been a lot of years when my math skills were at this level**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20166025v1
- Background: Coronavirus Disease 2019 (COVID-19) is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and spreads through droplet-mediated transmission on contaminated surfaces and in air. Mounting scientific evidence from observational studies suggests that face masks for the general public may reduce the spread of infections. However, results from randomized control trials (RCT) have been presented as inconclusive, and concerns related to the safety and efficacy of non-surgical face masks in non-clinical settings remain. This controversy calls for a meta-analysis which considers non-compliance in RCTs, the time-lag in benefits of universal masking, and possible adverse effects. Methods: We performed a meta-analysis of RCTs of non-surgical face masks in preventing viral respiratory infections in non-hospital and non-household settings at cumulative and maximum follow-up as primary endpoints. The search for RCTs yielded five studies published before May 29th, 2020. We pooled estimates from the studies and performed random-effects meta-analysis and mixed-effects meta-regression across studies, accounting for covariates in compliance vs. non-compliance in treatment. Results: Face masks decreased infections across all studies at maximum follow-up (p=0.0318$, RR=0.608 [0.387 - 0.956]), and particularly in studies without non-compliance bias. We found significant between-study heterogeneity in studies with bias (I^2=71.2%, p=0.0077). We also used adjusted meta-regression to account for heterogeneity. The results support a

significant protective effect of masking (p=0.0006, beta=0.0214, SE= 0.0062). No severe adverse effects were detected. Interpretation: The meta-analysis of existing randomized control studies found support for the efficacy of face masks among the general public. Our results show that face masks protect populations from infections and do not pose a significant risk to users. Recommendations and clear communication concerning the benefits of face masks should be provided to limit the number of COVID-19 and other respiratory infections. [**note: a meta-analysis of face mask studies from Finland. WEAR YOUR MASK when out and about.**] https://www.medrxiv.org/content/10.1101/2020.07.31.20166116v1

- As schools prepare for the start of the Fall 2020 semester, many are struggling to make decisions regarding whether or not to return to on-campus classes or whether to remain fully online. Unfortunately, there is no "one-size-fits-all" answer, and schools must balance their own risks against the costs of remote learning. We present a tool that integrates information about study body composition with predictions of COVID-19 infection rates in order to provide clarity and insight into the decisions facing colleges and universities nationwide. Our tool is freely available and currently hosted at the following location: https://bewicklab.shinyapps.io/covid-1/ [**note: this might be a useful tool to use in making a determination on school opening]** https://www.medrxiv.org/content/10.1101/2020.07.31.20165761v1
- Antibody testing is important for understanding patterns of exposure and potential immunity to SARS-CoV-2. Prior data on seroprevalence have been subject to variations in selection of individuals and nature as well as timing of testing in relation to exposures. Objective: We sought to determine the extent of SARS-CoV-2 seroprevalance and the factors associated with seroprevelance across a diverse cohort of healthcare workers. Design: Observational cohort study of healthcare workers, including SARS-CoV-2 serology testing and participant questionaires. Participants: A diverse and unselected population of adults (n=6,062) employed in a multi-site healthcare delivery system located in Los Angeles County, including individuals with direct patient contact and others with non-patient-oriented work functions. Exposure: Exposure and infection with the SARS-CoV-2 virus, as determined by seropositivity. Main Outcomes: Using Bayesian and multi-variate analyses, we estimated seroprevalence and factors associated with seropositivity and antibody titers, including pre-existing demographic and clinical characteristics; potential Covid-19 illness related exposures; and, symptoms consistent with Covid-19 infection. Results: We observed a seroprevalence rate of 4.1%, with anosmia as the most prominently associated self-reported symptom in addition to fever, dry cough, anorexia, and myalgias. After adjusting for potential confounders, pre-existing medical conditions were not associated with antibody positivity. However, seroprevalence was associated with younger age, Hispanic ethnicity, and African-American race, as well as presence of either a personal or household member having a prior diagnosis of Covid-19. Importantly, African American race and Hispanic ethnicity were associated with antibody positivity even after adjusting for personal Covid-19 diagnosis status, suggesting the contribution of unmeasured structural or societally factors. Notably, number of people, or children, in the home was not associated with antibody positivity. Conclusion and Relevance: The demographic factors associated with SARS-CoV-2 seroprevalence among our healthcare workers underscore the importance of exposure sources beyond the workplace. The size and diversity of our study population, combined with robust survey and modeling techniques, provide a vibrant picture of the demographic factors, exposures, and symptoms that can identify individuals with

susceptibility as well as potential to mount an immune response to Covid-19. [**note: large serology study on Los Angeles healthcare workers**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20163055v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check.

CLINICAL TRIAL RESULTS

- In May 2020 the Russian Ministry of Health granted fast-track marketing authorization to RNA polymerase inhibitor AVIFAVIR (favipiravir) for the treatment of COVID-19 patients. In the pilot stage of Phase II/III clinical trial, AVIFAVIR enabled SARS-CoV-2 viral clearance in 62.5% of patients within 4 days, and was safe and well-tolerated. **[note: this is the first report of a favipiravir trial that I've seen. Unfortunately it is probably underpowered with too few patients included. Treatment arm included antibiotics and anticoagulants (no drug names mentioned) and main endpoints were only viral clearance and return to basal temperature. We will need to see much better clinical trial data on this drug.**]
https://www.medrxiv.org/content/10.1101/2020.07.26.20154724v1
- Background Effective antiviral treatments are required to contain the ongoing coronavirus disease 2019 (COVID-19) pandemic. A previous report in 814 patients COVID-19 positive in Cuba provided preliminary therapeutic efficacy evidence with interferon alpha-2b (IFN alpha-2b) from March 11 to April 14, 2020. This study, re-evaluates the contribution of IFN-α2b on the evolution of all patients, after 98 days of the epidemic, in a period from March 11 to June 17, 2020. Method A prospective observational study was implemented to monitor a therapeutic intervention with IFN alpha-2b used in the national protocol for COVID-19 attending in Cuba. Were included patients with positive throat swab specimens by real time RT-PCR who gave informed consent and had no contraindications for IFN treatment. Patients received therapy as per the Cuban COVID protocol that included a combination of oral antivirals (lopinavir/ritonavir and chloroquine) with intramuscular or subcutaneous administration of IFN alpha-2b The primary endpoint was the proportion of patients discharged from hospital, secondary was the case fatality rate and several outcomes related to time variables were also evaluated. Results From March 11th until June 17th, 2295 patients had been confirmed SARS-CoV-2 positive in Cuba, 2165 were treated with Heberon Alpha R and 130 received the approved protocol without IFN. The proportion of fully recovered patients was higher in the IFN-treated compared with non-IFN treated group. Prior IFN treatment decreases the likelihood of intensive care and increases the survival after severe or critical diseases. The benefits of IFN were significantly supported by time variables analyzed. Conclusions This second report confirm the preliminary evidences from first for the therapeutic effectiveness of IFN alpha-2b for SARS-Cov-2 infection and postulated that Heberon Alpha R is the main component within the antiviral triad used as a therapeutic intervention in the Cuban protocol COVID-19. [**note: further data on interferon treatment from Cuba**] https://www.medrxiv.org/content/10.1101/2020.07.28.20157974v1

DRUG DEVELOPMENT

- An urgent global quest for effective therapies to prevent and treat COVID-19 disease is ongoing. We previously described REGN-COV2, a cocktail of two potent neutralizing antibodies

(REGN10987+REGN10933) targeting non-overlapping epitopes on the SARS-CoV-2 spike protein. In this report, we evaluate the in vivo efficacy of this antibody cocktail in both rhesus macaques and golden hamsters and demonstrate that REGN-COV-2 can greatly reduce virus load in lower and upper airway and decrease virus induced pathological sequalae when administered prophylactically or therapeutically. Our results provide evidence of the therapeutic potential of this antibody cocktail. [**note: good news with this animal data on the Regeneron mAb cocktail. Trials are underway for both treatment and prophylaxis**]
https://www.biorxiv.org/content/10.1101/2020.08.02.233320v1

- COVID-19 affects vulnerable populations including elderly individuals and patients with cancer. Natural Killer (NK) cells and innate-immune TRAIL suppress transformed and virally-infected cells. ACE2, and TMPRSS2 protease promote SARS-CoV-2 infectivity, while inflammatory cytokines IL-6, or G-CSF worsen COVID-19 severity. We show MEK inhibitors (MEKi) VS-6766, trametinib and selumetinib reduce ACE2 expression in human cells. Chloroquine or hydroxychloroquine increase cleaved active SP-domain of TMPRSS2, and this is potentiated by MEKi. In some human cells, remdesivir increases ACE2-promoter luciferase-reporter expression, ACE2 mRNA and protein, and ACE2 expression is attenuated by MEKi. We show elevated cytokines in COVID-19-(+) patient plasma (N=9) versus control (N=11). TMPRSS2, inflammatory cytokines G-CSF, M-CSF, IL-1alpha, IL-6 and MCP-1 are suppressed by MEKi alone or in combination with remdesivir. MEKi enhance NK cell (but not T-cell) killing of target-cells, without suppressing TRAIL-mediated cytotoxicity. We generated a pseudotyped SARS-CoV-2 virus with a lentiviral core but with the SARS-CoV-2 D614 or G614 SPIKE (S) protein on its envelope and used VSV-G lentivirus as a negative control. Our results show infection of human bronchial epithelial cells or lung cancer cells and that MEKi suppress infectivity of the SARS-CoV-2-S pseudovirus following infection. We show a drug class-effect with MEKi to promote immune responses involving NK cells, inhibit inflammatory cytokines and block host-factors for SARS-CoV-2 infection leading also to suppression of SARS-CoV-2-S pseudovirus infection of human cells in a model system. MEKi may attenuate coronavirus infection to allow immune responses and antiviral agents to control COVID-19 disease progression and severity. [**note: see the link explaining what a MEK inhibitor is. We are finding lots of drugs that may have plausible mechanisms but will they ever see a clinical trial?**]
https://www.biorxiv.org/content/10.1101/2020.08.02.230839v1
- We used two approaches to design proteins with shape and chemical complementarity to the receptor binding domain (RBD) of SARS-CoV-2 Spike protein near the binding site for the human ACE2 receptor. Scaffolds were built around an ACE2 helix that interacts with the RBD, or de novo designed scaffolds were docked against the RBD to identify new binding modes. In both cases, designed sequences were optimized first in silico and then experimentally for target binding, folding and stability. Nine designs bound the RBD with affinities ranging from 100pM to 10nM, and blocked bona fide SARS-CoV-2 infection of Vero E6 cells with IC50 values ranging from 35 pM to 35 nM; the most potent of these - 56 and 64 residue hyperstable proteins made using the second approach - are roughly six times more potent on a per mass basis (IC50 ~ 0.23 ng/ml) than the best monoclonal antibodies reported thus far. Cryo-electron microscopy structures of the SARS-CoV-2 spike ectodomain trimer in complex with the two most potent minibinders show that the structures of the designs and their binding interactions with the RBD are nearly identical to the computational models, and that all three RBDs in a single Spike protein can be

engaged simultaneously. These hyperstable minibinders provide promising starting points for new SARS-CoV-2 therapeutics, and illustrate the power of computational protein design for rapidly generating potential therapeutic candidates against pandemic threats. [**note: some good work at identifying minibidners that will block SARS-CoV-2**]
https://www.biorxiv.org/content/10.1101/2020.08.03.234914v1

- This paper presents the design and study of a first-in-class cyclic peptide inhibitor against the SARS-CoV-2 main protease (Mpro). The cyclic peptide inhibitor is designed to mimic the conformation of a substrate at a C-terminal autolytic cleavage site of Mpro. Synthesis and evaluation of a first-generation cyclic peptide inhibitor reveals that the inhibitor is active against Mpro in vitro and is non-toxic toward human cells in culture. The initial hit described in this manuscript, UCI-1, lays the groundwork for the development of additional cyclic peptide inhibitors against Mpro with improved activities. [**note: new work on designing a cyclic peptide inhibitor of the Mpro enzyme.**]
https://www.biorxiv.org/content/10.1101/2020.08.03.234872v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- T cells are involved in the early identification and clearance of viral infections and also support the development of antibodies by B cells. This central role for T cells makes them a desirable target for assessing the immune response to SARS-CoV-2 infection. Here, we combined two high-throughput immune profiling methods to create a quantitative picture of the T-cell response to SARS-CoV-2. First, at the individual level, we deeply characterized 3 acutely infected and 58 recovered COVID-19 subjects by experimentally mapping their CD8 T-cell response through antigen stimulation to 545 Human Leukocyte Antigen (HLA) class I presented viral peptides (class II data in a forthcoming study). Then, at the population level, we performed T-cell repertoire sequencing on 1,015 samples (from 827 COVID-19 subjects) as well as 3,500 controls to identify shared "public" T-cell receptors (TCRs) associated with SARS-CoV-2 infection from both CD8 and CD4 T cells. Collectively, our data reveal that CD8 T-cell responses are often driven by a few immunodominant, HLA-restricted epitopes. As expected, the T-cell response to SARS-CoV-2 peaks about one to two weeks after infection and is detectable for several months after recovery. As an application of these data, we trained a classifier to diagnose SARS-CoV-2 infection based solely on TCR sequencing from blood samples, and observed, at 99.8% specificity, high early sensitivity soon after diagnosis (Day 3-7 = 83.8% [95% CI = 77.6-89.4]; Day 8-14 = 92.4 [87.6-96.6]) as well as lasting sensitivity after recovery (Day 29+/convalescent = 96.7% [93.0-99.2]). These results demonstrate an approach to reliably assess the adaptive immune response both soon after viral antigenic exposure (before antibodies are typically detectable) as well as at later time points. This blood-based molecular approach to characterizing the cellular immune response has applications in vaccine development as well as clinical diagnostics and monitoring. [**note: this is a thorough multi-author paper on T cell response to SARS-CoV-2 infection at both individual and population levels.**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20165647v1

DIAGNOSTIC DEVELOPMENT

- Seroreactivity against human endemic coronaviruses has been linked to disease severity after SARS-CoV-2 infection. Assays that are capable of concomitantly detecting antibodies against

endemic coronaviridae such as OC43, 229E, NL63, and SARS-CoV-2 may help to elucidate this question. We set up a platform for serum-screening and developed a bead-based Western blot system, namely DigiWest, capable of running hundreds of assays using microgram amounts of protein prepared directly from different viruses. METHODS: The parallelized and miniaturised DigiWest assay was adapted for detecting antibodies using whole protein extract prepared from isolated SARS-CoV-2 virus particles. After characterisation and optimization of the newly established test, whole virus lysates of OC43, 229E, and NL63 were integrated into the system. RESULTS: The DigiWest-based immunoassay system for detection of SARS-CoV-2 specific antibodies shows a sensitivity of 87.2 % and diagnostic specificity of 100 %. Concordance analysis with the SARS-CoV-2 immunoassays available by Roche, Siemens, and Euroimmun indicates a comparable assay performance (Cohen's Kappa ranging from 0.8799-0.9429). In the multiplexed assay, antibodies against the endemic coronaviruses OC43, 229E, and NL63 were detected, displaying a high incidence of seroreactivity against these coronaviruses. CONCLUSION: The DigiWest-based immunoassay, which uses authentic antigens from isolated virus particles, is capable of detecting individual serum responses against SARS-CoV-2 with high specificity and sensitivity in one multiplexed assay. It shows high concordance with other commercially available serologic assays. The DigiWest approach enables a concomitant detection of antibodies against different endemic coronaviruses and will help to elucidate the role of these possibly cross-reactive antibodies. [**note: another potentially useful way to detect antibodies from a German research group**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20165019v1

- One-step RT-qPCR is the most widely applied method for COVID-19 diagnostics. Designing in-house one-step RT-qPCR kits is restricted by the patent-rights for the production of enzymes and the lack of information about the components of the commercial kits. Here, we provide a simple, economical, and powerful one-step RT-qPCR kit based on patent-free, specifically-tailored versions of Moloney Murine Leukemia Virus Reverse Transcriptase and Thermus aquaticus DNA polymerase termed the R3T (Rapid Research Response Team) One-step RT-qPCR. Our kit was routinely able to reliably detect as low as 10 copies of the synthetic RNAs of the SARS-CoV-2. More importantly, our kit successfully detected COVID-19 in clinical samples of broad viral titers with similar reliability and selectivity as that of the Invitrogen SuperScript III Platinum One-step RT-qPCR and TaqPath 1-Step RT-qPCR kits. Overall, our kit has shown robust performance in both of laboratory settings and the Saudi Ministry of Health-approved testing facility. [**note: another DIY diagnostics approach from Saudi Arabia!**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20165704v1

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2020-08-05

Today we will pay homage to one of the great pianists who just passed away, Leon Fleischer. Here is the Washington Post obituary. He began studying with the great Artur Schnabel when he was nine years old and his recordings of the five Beethoven piano concertos with the Cleveland Orchestra remain classic interpretations. Fleischer lost the use of his right hand in the early 1960s because of a nerve problem, recovering some use following BoTox injections 20 years later. Here is a late career performance of Bach's 'Sheep may Safely Graze' https://www.youtube.com/watch?v=GyOEOuUPvM0 and of course

we need to also look and listen to a performance when he could not use his right hand at all; here is the Ravel concerto for left hand and orchestra: https://www.youtube.com/watch?v=Jgj6jScPWK8

US COVID-19 STATISTICS - **Infection Rate: 1.4%; CFR: 3.2%** (IR unchanged; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

Are financial pressures forcing the wrong kind of schools to open in the fall? Maybe so according to The New York Times. Note to the presidents of colleges and universities: you also have to protect the support staff! In the absence of Federal action on testing, states are banding together to set up a strategy to make sure they have access to test kits and materials. Here is a nice summary of the immune system's response to COVID-19 and why it can go out of whack. Do we really need to do randomized controlled trials on convalescent plasma? If so, how many patients would need to be enrolled? There are a number of them that are registered, and I suspect if data gets pooled for analysis that will be sufficient. This points to another flaw in the US COVID-19 research response. This could be coordinated much better. Do you think you have COVID-19? This Times interactive article may help with the diagnosis. If I ever get my COVID-19 Scent Strip™ commercialized that will help as well. Maybe THIS will help you make a decision to get aboard an airplane for a trip away from home. Some very good news on the vaccine front from Novavax. You can always follow the progress on the Times Vaccine Tracker.

Here is the press release on the Novavax COVID-19 vaccine. The company uses a baculovirus production system to express the Spike protein and then combines it with a proprietary adjuvant. I have not seen the preprint of the data and don't see the presentation slides yet. I suspect that Sanofi/GSK will take a similar approach in their upcoming vaccine candidate. The GSK adjuvant technology is used in their shingles vaccine.

This STAT piece argues for continued drug development along with vaccines. Don't put all the eggs in one basket. Here is an obvious question: why isn't ventilation being discussed as part of school reopening?

Derek Lowe on the Regeneron mAb animal data. Do read the first comment on the animal dose and potential production problems if one needs to manufacture to that dosage.

MODELING

- There currently is substantial controversy about the role played by SARS-CoV-2 in aerosols in disease transmission, due in part to detections of viral RNA but failures to isolate viable virus from clinically generated aerosols. Methods - Air samples were collected in the room of two COVID-19 patients, one of whom had an active respiratory infection with a nasopharyngeal (NP) swab positive for SARS-CoV-2 by RT-qPCR. By using VIVAS air samplers that operate on a gentle water-vapor condensation principle, material was collected from room air and subjected to RT-qPCR and virus culture. The genomes of the SARS-CoV-2 collected from the air and of virus isolated in cell culture from air sampling and from a NP swab from a newly admitted patient in the room were sequenced. Findings - Viable virus was isolated from air samples collected 2 to 4.8m away from the patients. The genome sequence of the SARS-CoV-2 strain isolated from the material collected by the air samplers was identical to that isolated from the NP swab from the

patient with an active infection. Estimates of viable viral concentrations ranged from 6 to 74 TCID50 units/L of air. Interpretation - Patients with respiratory manifestations of COVID-19 produce aerosols in the absence of aerosol-generating procedures that contain viable SARS-CoV-2, and these aerosols may serve as a source of transmission of the virus. [**note: these Univ of Florida investigators find viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. I am not surprised.**]
https://www.medrxiv.org/content/10.1101/2020.08.03.20167395v1

- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the cause of Coronavirus Disease 2019 (COVID-19) and responsible for the current pandemic. Recent SARS-CoV-2 susceptibility and transmission studies in cats show that the virus can replicate in these companion animals and transmit to other cats. Here, we present an in-depth study of SARS-CoV-2 infection, associated disease and transmission dynamics in domestic cats. Six 4- to 5-month-old cats were challenged with SARS-CoV-2 via intranasal and oral routes simultaneously. One day post challenge (DPC), two sentinel contact cats were co-mingled with the principal infected animals. Animals were monitored for clinical signs, clinicopathological abnormalities and viral shedding throughout the 21 DPC observation period. Postmortem examinations were performed at 4, 7 and 21 DPC to investigate disease progression. Viral RNA was not detected in blood but transiently in nasal, oropharyngeal and rectal swabs and bronchoalveolar lavage fluid as well as various tissues. Tracheobronchoadenitis of submucosal glands with the presence of viral RNA and antigen was observed in airways of the infected cats on 4 and 7 DPC. Serology showed that both, principal and sentinel cats, developed SARS-CoV-2-specific and neutralizing antibodies to SARS-CoV-2 detectable at 7 DPC or 10 DPC, respectively. All animals were clinically asymptomatic during the course of the study and capable of transmitting SARS-CoV-2 to sentinels within 2 days of comingling. The results of this study are critical for our understanding of the clinical course of SARS-CoV-2 in a naturally susceptible host species, and for risk assessment of the maintenance of SARS-CoV-2 in felines and transmission to other animals and humans. [**note: cat owners may want to read this paper!**]
https://www.biorxiv.org/content/10.1101/2020.08.04.235002v1

NEWLY REGISTERED CLINICAL TRIALS

- The primary objective of the study is to determine if Prolastin plus SMT can reduce the proportion of participants dying or requiring intensive care unit (ICU) admission on or before Day 15 or who are dependent on invasive mechanical ventilation on Day 15 versus SMT alone in hospitalized participants with Coronavirus disease 2019 (COVID-19). [**note: this is a Spanish trial of alpha-1-antitrypsin**] NCT04495101
- This study is to support the ongoing clinical development of acalabrutinib (CALQUENCE®) in hospitalized COVID-19 patients. Because many COVID-19 patients may be unable to swallow capsules due to respiratory failure (eg, they may require endotracheal intubation for ventilator support and Naso Gastric tube placement), it is important to have a clinically acceptable method to administer acalabrutinib (capsules) via NG tube. Furthermore, many hospitalized patients are placed on high doses of proton pump inhibitors (PPIs) (also commonly known as antacid medication). This study is designed to determine the Pharmaco Kinetics (effect of body/ bodily systems on the drug), safety and tolerability of acalabrutinib suspension, when coadministered with a PPI, in participants with confirmed SARS-CoV-2 infection requiring hospitalization due to

respiratory failure, attributable to COVID-19 pneumonia and who have an Nasogastric (NG) tube in place. [**note: this is a very small trial sponsored by AstraZeneca and the second one registered for this drug.**] NCT04497948

- This is a randomized, placebo-controlled, two center, Phase I trial in healthy adult volunteer participants consisting of two phases, an unblinded dose escalation and a double blind treatment phase to investigate the safety, tolerability and immunogenicity of a novel measles-vector based vaccine candidate against SARS-CoV-2 infection (TMV-083). [**note: another COVID-19 vaccine, this time it is a measles based vector vaccine from Themis Bioscience that was working with Merck on this project. Merck acquired the company a couple of months ago. It will be interesting to see the data on this one. Of course I need to disclose that I am a Merck shareholder.**] NCT04497298
- Boswellia Serrata gum and Licorice extract are two nutritional agents that have pharmacological actions that could support the medical intervention for COVID-19. They have broad antiviral activity, anti-inflammatory, anti-lung injury, antibacterial activity, antithrombic formation, and immunomodulatory activity. [**note: this is an Egyptian study and I vaguely recall another one with this same plant product. It for all you alternative medicine fans.**] NCT04487964
- The goal of this project is to rapidly screen promising agents, in the setting of an adaptive platform trial, for treatment of critically ill COVID-19 patients. In this phase 2 platform design, agents will be identified with a signal suggesting a big impact on reducing mortality and the need for, as well as duration, of mechanical ventilation. [**note: this is the 'I-Spy' trial (I'm not making this up!) for seriously ill COVID-19 patients. The base treatment drug is remdesivir with or without cenicriviroc, icatibant, razuprotafib, or apremilast. The sponsor is a non-profit, Quantum Leap Healthcare Collaborative about which I know very little.**] NCT04488081
- The primary objective of this early Phase 1 study is to identify the V591 dose that achieves the target immune response in humans based on preclinical or early clinical data. [**note: this is the US arm of the Merck vaccine listed above. Merck has a second vaccine candidate using a recombinant vesicular stomatitis viral platform similar to the Ebola vaccine they developed.]** NCT04498247
- The study is a randomized controlled trail with an observational arm and aims at collecting information on the prevalence of COVID 19 infection in seasoned yoga practitioners by comparing it with the prevalence of COVID-19 infection prevalence rates among age and gender matched control participants who do not practice yoga. The study hypothesizes that yoga practice promotes protection and enhances recovery from the COVID-19 infection. To prove the hypothesis, we are collecting and comparing responses from seasoned yoga practitioners to age and gender matched controls participants (who do not practice yoga routinely) regarding their recovery from the COVID 19 infection. Based on validated questionnaires on perceived stress, anxiety, depression, well-being, mindfulness, joy disposition, and resilience in participants over the study duration, we also collect information on participant's mental and emotional predispositions. [**note: sponsor is Beth Israel Deaconess and they want to enroll 30K subjects!!**] NCT04498442
- The purpose of this study is to assess the potential for a non-invasive sensory based intervention to reduce the stress associated with COVID-19 testing. [**note: you might think I am making up some of these trials to see if you read this far down in the newsletter. I can tell you I do not.**

This trial is being sponsored by the Franklin School of Integrative Health Sciences. You all should take a look at their website. Maybe aromatherapy is the way to go!] NCT04495842

CLINICAL TRIAL RESULTS

- Background: Corona virus infection is a respiratory infection, compromising the normal breathing in critical patients by damaging the lungs. Researches are ongoing to find an efficient treatment strategy for this disease by either inactivating the virus or boosting the immune system of patient or by managing the cytokine storm. Aim: To evaluate the clinical outcomes of Substance P receptor Neurokinin 1 antagonist in Covid 19 patients against the usual treatments as controls. Patients and Methods: It is a randomized clinical trial, open label, having two arms, one receiving normal management and care while other receiving Neurokinin 1 Receptor antagonist, Aprepitant, in addition. Dexamethasone, a corticosteroid is also administered orally to both the groups. PCR positive, hospitalized patients with more than 18 years of age, both genders, moderate to critical phase were included. 18 patients were randomly allocated in both arms, having 10 in group A and 8 in group B. Lab investigations were performed in both the groups before and after the intervention. We report preliminary results for the comparison of Aprepitant 80 mg given once daily for 3 to 5 days vs routine management. The primary outcome was total in hospital days and duration of disease. Results: Mean age of patients in group A was 47.63 +12.07years while 60.90+ 9.75 years in group B. There were 3 males in group A and 8 in group B. There were 2 critical patients in group A and 5 in group B. Biochemical and hematological parameters in both groups didnot show much difference except the C reactive protein reduction in the intervention group, indicative of a reduced inflammation. Oxygen saturation also improved but more patients should be enrolled to get a statistically significant data. One patient was discharged from each group within 5 days and one patient expired in each. Conclusions: It is a pilot study but the findings give a strong clue for the therapeutic potential of Aprepitant. Patients who received a combination therapy of Aprepitant and Dexamethasone were recovered earlier and showed improved clinical outcomes, laboratory findings and reduced C reactive protein which is an inflammatory marker. We suggest here a study on larger sample size to get a deeper insight of its potential and efficacy. It may be more effective in severe to critical patients having respiratory difficulties. [**note: this is a very small trial in Brazil using the neurokinin-1 receptor antagonist aprepitant with dexamethasone. Clearly a larger trial is needed to show if this is of value in clinical settings. There is a trial sponsored by Heron Therapeutics here in the US - NCT04470622**]
  https://www.medrxiv.org/content/10.1101/2020.08.01.20166678v1
- Acute malignant catatonia with autonomic instability developed in a previously healthy man with PCR-verified SARS-CoV-2. CT and MRI were normal, EEG showed slowing and cerebrospinal fluid showed a subtle indication of inflammation. There were no signs of pathology in other organs. 18F-FDG-PET conveyed high bilateral uptake in the striatum. While commercial tests were negative, immunohistochemical staining of mouse brain revealed anti-neuronal IgG antibodies against neuronal targets in the hippocampus, thalamus, striatum and cortex. Early treatment with plasmapheresis and corticosteroid reversed disease progression and may have prevented large-scale neurological damage. We are not aware of other types of encephalitis with such distinct pyramidal tract symptoms and raise the possibility that this may be a novel

form of autoimmune encephalitis induced by infection with SARS-CoV-2. [**note: this is a single patient in Sweden. Let's hope it stays at this number as this can get out of control if not promptly treated.**] https://www.medrxiv.org/content/10.1101/2020.07.23.20160770v1

- Coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has a broad clinical presentation ranging from asymptomatic infection to fatal disease. Different features associated with the immune response to SARS-CoV-2, such as hyperinflammation and reduction of peripheral CD8+ T cell counts are strongly associated with severe disease. Here, we confirm the reduction in peripheral CD8+ T cells both in relative and absolute terms and identify T cell apoptosis and migration into inflamed tissues as possible mechanisms driving peripheral T cell lymphopenia. Furthermore, we find evidence of elevated serum interleukin-7, thus indicating systemic T cell paucity and signs of increased T cell proliferation in patients with severe lymphopenia. Following T cell lymphopenia in our pseudo-longitudinal time course, we observed expansion and recovery of poly-specific antiviral T cells, thus arguing for lymphopenia-induced T cell proliferation. In summary, this study suggests that extensive T cell loss and subsequent T cell proliferation are characteristic of severe COVID-19. [**note: more immune markers for severe COVID-19. Someone will figure out how to control the cascade and I hope it's soon!**]
https://www.biorxiv.org/content/10.1101/2020.08.04.236521v1

DRUG DEVELOPMENT

- Background and objective: The outbreak of COVID-19 has become a global health concern. In this study, we evaluate the effectiveness and safety of convalescent plasma therapy in patients with severe and critically ill COVID-19. Methods: Sixteen COVID-19 patients received transfusion of anti-COVID-19 antibody-positive convalescent plasma. The main outcome was time for viral nucleic acid amplification (NAA) test turning negative. Clinical laboratory parameters were measured at the baseline (d0) before plasma transfusion, and day 1 (d1), day 3 (d3) after transfusion as well. Results: Among the 16 patients, 10 of them had a consistently positive result of viral NAA test before convalescent plasma transfusion. Eight patients (8/10) became negative from day 2 to day 8 after transfusion. Severe patients showed a shorter time for NAA test turning negative after transfusion (mean rank 2.17 vs 5.90, P = 0.036). Two critically ill patients transfused plasma with lower antibody level remained a positive result of NAA test. CRP level demonstrated a decline 1 day after convalescent plasma treatment, compared with the baseline (P = 0.017). No adverse events were observed during convalescent plasma transfusion. Conclusions: Viral NAA test of most patients with COVID-19 who received convalescent plasma transfusion turned negative on the 2nd to 8th days after transfusion, and the negative time of severe patients was shorter than that of critically ill patients. [**note: positive study using convalescent plasma in China}**
https://www.medrxiv.org/content/10.1101/2020.08.02.20166710v1
- Severe acute respiratory syndrome coronavirus (SARS-CoV-2) has infected more than 16,000,000 people and has caused the death of more than 650,000 individuals since December 2019. A safe and effective vaccine that can provide herd immunity against SARS-CoV-2 is urgently needed to stop the spread of this virus among humans. Many human viral vaccines are live attenuated forms of viruses that elicit humoral and cellular immunity. Here, we describe the development of a cold-adapted live attenuated vaccine (SARS-CoV-2/human/Korea/CNUHV03-

CA22 degree celsius/2020) by gradually adapting the growth of SARS-CoV-2 from 37 degree celsius to 22 degree celsius in Vero cells. This vaccine can be potentially administered to humans through nasal spray. Its single dose was observed to strongly induce the neutralising antibody (over 640), cellular immunity, and mucosal IgA antibody in intranasally immunised K18-hACE2 mice, which are very susceptible to SARS-CoV-2 and SARS-CoV infection. The one-dose vaccinated mice were completely protected from SARS-CoV-2 infection and did not show loss of body weight, death, and the presence of virus in tissues, such as the nasal turbinates, brain, lungs, and kidneys. Taken together, the cold-adapted live attenuated SARS-CoV-2 vaccine developed by us may contribute to saving of human lives from the threat of SARS-CoV-2. [**note: from South Korea, a live attenuated SARS-CoV-2 vaccine that can be delivered intranasally. I don't know what the possibility of scale up with this approach is and of course they will need to assure that the process is replicable such that viable virus is not present. These kinds of approaches can lead to strong immunity.**]
https://www.biorxiv.org/content/10.1101/2020.08.04.235689v1

- The technical design of our SARS-CoV-2 inactivated vaccine provides a viral antigen that enables the exposure of more than one structural protein based upon the antibody composition of COVID-19 patients' convalescent serum. This design led to valid immunity with increasing neutralizing antibody titers and a CTL response detected post-immunization of this vaccine by two injections in rhesus macaques. Further, this elicited immunoprotection in macaques enables not only to restrain completely viral replication in tissues of immunized animals, compared to the adjuvant control and those immunized by an RBD peptide vaccine, but also to significantly alleviate inflammatory lesion in lung tissues in histo-pathologic detection, compared to the adjuvant control with developed interstitial pneumonia. The data obtained from these macaques immunized with the inactivated vaccine or RBD peptide vaccine suggest that immunity with a clinically protective effect against SARS-CoV-2 infection should include not only specific neutralizing antibodies but also specific CTL responses against at least the S and N antigens. [**note: this is animal challenge data for the Chinese inactivated SARS-CoV-2 vaccine. It's prepared using a traditional formaldehyde inactivation step. I believe this is in clinical trials.]** https://www.biorxiv.org/content/10.1101/2020.08.04.235747v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- In February 2020 a substitution at the interface between SARS-CoV-2 Spike protein subunits, Spike D614G, was observed in public databases. The Spike 614G variant subsequently increased in frequency in many locations throughout the world. Global patterns of dispersal of Spike 614G are suggestive of a selective advantage of this variant, however the origin of Spike 614G is associated with early colonization events in Europe and subsequent radiations to the rest of the world. Increasing frequency of 614G may therefore be due to a random founder effect. We investigate the hypothesis for positive selection of Spike 614G at the level of an individual country, the United Kingdom, using more than 25,000 whole genome SARS-CoV-2 sequences collected by COVID-19 Genomics UK Consortium. Using phylogenetic analysis, we identify Spike 614G and 614D clades with unique origins in the UK and from these we extrapolate and compare growth rates of co-circulating transmission clusters. We find that Spike 614G clusters are introduced in the UK later on average than 614D clusters and grow to larger size after adjusting for time of introduction. *Phylodynamic analysis does not show a significant increase in*

growth rates for clusters with the 614G variant, but population genetic modelling indicates that 614G increases in frequency relative to 614D in a manner consistent with a selective advantage. We also investigate the potential influence of Spike 614D versus G on virulence by matching a subset of records to clinical data on patient outcomes. We do not find any indication that patients infected with the Spike 614G variant have higher COVID-19 mortality, but younger patients have slightly increased odds of 614G carriage. Despite the availability of a very large data set, well represented by both Spike 614 variants, not all approaches showed a conclusive signal of higher transmission rate for 614G, but significant differences in growth, size, and composition of these lineages indicate a need for continued study. [**note: here is a large phylogenetic study of the European mutation of SARS-CoV-2. They did not observe higher mortality but more research is needed.**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20166082v1

DIAGNOSTIC DEVELOPMENT

- RT-qPCR on nasopharyngeal swabs is currently the reference COVID-19 diagnosis method. We developed a multiplex RT-ddPCR assay, targeting six SARS-CoV-2 genomic regions, and evaluated it on nasopharyngeal swabs and saliva samples collected from 130 COVID-19 positive or negative ambulatory individuals, who presented symptoms suggestive of mild or moderate Sars-CoV2 infection. The 6-plex RT-ddPCR assay was shown to have 100% sensitivity on nasopharyngeal swabs and a higher sensibility than RT-qPCR on saliva (85% versus 62%). Saliva samples from 2 individuals with negative results on nasopharyngeal swabs were found to be positive. These results show that multiplex RT-ddPCR should represent an alternative and complementary tool for the diagnosis of COVID-19, in particular to control RT-qPCR ambiguous results, and its application to saliva an appropriate strategy for repetitive sampling and testing individuals for whom nasopharyngeal swabbing is not possible. [**note: this one is from France and a slightly different PCR test.**]
https://www.medrxiv.org/content/10.1101/2020.08.02.20166694v1
- Current bottlenecks for improving accessibility and scalability of SARS-CoV-2 testing include diagnostic assay costs, complexity, and supply chain shortages. To resolve these issues, we developed SalivaDirect. The critical component of our approach is to use saliva instead of respiratory swabs, which enables non-invasive frequent sampling and reduces the need for trained healthcare professionals during collection. Furthermore, we simplified our diagnostic test by (1) not requiring nucleic acid preservatives at sample collection, (2) replacing nucleic acid extraction with a simple proteinase K and heat treatment step, and (3) testing specimens with a dualplex quantitative reverse transcription PCR (RT-qPCR) assay. We validated SalivaDirect with reagents and instruments from multiple vendors to minimize the risk for supply chain issues. Regardless of our tested combination of reagents and instruments from different vendors, we found that SalivaDirect is highly sensitive with a limit of detection of 6-12 SARS-CoV-2 copies/µL. When comparing paired nasopharyngeal swabs and saliva specimens using the authorized ThermoFisher Scientific TaqPath COVID-19 combo kit and our SalivaDirect protocol, we found high agreement in testing outcomes (>94%). Being flexible and inexpensive ($1.29-$4.37/sample), SalivaDirect is a viable and accessible option to help alleviate SARS-CoV-2 testing demands. We submitted SalivaDirect as a laboratory developed test to the US Food and Drug Administration for Emergency Use Authorization on July 14th, 2020, and current details can be

found on our website (covidtrackerct.com/about-salivadirect/). [**note: this looks like a good approach to saliva testing for SARS-CoV-2. Let us hope FDA agrees. Also cool is that the research was supported by the National Basketball Association!!!!**]
https://www.medrxiv.org/content/10.1101/2020.08.03.20167791v1

- The SARS-CoV-2 pandemic has swept the world and poses a significant global threat to lives and livelihoods, with over 16 million confirmed cases and at least 650 000 deaths from COVID-19 in the first 7 months of the pandemic. Developing tools to measure seroprevalence and understand protective immunity to SARS-CoV-2 is a priority. We aimed to develop a serological assay using plant-derived recombinant viral proteins, which represent important tools in less-resourced settings. Methods: We established an indirect enzyme-linked immunosorbent assay (ELISA) using the S1 and receptor-binding domain (RBD) portions of the spike protein from SARS-CoV-2, expressed in Nicotiana benthamiana. We measured antibody responses in sera from South African patients (n=77) who had tested positive by PCR for SARS-CoV-2. Samples were taken a median of six weeks after the diagnosis, and the majority of participants had mild and moderate COVID-19 disease. In addition, we tested the reactivity of pre-pandemic plasma (n=58) and compared the performance of our in-house ELISA with a commercial assay. We also determined whether our assay could detect SARS-CoV-2-specific IgG and IgA in saliva. Results: We demonstrate that SARS-CoV-2-specific immunoglobulins are readily detectable using recombinant plant-derived viral proteins, in patients who tested positive for SARS-CoV-2 by PCR. Reactivity to S1 and RBD was detected in 51 (66%) and 48 (62%) of participants, respectively. Notably, we detected 100% of samples identified as having S1-specific antibodies by a validated, high sensitivity commercial ELISA, and OD values were strongly and significantly correlated between the two assays. For the pre-pandemic plasma, 1/58 (1.7%) of samples were positive, indicating a high specificity for SARS-CoV-2 in our ELISA. SARS-CoV-2-specific IgG correlated significantly with IgA and IgM responses. Endpoint titers of S1- and RBD-specific immunoglobulins ranged from 1:50 to 1:3200. S1-specific IgG and IgA were found in saliva samples from convalescent volunteers. Conclusions: We demonstrate that recombinant SARS-CoV-2 proteins produced in plants enable robust detection of SARS-CoV-2 humoral responses. This assay can be used for seroepidemiological studies and to measure the strength and durability of antibody responses to SARS-CoV-2 in infected patients in our setting. [**note: more cool diagnostic approach, this time from South Africa. They use recombinant tobacco plants to produce the SARS-CoV-2 antigens to make their diagnostic test.**]
https://www.medrxiv.org/content/10.1101/2020.08.04.20167940v1
- Rapid point-of-care tests (POCTs) for SARS-CoV-2-specific antibodies vary in performance. A critical need exists to perform head-to-head comparison of these assays. Methods. Performance of fifteen different lateral flow POCTs for the detection of SARS-CoV-2-specific antibodies was performed on a well characterized set of 100 samples. Of these, 40 samples from known SARS-CoV-2-infected, convalescent individuals (average of 45 days post symptom onset) were used to assess sensitivity. Sixty samples from the pre-pandemic era (negative control), that were known to have been infected with other respiratory viruses (rhinoviruses A, B, C and/or coronavirus 229E, HKU1, NL63 OC43) were used to assess specificity. The timing of seroconversion was assessed on five POCTs on a panel of 272 longitudinal samples from 47 patients of known time since symptom onset. Results. For the assays that were evaluated, the sensitivity and specificity for any reactive band ranged from 55%-97% and 78%-100%, respectively. When assessing the

performance of the IgM and the IgG bands alone, sensitivity and specificity ranged from 0%-88% and 80%-100% for IgM and 25%-95% and 90%-100% for IgG. Longitudinal testing revealed that median time post symptom onset to a positive result was 7 days (IQR 5.4, 9.8) for IgM and 8.2 days (IQR 6.3 to 11.3). Conclusion. The testing performance varied widely among POCTs with most variation related to the sensitivity of the assays. The IgM band was most likely to misclassify pre-pandemic samples. The appearance of IgM and IgG bands occurred almost simultaneously. [**note: this is large comparison of point of care serology test kits. I'm seeing more of this research and likely won't be covering it. We need to have quick diagnostics and sooner or later one will emerge.**]
https://www.medrxiv.org/content/10.1101/2020.07.31.20166041v1

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2020-08-06

This clip from the Australian collective Stringspace came up on my YouTube feed yesterday. This is an interesting concept and looking at their videos they do covers from all different types of music. Perhaps the little elves who reside inside the YouTube brain know that I like Bossa Nova and are constantly finding new arrangements of top Antonio Carlos Jobim compositions are working their hearts out to please me. It doesn't matter as I found this version of Desafinado quite arresting: https://www.youtube.com/watch?v=-YkK5eSlX7M If I am ever in Australia and planning an event needing music, I will contact these folks.

US COVID-19 STATISTICS - **Infection Rate: 1.5%; CFR: 3.2%** (IR up 0.1%; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

A scene from Paris. One person is wearing a mask. Let's hope the others are all good virus-free friends.

Ed Yong of The Atlantic has a good overview of the immune system and COVID-19.

CDC issued a medical alert for acute flaccid myelitis that affects mostly children. This condition is caused by enteroviruses and it is unclear what impact COVID-19 distancing will have. It may be that cases are fewer this year.

The New York Times gives you all the info about enrolling in a COVID-19 vaccine trial! Times reporter, David Leonhardt discusses the unique US failure in controlling the virus. Germany is testing anyone returning to the country from a COVID-19 hot zone.

Here is a Tennessee school district that just opened for instruction courtesy of The Washington Post. There is no mandatory mask policy; why can't they just tell students masks are fun. If they still taught home economics, the sewing class could be put to work making designer masks. The FDA Commissioner says only a safe and effective vaccine will be approved. I will hold him to this promise!

Here is Tweet from Ohio Governor Mike DeWine about a single church member who infected at least 91 different people at a service; classic example of a superspreader. You should be able to click on the image to see how the spread tracked.

STAT have a story on a California company who are developing a LAMP COVID-19 test. It's an interesting read and goes into a bit of detail on how these types of tests work.

Medscape offer an opinion piece on how doctors can fight back against COVID conspiracy theories. In my opinion, the simplest approach is to target an audience of one key person. 😉 Here is an interesting report on how skin eruptions may help identify patients with severe COVID-19 who are more likely to develop coagulopathies.

MODELING

- What determines the success of a COVID-19 Test & Trace policy? We use an SEIR agent-based model on a graph, with realistic epidemiological parameters. Simulating variations in certain parameters of Testing & Tracing, we find that important determinants of successful containment are: (i) the time from symptom onset until a patient is self-isolated and tested, and (ii) the share of contacts of a positive patient who are successfully traced. Comparatively less important is (iii) the time of test analysis and contact tracing. When the share of contacts successfully traced is higher, the Test & Trace Time rises somewhat in importance. These results are robust to a wide range of values for how infectious presymptomatic patients are, to the amount of asymptomatic patients, to the network degree distribution and to base epidemic growth rate. We also provide mathematical arguments for why these simulation results hold in more general settings. Since real world Test & Trace systems and policies could affect all three parameters, Symptom Onset to Test Time should be considered, alongside test turnaround time and contact tracing coverage, as a key determinant of Test & Trace success. [**note: a simplified approach to determining success of track and trace. If it is so simple, why are so many failing?**] https://www.medrxiv.org/content/10.1101/2020.08.05.20168799v1

- To mitigate the COVID-19 pandemic, it is key to slow down the spreading of the life-threatening coronavirus (SARS-CoV-2). This spreading mainly occurs through virus-laden droplets expelled at speaking, screaming, shouting, singing, coughing, sneezing, or even breathing [1-7]. To reduce infections through such respiratory droplets, authorities all over the world have introduced the so-called "2-meter distance rule" or "6-foot rule". However, there is increasing empirical evidence, e.g. through the analysis of super-spreading events [6, 8-11], that airborne transmission of the coronavirus over much larger distances plays a major role [1-3, 7, 12-15], with tremendous implications for the risk assessment of coronavirus transmission. It is key to better and fundamentally understand the environmental ambient conditions under which airborne transmission of the coronavirus is likely to occur, in order to be able to control and adapt them. Here we employ direct numerical simulations of a typical respiratory aerosol in a turbulent jet of the respiratory event within a Lagrangian-Eulerian approach [16-18] with 5000 droplets, coupled to the ambient velocity, temperature, and humidity fields to allow for exchange of mass and heat [19] and to realistically account for the droplet evaporation under different ambient conditions. We found that for an ambient relative humidity of 50% the lifetime of the smallest droplets of our study with initial diameter of 10 µm gets extended by a factor of more than 30 as compared to what is suggested by the classical picture of Wells [20, 21], due to collective effects during droplet evaporation and the role of the respiratory humidity [22], while the larger droplets basically behave ballistically. With increasing ambient relative humidity the extension of the lifetimes of the small droplets further increases and goes up to 150 times for 90% relative humidity, implying more than two meters advection range of the respiratory droplets within one second. Smaller droplets live even longer and travel further. *Our results may explain why COVID-19 superspreading events can occur for large ambient relative humidity such as in cooled-down meat-processing plants [10] or in pubs with poor ventilation. We anticipate our tool and approach to be starting points for larger parameter studies and for optimizing ventilation and indoor humidity controlling concepts, which in the upcoming autumn and winter both will be key in mitigating the COVID-19 pandemic*. [**note: from a Dutch group, a model for viral lifetime in respiratory droplets. Large ambient humidity may be a factor.**] https://www.medrxiv.org/content/10.1101/2020.08.04.20168468v1
- Importance: A seroprevalence study can estimate the percentage of people with SARS-CoV-2 antibodies in the general population. Most existing reports have used a convenience sample, which may bias their estimates. Objective: To estimate the seroprevalence of antibodies against SARS-CoV-2 based on a random sample of adults living in Connecticut between March 1 and June 1, 2020. Design: Cross-sectional. Setting: We sought a representative sample of Connecticut residents who completed a survey between June 4 and June 23, 2020 and underwent serology testing for SARS-CoV-2-specific IgG antibodies between June 10 and July 6, 2020. Participants: 505 respondents, aged ≥18 years, residing in non-congregate settings who completed both the survey and the serology test. Main outcomes and measures: We estimated the seroprevalence of SARS-CoV-2-specific IgG antibodies among the overall population and across pre-specified subgroups. We also assessed the prevalence of symptomatic illness, risk factors for virus exposure, and self-reported adherence to risk mitigation behaviors among this population. Results: Of the 505 respondents (mean age 50 [±17] years; 54% women; 76% non-Hispanic White individuals) included, 32% reported having at least 1 symptom suggestive of COVID-19 since March 1, 2020. Overall, 18 respondents had SARS-CoV-2-specific antibodies,

resulting in the state-level weighted seroprevalence of 3.1 (90% CI 1.4-4.8). Individuals who were asymptomatic had significantly lower seroprevalence (0.6% [90% CI 0.0-1.5]) compared with the overall state estimate, while those who reported having had ≥1 and ≥2 symptoms had a seroprevalence of 8.0% (90% CI 3.1-12.9) and 13.0% (90% CI 3.5-22.5), respectively. All 9 of the respondents who reported previously having a positive coronavirus test were positive for SARS-CoV-2-specific IgG antibodies. Nearly two-third of respondents reported having avoided public places (74%) and small gatherings of family or friends (75%), and 97% reported wearing a mask outside their home, at least part of the time. Conclusions and relevance: *These estimates indicate that most people in Connecticut do not have detectable levels of antibodies against SARS-CoV-2. There is a need for continued adherence to risk mitigation behaviors among Connecticut residents, to prevent resurgence of COVID-19 in this region*. [**note: this will be of interest to my Connecticut readers. The sampling technique is flawed so I would not put a great deal of faith in the conclusion.**]
https://www.medrxiv.org/content/10.1101/2020.08.04.20168203v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not check.

CLINICAL TRIAL RESULTS

- There is no proven prognostic marker or adequate number of studies in patients hospitalized for coronavirus disease 2019 (COVID19). We conducted a retrospective cohort study of patients hospitalized with COVID19 from March 14 to June 17, 2020, at Sao Paulo Hospital. SARSCoV2 viral load was assessed using the cycle threshold (Ct) values obtained from an RTPCR assay applied to the nasopharyngeal swab samples. Disease severity and patient outcomes were compared. Among the 875 patients, 50.1% (439/875) had mild, 30.4% (266/875) moderate, and 19.5% (170/875) severe disease. A Ct value of <25 (472/875) indicated a high viral load, which was independently associated with mortality (OR: 0,34; 95% CI: 0,217 to 0,533; p < 0.0001). Admission SARSCoV2 viral load is an important surrogate biomarker of infectivity and is independently associated with mortality among patients hospitalized with COVID19. [**note: from hard hit Brazil, a study on viral load and disease severity.**]
https://www.medrxiv.org/content/10.1101/2020.08.04.20164061v1

DRUG DEVELOPMENT

- COVID-19 disease caused by the SARS-CoV-2 virus is characterized by dysregulation of effector T cells and accumulation of exhausted T cells. T cell responses to viruses can be corrected by adoptive cellular therapy using donor-derived virus-specific T cells. Here we show that SARS-CoV-2-exposed blood donations contain CD4 and CD8 memory T cells specific for SARS-CoV-2 spike, nucleocapsid and membrane antigens. These peptides can be used to isolate virus-specific T cells in a GMP-compliant process. These T cells can be rapidly expanded using GMP-compliant reagents for use as a therapeutic product. Memory and effector phenotypes are present in the selected virus-specific T cells, but our method rapidly expands the desirable central memory phenotype. A manufacturing yield ranging from 1010 to 1011 T cells can be obtained within 21 days culture. Thus, multiple therapeutic doses of virus-specific T cells can be rapidly generated from convalescent donors for treatment of COVID-19 patients. [**note: a big shout out to these**

Scottish researchers for coming up with a way to expand T cells from SARS-CoV-2 exposed blood donations. This might have useful clinical properties.]
https://www.biorxiv.org/content/10.1101/2020.08.05.237867v1

- Antiviral therapy is urgently needed to combat the coronavirus disease 2019 (COVID-19) pandemic, which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The protease inhibitor camostat mesylate inhibits SARS-CoV-2 infection of lung cells by blocking the virus-activating host cell protease TMPRSS2. Camostat mesylate has been approved for treatment of pancreatitis in Japan and is currently being repurposed for COVID-19 treatment. However, potential mechanisms of viral resistance as well as camostat mesylate metabolization and antiviral activity of metabolites are unclear. Here, we show that SARS-CoV-2 can employ TMPRSS2-related host cell proteases for activation and that several of them are expressed in viral target cells. However, entry mediated by these proteases was blocked by camostat mesylate. The camostat metabolite GBPA inhibited the activity of recombinant TMPRSS2 with reduced efficiency as compared to camostat mesylate and was rapidly generated in the presence of serum. Importantly, the infection experiments in which camostat mesylate was identified as a SARS-CoV-2 inhibitor involved preincubation of target cells with camostat mesylate in the presence of serum for 2 h and thus allowed conversion of camostat mesylate into GBPA. Indeed, when the antiviral activities of GBPA and camostat mesylate were compared in this setting, no major differences were identified. Our results indicate that use of TMPRSS2-related proteases for entry into target cells will not render SARS-CoV-2 camostat mesylate resistant. Moreover, the present and previous findings suggest that the peak concentrations of GBPA established after the clinically approved camostat mesylate dose (600 mg/day) will result in antiviral activity. [**note: this is a multi-national group who have figured out the mechanism of action for camostat. There are a handful of clinical trials going on but I've not seen any data.**] https://www.biorxiv.org/content/10.1101/2020.08.05.237651v1
- The coronavirus disease 2019 (COVID-19) pandemic has created an urgent need for therapeutics that inhibit the SARS-CoV-2 virus and suppress the fulminant inflammation characteristic of advanced illness. Here, we describe the anti-COVID-19 potential of PTC299, an orally available compound that is a potent inhibitor of dihydroorotate dehydrogenase (DHODH), the rate-limiting enzyme of the de novo pyrimidine biosynthesis pathway. In tissue culture, PTC299 manifests robust, dose-dependent, and DHODH-dependent inhibition of SARS CoV-2 replication (EC50 range, 2.0 to 31.6 nM) with a selectivity index >3,800. PTC299 also blocked replication of other RNA viruses, including Ebola virus. Consistent with known DHODH requirements for immunomodulatory cytokine production, PTC299 inhibited the production of interleukin (IL)-6, IL-17A (also called IL-17), IL-17F, and vascular endothelial growth factor (VEGF) in tissue culture models. The combination of anti-SARS-CoV-2 activity, cytokine inhibitory activity, and previously established favorable pharmacokinetic and human safety profiles render PTC299 a promising therapeutic for COVID-19. [**note: this is an experimental drug from PTC Therapeutics that inhibits dihydroorotate dehydrogenase. The company has a multi-site clinical trial underway.**]
https://www.biorxiv.org/content/10.1101/2020.08.05.238394v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Repeat molecular testing for SARS-CoV-2 may result in scenarios including multiple positive results, positive test results after negative tests, and repeated false negative results in

symptomatic individuals. Consecutively collected specimens from a retrospective cohort of COVID-19 patients at the Johns Hopkins Hospital were assessed for RNA and infectious virus shedding. Whole genome sequencing confirmed the virus genotype in patients with prolonged viral RNA shedding and droplet digital PCR (ddPCR) was used to assess the rate of false negative standard of care PCR results. Recovery of infectious virus was associated with Ct values of 18.8 ± 3.4. Prolonged viral RNA shedding was associated with recovery of infectious virus in specimens collected up to 20 days after the first positive result in patients who were symptomatic at the time of specimen collection. The use of Ct values and clinical symptoms provides a more accurate assessment of the potential for infectious virus shedding. [**note: the ability of the SARS-CoV-2 virus to linger is a vexing issue. Here is some data showing infectious virus recover 20 days post infection.**] https://www.medrxiv.org/content/10.1101/2020.08.05.20168963v1

- SARS-CoV-2 is a coronavirus responsible for the COVID-19 pandemic. In order to understand its pathogenicity, antigenic potential and to develop diagnostic and therapeutic tools, it is essential to portray the full repertoire of its expressed proteins. The SARS-CoV-2 coding capacity map is currently based on computational predictions and relies on homology to other coronaviruses. Since coronaviruses differ in their protein array, especially in the variety of accessory proteins, it is crucial to characterize the specific collection of SARS-CoV-2 translated open reading frames (ORF)s in an unbiased and open-ended manner. Utilizing a suit of ribosome profiling techniques, we present a high-resolution map of the SARS-CoV-2 coding regions, allowing us to accurately quantify the expression of canonical viral ORFs and to identify 23 novel unannotated viral ORFs. These ORFs include several in-frame internal ORFs lying within existing ORFs, resulting in N-terminally truncated products, as well as internal out-of-frame ORFs, which generate novel polypeptides, such as a 97 amino acid (aa) poly-peptide that is translated from the ORF-N transcript. Finally, we detected a prominent initiation at a CUG codon located in the 5'UTR. Although this codon is shared by all SARS-CoV-2 transcripts, the initiation was specific to the genomic RNA, indicating that the genomic RNA harbors unique features that may affect ribosome engagement. Overall, our work reveals the full coding capacity of SARS-CoV-2 genome, providing a rich resource, which will form the basis of future functional studies and diagnostic efforts. [**note: from Israel, the coding capacity of SARS-CoV-2.**] https://www.biorxiv.org/content/10.1101/2020.05.07.082909v3

DIAGNOSTIC DEVELOPMENT

- Efforts to contain the spread of SARS-CoV-2 have spurred the need for reliable, rapid, and cost-effective diagnostic methods which can easily be applied to large numbers of people. However, current standard protocols for the detection of viral nucleic acids while sensitive, require a high level of automation, sophisticated laboratory equipment and trained personnel to achieve throughputs that allow whole communities to be tested on a regular basis. Here we present Cap-iLAMP (capture and improved loop-mediated isothermal amplification). This method combines a hybridization capture-based RNA extraction of non-invasive gargle lavage samples to concentrate samples and remove inhibitors with an improved colorimetric RT-LAMP assay and smartphone-based color scoring. Cap-iLAMP is compatible with point-of-care testing and enables the detection of SARS-CoV-2 positive samples in less than one hour. In contrast to direct addition of the sample to improved LAMP (iLAMP), Cap-iLAMP does not result in false positives and single infected samples can be detected in a pool among 25 uninfected samples, thus

reducing the technical cost per test to ~1 Euro per individual. [**note: another approach to a cheap POC diagnostic. One would think that there would be a huge effort to get this type of approach deployed ASAP. Why hasn't it happened?**] https://www.medrxiv.org/content/10.1101/2020.08.04.20168617v1

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2020-08-07

I want to stay with Stringspace for a couple of more days as there are some great classical covers. Here is the Mahler Symphony #5 Adagietto arranged for string quartet. I've always found this to be both haunting and soothing. I cannot say anything more about this than just listen: https://www.youtube.com/watch?v=8UgGLEccLWE Here is the full orchestral version from the World Orchestra for Peace conducted by Valery Gergiev: https://www.youtube.com/watch?v=CFQQsu6VBYA

US COVID-19 STATISTICS - **Infection Rate: 1.5%; CFR: 3.2%** (IR up 0.1%; CFR down 0.1%; **note:** the CFR for this current outbreak continues to hover at 2%)

I don't know what to make of this Oak Ridge lab paper using their supercomputer to come up with some COVID-19 drug candidates (scroll down in the article for the drugs they identified). HERE is the full paper. There have been a lot of computer driven drug discovery papers so far this year and I don't think many of the identified compounds have been taken into clinical trials.

This Washington Post article gives us cause for hope. Here is a cautionary tale about a high school in Georgia but it doesn't end as you think it might; on the other hand it still could end up as you think it might (how is that for being cryptic?). Let us hope the annual Sturgis motorcycle rally does not turn into a superspreader event.

From the frontlines, The New York Times covers personal experiences with school reopenings.

Derek Lowe comments on the Novavax human phase 1 data. HERE is a link to the Novavax data.

Here is a pretty cool invention, a long lasting sanitizing skin protectant. Herein, we describe a skin protectant, IonLASTTM, based on an ionic liquid/deep eutectic solvent, formed by GRAS materials, choline and geranic acid (CAGE, CG-101), that provides protection for at least 4h after a single application. IonLASTTM was formulated as a gel that facilitates easy application on the skin. Tolerance of CG-101 was substantiated through a study in human volunteers. In vitro studies confirmed that IonLASTTM effectively inactivates a human coronavirus hCoV229E.

I was totally unaware of this vaccine controversy that STAT discusses. Clearly this proponent of mass vaccination following small trials doesn't understand safety issues. Of course, the Russians are plunging forward with a mass vaccine effort and I guess we will see what happens there, or we might not.

The Lancet has an interesting comment piece on the elimination of COVID-19: what it would look like and is it possible?

JAMA have a viewpoint on rethinking clinical trial reform during the COVID-19 pandemic. This is a good paper to read as the authors, "*…examine several changes precipitated by the current crisis that represent potential areas of improvement for the future*." There is also an important paper from South Korea that looks at viral presence by PCR in both symptomatic and asymptomatic individuals. Keeping

with the Rumsfeld Paradigm, "*In a recent study, viral shedding from sputum has been shown to extend beyond symptom duration. It is important to note that detection of viral RNA does not equate infectious virus being present and transmissible. For a better understanding of the viral shedding and potential transmissibility of asymptomatic infection, large rigorous epidemiologic and experimental studies are needed*."

Nature have an article on how the pandemic plays out. It's a bit of a downer article but being the optimist, I think a lot of stuff will be figured out by the end of this year. I like the final quote in the article, "There is so much we still don't know about this virus….until we have better data, we're just going to have a lot of uncertainty." As a Rumsfeld Paradigm believer, I could not say this any better!!

The final downer article of the day is this one from Kaiser Health News on the impact of obesity and COVID-19.

Enough for today!

MODELING

- Background. No versatile web app exists that allows epidemiologists and managers around the world to fully analyze the impacts of COVID-19 mitigation. The NMB-DASA web app presented here fills this gap. Methods. Our web app uses a model that explicitly identifies a contact class of individuals, symptomatic and asymptomatic classes and a parallel set of response class, subject to lower contact pathogen contact rates. The user inputs a CSV file containing incidence and mortality time series. A default set of parameters is available that can be overwritten through input or online entry, and a subset of these can be fitted to the model using an MLE algorithm. The end of model-fitting and forecasting intervals are specifiable and changes to parameters allows counterfactual and forecasted scenarios to be explored. Findings. We illustrate the app in the context of the current COVID-19 outbreak in Israel, which can be divided into four distinct phases: an initial outbreak; a social distancing, a social relaxation, and a second wave mitigation phase. Our projections beyond the relaxation phase indicate that an 85% drop in social relaxation rates are needed just to stabilize the current incidence rate and that at least a 95% drop is needed to quell the outbreak. Interpretation. Our analysis uses only incidence and mortality rates. In the hands of policy makers and health officers, we believe our web app provides an invaluable tool for evaluating the impacts of different outbreak mitigation policies and measures. [**note: these Univ of California researchers have developed a predictive web app that allows for the analysis of COVID-19 mitigation. It is tested against the current outbreak in Israel.**] https://www.medrxiv.org/content/10.1101/2020.08.06.20155804v1
- Covid-19 infection case predictions (total cases) are made for August through December 2020 for 10 US States (NY, WA, GA, IL, MN, FL, OH, MI, CA, and NC). A two-parameter model based on social distance index (SDI) and disease transmission efficiency (G) parameters is used to characterize SARS-CoV-2 disease spread. Current lack of coherent and coordinated US policy causes the US to follow a linear infection growth path with a limit cycle behavior that modulates the US between accelerating and decaying infection growth on either side of a linear growth path boundary. Four prediction cases are presented: 1) No school re-openings; fall season temperature effect 2) No school re-openings; no fall season temperature effect 3) School re-

openings; fall season temperature effect 4) School re-openings; no fall season temperature effect Fall outdoor temperatures, in contrast to the 1918 pandemic, are predicted to be beneficial for dampening SARS-CoV-2 transmission in States as they pass through swing season temperature range of 70F to 50F. Physical re-opening of schools in September are predicted to accelerate infections. States with low current infectious case numbers (eg, NY) are predicted to be minimally impacted while States with high current infectious case numbers (eg, CA and FL) will be significantly impacted by school re-openings. Updated infection predictions will be posted monthly (Sept, Oct, Nov, Dec) with adjustments based on actual trends in SDI and G. Assessments related to outdoor temperature impact, school re-openings, and other public gathering re-openings will be discussed in updated reports. [**note: this model is developed by a Univ of Illinois engineering professor and shows impacts in 10 states based on outdoor temperature and school reopenings. There are some very good diagrams here relating to impact.**] https://www.medrxiv.org/content/10.1101/2020.08.06.20169896v1

- Objectives: To improve understanding of transition from viral infection to viral clearance, and antibody response in pediatric patients with SARS-CoV-2 infection. Study design: This retrospective analysis of children tested for SARS-CoV-2 by RT-PCR and IgG antibody at a quaternary-care, free-standing pediatric hospital between March 13th, 2020 to June 21st, 2020 included 6369 patients who underwent PCR testing and 215 patients who underwent antibody testing. During the initial study period, testing focused primarily on symptomatic children; the later study period included asymptomatic patients who underwent testing as preadmission or preprocedural screening. We report the proportion of positive and negative tests, time to viral clearance, and time to seropositivity. Results: The rate of positivity varied over time due to viral circulation in the community and transition from targeted testing of symptomatic patients to more universal screening of hospitalized patients. Median duration of viral shedding (RT-PCR positivity) was 19.5 days and RT-PCR negativity from positivity was 25 days. *Of note, patients aged 6 to 15 years demonstrated a longer period of RT-PCR negativity from positivity, compared to patients aged 16 to 22 years (median=32 versus 18 days, p=0.015). Median time to seropositivity from RT-PCR positivity was 18 days while median time to reach adequate levels of neutralizing antibodies (defined as equivalent to 160 titer) was 36 days. Conclusions: The majority of patients demonstrated a prolonged period of viral shedding after infection with SARS CoV-2. Whether this correlates with persistent infectivity is unknown. Only 17 of 33 patients demonstrated neutralizing antibodies, suggesting that some patients may not mount significant immune responses to infection. It remains unknown if IgG antibody production correlates with immunity and how long measurable antibodies persist and protect against future infection*. [**note: the more we know, the more we don't know, and there is always the Rumsfeld Paradigm to deal with. We do need good data on children with respect to viral shedding. Do they represent a transmission risk? I guess with school reopenings this question will be answered on the societal level and perhaps not in a good way.**] https://www.medrxiv.org/content/10.1101/2020.08.06.20162446v1

NEWLY REGISTERED CLINICAL TRIALS

- It's boring to check this every day so today I didn't check for new trials.

CLINICAL TRIAL RESULTS

- Severe cases of COVID-19 are characterized by a strong inflammatory process that may ultimately lead to organ failure and patient death. The NLRP3 inflammasome is a molecular platform that promotes inflammation via cleavage and activation of key inflammatory molecules including active caspase-1 (Casp1p20), IL-1β and IL-18. Although the participation of the inflammasome in COVID-19 has been highly speculated, the inflammasome activation and participation in the outcome of the disease is unknown. Here we demonstrate that the NLRP3 inflammasome is activated in response to SARS-CoV-2 infection and it is active in COVID-19, influencing the clinical outcome of the disease. Studying moderate and severe COVID-19 patients, we found active NLRP3 inflammasome in PBMCs and tissues of post-mortem patients upon autopsy. Inflammasome-derived products such as Casp1p20 and IL-18 in the sera correlated with the markers of COVID-19 severity, including IL-6 and LDH. Moreover, higher levels of IL-18 and Casp1p20 are associated with disease severity and poor clinical outcome. Our results suggest that the inflammasome is key in the pathophysiology of the disease, indicating this platform as a marker of disease severity and a potential therapeutic target for COVID-19. [**note: from Brazil, some further work on the inflammatory process that leads to severe COVID-19. It gets more complicated with each finding.**]
  https://www.medrxiv.org/content/10.1101/2020.08.05.20168872v1

DRUG DEVELOPMENT

- Vaccine efforts against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) responsible for the current COVID-19 pandemic are focused on SARS-CoV-2 spike glycoprotein, the primary target for neutralizing antibodies. Here, we performed cryo-EM and site-specific glycan analysis of one of the leading subunit vaccine candidates from Novavax based on a full-length spike protein formulated in polysorbate 80 (PS 80) detergent. Our studies reveal a stable prefusion conformation of the spike immunogen with slight differences in the S1 subunit compared to published spike ectodomain structures. Interestingly, we also observed novel interactions between the spike trimers allowing formation of higher order spike complexes. This study confirms the structural integrity of the full-length spike protein immunogen and provides a basis for interpreting immune responses to this multivalent nanoparticle immunogen. [**note: this is the Novavax antigen and research is from the company and Scripps Institute.**]
  https://www.biorxiv.org/content/10.1101/2020.08.06.234674v1
- There are three key SARS-CoV-2 enzymes potentially targetable with antivirals: papain-like protease (PLpro), main protease (Mpro), and RNA-dependent RNA polymerase. Of these, PLpro is an especially attractive target because it plays an essential role in several viral replication processes, including cleavage and maturation of viral polyproteins, assembly of the replicasetranscriptase complex (RTC), and disruption of host viral response machinery to facilitate viral proliferation and replication. Moreover, this enzyme is conserved across different coronaviruses and promising inhibitors have already been discovered for its SARS-CoV variant. Here we report a substantive body of structural, biochemical, and virus replication studies that identify several inhibitors of the enzyme from SARS-CoV-2 in both wild-type and mutant forms. These efforts include the first structures of wild-type PLpro, the active site C111S mutant, and their complexes with inhibitors, determined at 1.60-2.70 Angstroms. This collection of structures provides fundamental molecular and mechanistic insight to PLpro, and critically, illustrates details for inhibitors recognition and interactions. All presented compounds inhibit the

peptidase activity of PLpro in vitro, and some molecules block SARS-CoV-2 replication in cell culture assays. These collated findings will accelerate further structure-based drug design efforts targeting PLpro, with the ultimate goal of identifying high-affinity inhibitors of clinical value for SARS-CoV-2. [**note: some really good crystallography on one of the key viral proteases from Univ of Chicago. There are some decent inhibitors used in the study that might be structures for drug development**] https://www.biorxiv.org/content/10.1101/2020.08.06.240192v1

- The design of an immunogenic scaffold that serves a role in treating a pathogen, and can be rapidly and predictively modeled, has remained an elusive feat. Here, we demonstrate that SARS-BLOCK™ synthetic peptide scaffolds act as antidotes to SARS-CoV-2 spike protein-mediated infection of human ACE2-expressing cells. Critically, SARS-BLOCK™ peptides are able to potently and competitively inhibit SARS-CoV-2 S1 spike protein receptor binding domain (RBD) binding to ACE2, the main cellular entry pathway for SARS-CoV-2, while also binding to neutralizing antibodies against SARS-CoV-2. In order to create this potential therapeutic antidote-vaccine, we designed, simulated, synthesized, modeled epitopes, predicted peptide folding, and characterized behavior of a novel set of synthetic peptides. The biomimetic technology is modeled off the receptor binding motif of the SARS-CoV-2 coronavirus, and modified to provide enhanced stability and folding versus the truncated wildtype sequence. These novel peptides attain single-micromolar binding affinities for ACE2 and a neutralizing antibody against the SARS-CoV-2 receptor binding domain (RBD), and demonstrate significant reduction of infection in nanomolar doses. *We also demonstrate that soluble ACE2 abrogates binding of RBD to neutralizing antibodies, which we posit is an essential immune-evasive mechanism of the virus. SARS-BLOCK™ is designed to uncloak the viral ACE2 coating mechanism, while also binding to neutralizing antibodies with the intention of stimulating a specific neutralizing antibody response*. Our peptide scaffolds demonstrate promise for future studies evaluating specificity and sensitivity of immune responses to our antidote-vaccine. In summary, SARS-BLOCK™ peptides are a promising COVID-19 antidote designed to combine the benefits of a therapeutic and vaccine, effectively creating a new generation of prophylactic and reactive antiviral therapeutics whereby immune responses can be enhanced rather than blunted. [**note: looks like today is drug discovery day! This is an interesting approach from a UCSF group, identifying some peptides that might have a dual function. Their hypothesis that soluble ACE2 may be an immune-evasive mechanism for the virus is intriguing. I suspect that this will turn out to be an important paper that leads to a new approach for treatment.**] https://www.biorxiv.org/content/10.1101/2020.08.06.238915v2
- Currently, the most efficacious therapeutic against SARS-CoV-2 infection is the Remdesivir (RDV), an adenine-like ribonucleotide analogue that is very efficiently incorporated by the SARS-CoV-2 replicase. Understanding why RDV is so well incorporated will facilitate development of even more effective therapeutics. Here, we have applied a high-throughput, single-molecule, magnetic-tweezers platform to study thousands of cycles of nucleotide addition by the SARS-CoV-2 replicase in the absence and presence of RDV, a Favipiravir-related analog (T-1106), and the endogenously produced ddhCTP. Our data are consistent with two parallel catalytic pathways of the replicase: a high-fidelity catalytic (HFC) state and a low-fidelity catalytic (LFC) state, the latter allowing the slow incorporation of both cognate and non-cognate nucleotides. ddhCTP accesses HFC, T-1106 accesses LFC as a non-cognate nucleotide, while RDV efficiently accesses both LFC pathway. In contrast to previous reports, we provide unequivocal evidence

against RDV functioning as a chain terminator. We show that RDV incorporation transiently stalls the replicase, only appearing as termination events when traditional, gel-based assays are used. The efficiency of ddhCTP utilization by the SARS-CoV-2 replicase suggests suppression of its synthesis during infection, inspiring new therapeutic strategies. Use of this experimental paradigm will be essential to the development of therapeutic nucleotide analogs targeting polymerases. [**note: a look at more ribonucleotide inhibitors.**]
https://www.biorxiv.org/content/10.1101/2020.08.06.240325v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Understanding humoral immune responses to SARS-CoV-2 infection will play a critical role in the development of vaccines and antibody-based interventions. We report systemic and mucosal antibody responses in convalescent individuals who experienced varying disease severity. Robust antibody responses to diverse SARS-CoV-2 antigens and evidence of elevated responses to endemic CoV were observed among convalescent donors. SARS-CoV-2-specific IgA and IgG responses were often negatively correlated, particularly in mucosal samples, suggesting subject-intrinsic biases in isotype switching. Assessment of antibody-mediated effector functions revealed an inverse correlation between systemic and mucosal neutralization activity and site-dependent differences in the isotype of neutralizing antibodies. Serum neutralization correlated with systemic anti-SARS-CoV-2 IgG and IgM response magnitude, while mucosal neutralization was associated with nasal SARS-CoV-2-specific IgA. These findings begin to map how diverse Ab characteristics relate to Ab functions and outcomes of infection, informing public health assessment strategies and vaccine development efforts. [**note: more on the antibody response in convalescent individuals.**]
https://www.medrxiv.org/content/10.1101/2020.08.05.20168971v1

DIAGNOSTIC DEVELOPMENT

- The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is due to the high rates of transmission by individuals who are asymptomatic at the time of transmission. Frequent, widespread testing of the asymptomatic population for SARS-CoV-2 is essential to suppress viral transmission and is a key element in safely reopening society. Despite increases in testing capacity, multiple challenges remain in deploying traditional reverse transcription and quantitative PCR (RT-qPCR) tests at the scale required for population screening of asymptomatic individuals. We have developed SwabSeq, a high-throughput testing platform for SARS-CoV-2 that uses next-generation sequencing as a readout. SwabSeq employs sample-specific molecular barcodes to enable thousands of samples to be combined and simultaneously analyzed for the presence or absence of SARS-CoV-2 in a single run. Importantly, SwabSeq incorporates an in vitro RNA standard that mimics the viral amplicon, but can be distinguished by sequencing. This standard allows for end-point rather than quantitative PCR, improves quantitation, reduces requirements for automation and sample-to-sample normalization, enables purification-free detection, and gives better ability to call true negatives. We show that SwabSeq can test nasal and oral specimens for SARS-CoV-2 with or without RNA extraction while maintaining analytical sensitivity better than or comparable to that of fluorescence-based RT-qPCR tests. SwabSeq is simple, sensitive, flexible, rapidly scalable, inexpensive enough to test widely and frequently, and can provide a turn around time of 12 to 24 hours. [**note: this is another good approach to**

scaling up viral testing. There is so much exciting research in the diagnostic space but we still need to step on the gas pedal to get this commercialized and deployed.]
https://www.medrxiv.org/content/10.1101/2020.08.04.20167874v1

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2020-08-08

The great thing about doing these daily music selections is finding excellent performers that I've not heard of. Nashville-based pianist Beegie Adair is one such artist. She has been associated with the Nashville Jazz Workshop for some time. Here she is with vocalist Monica Ramey performing 'Make Someone Happy' https://www.youtube.com/watch?v=fEmG6_lNL9A I just love 'The Great American Songbook!!!' How much do I love these songs? I went to Amazon Music and bought two of Ms. Ramey's albums!

US COVID-19 STATISTICS - **Infection Rate: 1.5%; CFR: 3.2%** (IR up no change; CFR no change; **note:** the CFR for this current outbreak continues to hover at 2%)

Herd immunity? The Washington Post explains it all. Special education students are most at risk with the shutdown of schools. Of course there is more on the politics of a COVID-19 vaccine. Hispanic and Black children are at risk for hospitalization; CDC report on this finding is HERE.

The New York Time writes about outbreaks in European nursing homes. Maybe superspreading events can be controlled since the window for viral spreading appears to be narrow. Here is the full paper that the article references: "a coupled within-host and between-host mathematical model to identify viral shedding levels required for transmission of SARS-CoV-2 and influenza, and to explain why super-spreading events occur more commonly during SARS-CoV-2 infection." It is well wort reading and of course the key lesson is to avoid poorly ventilated spaces with lots of people! Governor Cuomo gives the go-ahead for schools to reopen based on local decisions.

The Guardian weighs in on the spread of COVID-19 in the US.

Science have a review of the role interferons play in viral pneumonia; this is a very good primer on this topic.

The Lancet have several letters and authors reply on the use of anakinra for severe COVID-19. This IL-1 receptor antagonist is in clinical trials.

JAMA have a viewpoint on COVID-19 vaccine regulatory safeguards by senior management of the FDA. There is some good clarification of FDA's COVID-19 vaccine guidance.

According to Kaiser Health News, COVID-19 vaccine trials are attracting a lot of volunteers! Here is a useful article on when to visit your favorite store, restaurant, or salon.

MODELING

- Coronavirus disease-19 (COVID-19) emerged in November, 2019 in China and rapidly became pandemic. As with other coronaviruses, a preponderance of evidence suggests the virus originated in horseshoe bats (Rhinolophus spp.) and likely underwent a recombination event in

an intermediate host prior to entry into human populations. A significant concern is that SARS-CoV-2 could become established in secondary reservoir hosts outside of Asia. To assess this potential, we challenged deer mice (Peromyscus maniculatus) with SARS-CoV-2 and found robust virus replication in the upper respiratory tract, lungs and intestines, with detectable viral RNA for up to 21 days in oral swabs and 14 days in lungs. Virus entry into the brain also occurred, likely via gustatory-olfactory-trigeminal pathway with eventual compromise to the blood brain barrier. Despite this, no conspicuous signs of disease were observed and no deer mice succumbed to infection. Expression of several innate immune response genes were elevated in the lungs, notably IFNα, Cxcl10, Oas2, Tbk1 and Pycard. Elevated CD4 and CD8β expression in the lungs was concomitant with Tbx21, IFNγ and IL-21 expression, suggesting a type I inflammatory immune response. Contact transmission occurred from infected to naive deer mice through two passages, showing sustained natural transmission. In the second deer mouse passage, an insertion of 4 amino acids occurred to fixation in the N-terminal domain of the spike protein that is predicted to form a solvent-accessible loop. Subsequent examination of the source virus from BEI Resources indicated the mutation was present at very low levels, demonstrating potent purifying selection for the insert during in vivo passage. Collectively, this work has determined that deer mice are a suitable animal model for the study of SARS-CoV-2 pathogenesis, and that they have the potential to serve as secondary reservoir hosts that could lead to periodic outbreaks of COVID-19 in North America. [**note: the virus is going to find a lot more animal hosts in addition to these deer mice. Viruses just want a place to replicate which is their only goal in life.**] https://www.biorxiv.org/content/10.1101/2020.08.07.241810v1

- The primary aim was to evaluate whether randomly sampling and testing a set number of individuals for active or past COVID-19 while adjusting for misclassification error captures a simulated prevalence. The secondary aim was to quantify the impact of misclassification error bias on publicly reported case data in Maryland. Methods: Using a stratified random sampling approach, 50,000 individuals were selected from a simulated Maryland population to estimate the prevalence of active and past COVID-19. Data from the 2014-2018 and 2018 American Community Surveys were used. The simulated prevalence was 0.5% and 1.0% for active and past COVID-19, respectively. Bayesian models, informed by published validity estimates, were used to account for misclassification error when estimating the prevalence of active and past COVID-19. Results: Failure to account for misclassification error overestimated the simulated prevalence for active and past COVID-19. Adjustment for misclassification error decreased the point estimate for active and past COVID-10 prevalence by 55% and 29%, respectively. Adjustment for sampling method and misclassification error only captured the simulated past COVID-19 prevalence. The simulated active COVID-19 prevalence was only captured when set to 0.7% and above. Adjustment for misclassification error for publicly reported Maryland data increased the estimated average daily cases by 8%. Conclusions: *Random sampling and testing of COVID-19 is needed but must be accompanied by adjustment for misclassification error to avoid over- or underestimating the prevalence. This approach bolsters disease control efforts. Implementing random testing for active COVID-19 may be best in a smaller geographic area with highly prevalent cases.* [**note: here is some follow-up work from MITRE Corporation from a paper I recently linked.**] https://www.medrxiv.org/content/10.1101/2020.08.06.20169656v1
- In order to prepare for and control the continued spread of the COVID-19 pandemic while minimizing its economic impact, the world needs to be able to estimate and predict COVID-19's

spread. Unfortunately, we cannot directly observe the prevalence or growth rate of COVID-19; these must be inferred using some kind of model. We propose a hierarchical Bayesian extension to the classic susceptible-exposed-infected-removed (SEIR) compartmental model that adds compartments to account for isolation and death and allows the infection rate to vary as a function of both mobility data collected from mobile phones and a latent time-varying factor that accounts for changes in behavior not captured by mobility data. Since confirmed-case data is unreliable, we infer the model's parameters conditioned on deaths data. We replace the exponential-waiting-time assumption of classic compartmental models with Erlang distributions, which allows for a more realistic model of the long lag between exposure and death. The mobility data gives us a leading indicator that can quickly detect changes in the pandemic's local growth rate and forecast changes in death rates weeks ahead of time. This is an analysis of observational data, so any causal interpretations of the model's inferences should be treated as suggestive at best; nonetheless, the model's inferred relationship between different kinds of trips and the infection rate do suggest some possible hypotheses about what kinds of activities might contribute most to COVID-19's spread. [**note: Google is not just a search tool, they have a good group of researchers who have been working on a variety of health issues. Here is a modeling paper that is interesting to read.**]
https://www.medrxiv.org/content/10.1101/2020.08.06.20169664v1

- Background Large-scale school closures have been implemented worldwide to curb the spread of COVID-19. However, the impact of school closures and re-opening on epidemic dynamics remains unclear. Methods We simulated COVID-19 transmission dynamics using an individual-based stochastic model, incorporating social-contact data of school-aged children during shelter-in-place orders derived from Bay Area (California) household surveys. We simulated transmission under observed conditions and counterfactual intervention scenarios between March 17-June 1, and evaluated various fall 2020 K-12 reopening strategies. Findings Between March 17-June 1, assuming children <10 were half as susceptible to infection as older children and adults, we estimated school closures averted a similar number of infections (13,842 cases; 95% CI: 6,290, 23,040) as workplace closures (15,813; 95% CI: 9,963, 22,617) and social distancing measures (7,030; 95% CI: 3,118, 11,676). School closure effects were driven by high school and middle school closures. Under assumptions of moderate community transmission, we estimate that fall 2020 school reopenings will increase symptomatic illness among high school teachers (an additional 40.7% expected to experience symptomatic infection, 95% CI: 1.9, 61.1), middle school teachers (37.2%, 95% CI: 4.6, 58.1), and elementary school teachers (4.1%, 95% CI: -1.7, 12.0). Results are highly dependent on uncertain parameters, notably the relative susceptibility and infectiousness of children, and extent of community transmission amid re-opening. The school-based interventions needed to reduce the risk to fewer than an additional 1% of teachers infected varies by grade level. A hybrid-learning approach with halved class sizes of 10 students may be needed in high schools, while maintaining small cohorts of 20 students may be needed for elementary schools. Interpretation *Multiple in-school intervention strategies and community transmission reductions, beyond the extent achieved to date, will be necessary to avoid undue excess risk associated with school reopening. Policymakers must urgently enact policies that curb community transmission and implement within-school control measures to simultaneously address the tandem health crises posed by COVID-19 and adverse child health and development consequences of long-term school closures*. [**note: from Cal Berkeley, some**

good thoughts on school reopenings. I really do wonder how much critical thinking is going into these decisions. Time will tell.]
https://www.medrxiv.org/content/10.1101/2020.08.06.20169797v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a multisite, double-blind, randomized, placebo-controlled, dose-escalating, phase 1 trial of NT-I7 with standard of care (SOC) versus placebo with SOC to evaluate the safety and efficacy of NT-I7 in adults with mild coronavirus disease 2019 (COVID-19). After determination of eligibility and baseline assessment, a single dose of the study agent (NT-I7 or placebo) will be administered after 1:1 randomization, along with SOC. Research blood collection will occur at baseline, days 3, 7, 14, 30, 60 and 90 days after administration. Primary and secondary evaluations will include assessment of adverse events (AEs), absolute lymphocyte count (ALC), and trajectory of other lymphocytes subsets: CD4, CD8, natural killer (NK), B, and mucosal-associated-invariant T (MAIT) cells. The final study visit will be at day 90 after the study agent administration. The investigators hypothesize that NT-I7 is safe for administration and preserves lymphocyte homeostasis in patients with mild COVID-19. [**note: this is a long-acting recombinant IL-7 that is supposed to amplify T cells and the sponsor is NeoImmune Tech**] NCT04501796
- This is an interventional, multicenter, 2-arm, parallel-group, randomized, double-blind, placebo controlled, dose-escalation, safety and efficacy study of F-652 treatment versus placebo in patients aged 18 years or older with a COVID-19 diagnosis confirmed by PCR. Eligible patients will have moderate to severe COVID-19 symptoms within 5 days post hospitalization and a positive COVID-19 testing. [**note: this is an IL-22—IgG2 fusion protein and the sponsor is Generon (Shanghai) Corp.**] NCT04498377

CLINICAL TRIAL RESULTS

- Paediatric inflammatory multisystem inflammatory syndrome temporally associated with SARS-CoV-2 infection (PIMS-TS) is a new disease with overlapping features of Kawasaki disease (KD) and toxic shock syndrome. Unbiased single cell RNA sequencing analysis of peripheral blood mononuclear cells from PIMS-TS and KD patients shows monocytes are the main source of pro-inflammatory cytokines and large changes in the frequency of classical, intermediate and non-classical monocytes occur in both diseases. [**note: information on multisystem inflammatory syndrome in children linked to monocytes as the main source of pro-inflammatory cytokines.**]
https://www.medrxiv.org/content/10.1101/2020.08.06.20164848v1
- Gastrointestinal (GI) symptoms of SARS-CoV2/COVID-19 in the form of anorexia,nausea, vomiting, abdominal pain and diarrhea are usually preceeded by respiratory manifestations and are associated with a poor prognosis. Hematochezia is an uncommon clinical presentation of COVID-19 disease and we hypothesize that older patients with significant comorbidites (obesity and cardiovascular) and prolonged hospitalization are suspectible to ischemic injury to the bowel. We reviewed the clinical course, key laboratory data including acute phase reactants, drug/medication history in two elderly male patients admitted for COVID-19 respiratory failure. Both patients had a complicated clinical course and suffered from hematochezia and acute blood loss anemia requiring blood transfusion around day 40 of their hospitalization. Colonoscopic impressions were correlated with the histopathological findings in the colonic

biopies and changes compatible with ischemia to nonspecific acute inflammation, edema and increased eosinophils in the lamina propria were noted.Both patients were on anticoagulants, multiple antibiotics and antifungal agents due to respiratory infections at the time of lower GI bleeding. Hematochezia resolved spontaneously with supportive care. Both patients eventually recovered and were discharged. Elderly patients with significant comorbid conditions are uniquely at risk for ischemic injury to the bowel. Hypoxic conditions due to COVID-19 pneumonia and respiratory failure, compounded by preexisting cardiovascular complications, and/or cytokine storm orchestrated by the viral infection leading to alteration in coagulation profile and/or drug/medication injury can be difficult to distinguish in these critically ill patients. *Presentation of hematochezia may further increase the mortality and morbidity of COVID-19 patients, and prompt consultation and management by gastroenterology is therefore warranted*. [**note: this is from NYU where several of my readers have trained and looks at very rare condition in two elderly male patients.**]
https://www.medrxiv.org/content/10.1101/2020.07.29.20164558v1

DRUG DEVELOPMENT

- Nothing to report today.

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Coronavirus genome replication is associated with virus-induced cytosolic double-membrane vesicles, which may provide a tailored micro-environment for viral RNA synthesis in the infected cell. However, it is unclear how newly synthesized genomes and mRNAs can travel from these sealed replication compartments to the cytosol to ensure their translation and the assembly of progeny virions. Here, we used cellular electron cryo-microscopy to visualize a molecular pore complex that spans both membranes of the double-membrane vesicle and would allow export of RNA to the cytosol. A hexameric assembly of a large viral transmembrane protein was found to form the core of the crown-shaped complex. This coronavirus-specific structure likely plays a critical role in coronavirus replication and thus constitutes a potential drug target. [**note: this is another unraveling of how the virus acts upon cell entry.**]
https://science.sciencemag.org/content/early/2020/08/05/science.abd3629
- Serology testing is explored for epidemiological research and to inform individuals after suspected infection. During the COVID-19 pandemic, frontline healthcare professionals (HCP) may be at particular risk for infection. No longitudinal data on functional seroconversion in HCP in regions with low COVID-19 prevalence and low pre-test probability exist. Methods: In a large German university hospital, we performed weekly questionnaire assessments and anti-SARS-CoV-2 IgG measurements with various commercial tests, a novel surrogate virus neutralization test, and a neutralization assay using live SARS-CoV-2. Results: From baseline to week six, n=1,080 screening measurements for anti-SARS CoV-2 (S1) IgG from n=217 frontline HCP (65% female) were performed. Overall, 75.6% of HCP reported at least one symptom of respiratory infection. Self-perceived infection probability declined over time (from mean 20.1% at baseline to 12.4 % in week six, $p<0.001$). In sera of convalescent PCR-confirmed COVID-19 patients, we measured high anti-SARS-CoV-2 IgG levels, obtained highly concordant results from ELISAs using e.g. the S1 spike protein domain and the nucleocapsid protein (NCP) as targets, and confirmed antiviral neutralization. However, in HCP the cumulative incidence for anti-SARS-CoV-2 (S1) IgG

was 1.86% for positive and 0.93% for equivocal positive results over the six week study period. Except for one HCP, none of the eight initial positive results were confirmed by alternative serology tests or showed in vitro neutralization against live SARS CoV-2. The only true seroconversion occurred without symptoms and mounted strong functional humoral immunity. Thus, the confirmed cumulative incidence for neutralizing anti-SARS-CoV-2 IgG was 0.47%. Conclusion: When assessing anti-SARS-CoV-2 immune status in individuals with low pre-test probability, we suggest confirming positive results from single measurements by alternative serology tests or functional assays. Our data highlight the need for a methodical serology screening approach in regions with low SARS-CoV-2 infection rates. [**note: serology testing from a German teaching hospital showing the need for adaptive testing approaches.**] https://www.medrxiv.org/content/10.1101/2020.08.06.20169250v1

- Most individuals infected with SARS-CoV-2 develop neutralizing antibodies that target the viral spike protein. Here we quantify how levels of these antibodies change in the months following SARS-CoV-2 infection by examining longitudinal samples collected between ≈30 and 152 days post-symptom onset from a prospective cohort of 34 recovered individuals with asymptomatic, mild, or moderate-severe disease. Neutralizing antibody titers declined an average of about four-fold from one to four months post-symptom onset. Importantly, our data are consistent with the expected early immune response to viral infection, where an initial peak in antibody levels is followed by a decline to a lower plateau. Additional studies of long-lived B-cells and antibody titers over longer time frames are necessary to determine the durability of immunity to SARS-CoV-2. [**note: ongoing serology of recovered SARS-CoV-2 infected individuals from Seattle, the first epicenter in the US. I expect other sites such as New York will be doing similar research.**] https://www.medrxiv.org/content/10.1101/2020.08.06.20169367v1

DIAGNOSTIC DEVELOPMENT

- The progress of the COVID-19 pandemic profoundly impacts the health of communities around the world, with unique impacts on colleges and universities. Transmission of SARS-CoV-2 by asymptomatic people is thought to be the underlying cause of a large proportion of new infections. However, the local prevalence of asymptomatic and pre-symptomatic carriers of SARS-CoV-2 is influenced by local public health restrictions and the community setting. Objectives: This study has three main objectives. First, we looked to establish the prevalence of asymptomatic SARS-CoV-2 infection on a university campus in California. Second, we sought to assess the changes in viral prevalence associated with the shifting community conditions related to non-pharmaceutical interventions (NPIs). Third, we aimed to compare the performance of CRISPR- and PCR-based assays for large-scale virus surveillance sampling in COVID-19 asymptomatic persons. Methods: We enrolled 1,808 asymptomatic persons for self-collection of oropharyngeal (OP) samples to undergo SARS-CoV-2 testing. We compared viral prevalence in samples obtained in two time periods: May 28th-June 11th; June 23rd-July 2nd. We detected viral genomes in these samples using two assays: CREST, a CRISPR-based method recently developed at UCSB, and the RT-qPCR test recommended by US Centers for Disease Control and Prevention (CDC). Results: Of the 1,808 participants, 1,805 were affiliates of the University of California, Santa Barbara, and 1,306 were students. None of the tests performed on the 732 samples collected between late May to early June were positive. In contrast, tests performed on the 1076 samples collected between late June to early July, revealed nine positive cases. This

change in prevalence met statistical significance, p = 0.013. One sample was positive by RT-qPCR at the threshold of detection, but negative by both CREST and CLIA-confirmation testing. With this single exception, there was perfect concordance in both positive and negative results obtained by RT-qPCR and CREST. The estimated prevalence of the virus, calculated using the confirmed cases, was 0.74%. The average age of our sample population was 28.33 (18-75) years, and the average age of the positive cases was 21.7 years (19-30). Conclusions: Our study revealed that there were no COVID-19 cases in our study population in May/June. Using the same methods, we demonstrated a substantial shift in prevalence approximately one month later, which coincided with changes in community restrictions and public interactions. This increase in prevalence, in a young and asymptomatic population which would not have otherwise accessed COVID-19 testing, indicated the leading wave of a local outbreak, and coincided with rising case counts in the surrounding county and the state of California. Our results substantiate that large, population-level asymptomatic screening using self-collection may be a feasible and instructive aspect of the public health approach within large campus communities, and the almost perfect concordance between CRISPR- and PCR-based assays indicate expanded options for surveillance testing [**note: as this paper comes from my alma mater, UC Santa Barbara, it is mandatory reading! The researchers show the utility of using CRISPR to assay for COVID-19 and that it compares almost perfectly with PCR-based assays. Good work Gauchos!!!**] https://www.medrxiv.org/content/10.1101/2020.08.06.20169771v1

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2020-08-09

For this reflection Sunday, let us have a listen to Hector Berlioz's 'Symphonie Fantastique.' I always enjoyed playing this on Halloween just to hear the witches sabbath movement. Here is the orchestra of Radio France conducted by Myung-Whun Chung: https://www.youtube.com/watch?v=5HgqPpjIH5c

Could asymptomatic people hold the clue to solving the COVID-19 mystery? This Washington Post article has some perspectives. Some athletes may have lasting cardiovascular effects from the virus. Finally, an inside view of White House deliberations on how to respond to the COVID-19 crisis.

This New York Times op-ed by Michael Osterholm and Neal Kashkari is a real downer. I don't think there is the will for the type of shut down they advocate. Here is a nice story on a Los Angeles contact tracer. Of course you have always wondered how to move an elephant in the midst of a pandemic, right?

The New Yorker offers a first person case study of COVID-19.

Unrelated to COVID-19, the OHDSI-COVID-19 consortium looked across several healthcare record sets to see if HCQ caused suicidal ideation in rheumatoid arthritis patients. The good news is that it does not! It is still not an effective treatment for COVID-19.

Not a lot of preprints to review today.

MODELING

- The COVID-19 pandemic has brought into sharp focus the need to understand respiratory virus transmission mechanisms. In preparation for an anticipated influenza pandemic, a substantial body of literature has developed over the last few decades showing that the short-range aerosol

route is an important, though often neglected transmission path. We develop a simple mathematical model for COVID-19 transmission via aerosols, apply it to known outbreaks, and present quantitative guidelines for ventilation and occupancy in the workplace. [**note: this is a very readable paper from an MIT physicist. The analysis and recommendations are quite good and he discusses building air handling requirements. This is well worth downloading and reading!**] https://www.medrxiv.org/content/10.1101/2020.05.21.20108894v3

- Serological assays can detect anti-SARS-CoV-2 antibodies, but their sensitivity often comes at the expense of specificity. Here we developed a Tripartite Automated Blood Immunoassay (TRABI) to assess the IgG response against SARS-CoV-2. Calibration was performed with 90 prepandemic and 55 virologically and clinically confirmed COVID-19 samples. Posterior probabilities were calculated from 3x8 measurements of logarithmically diluted samples against the ectodomain and the receptor-binding domain of the spike protein and the nucleocapsid protein. We then performed 948'528 assays on 5'503 prepandemic and 34'019 copandemic samples from hospital patients and healthy blood donors. The seroprevalence increased in March 2020 (0.3%; CI95%: 0.1% - 0.5%) among hospital patients but plateaued in April at 1.1-1.3%, and dropped to 0.3-0.7% in July. A dynamic transmission model describing SARS-CoV-2 transmission and seroconversion in the general population of the Canton of Zurich yielded an infection fatality ratio of 0.6% (CI95%: 0.4%-0.8%), similarly to other European areas. While the evolution of seroprevalence points to a high effectiveness of containment measures, our data highlight that antibody waning warrants a continuous seromonitoring to reliably estimate the prevalence in a population. [**note: here is a good Swiss serology study of the Zurich metro area.**] https://www.medrxiv.org/content/10.1101/2020.05.31.20118554v4

NEWLY REGISTERED CLINICAL TRIALS

- Did not check.

CLINICAL TRIAL RESULTS

- Objectives To investigate if the anxiety associated with COVID 19 is a promoting factor to tinnitus. Methods A retrospective research design was used to compare the clinical characteristics of tinnitus between the patients in 2020 under pandemic pressure and those from the matching period in 2019. While anxiety was quantified using the Zung Self-rating Anxiety Scale (SAS), tinnitus severity was evaluated using the Tinnitus Handicap Inventory (THI) questionnaire and the test of minimum masking level (MML). The assessments were repeated after the sound therapy plus educational counselling (STEC) and compared with EC alone therapy. Results A large increase in anxiety was evident in 2020 in both case rate and SAS. The treatment of both methods was less effective in 2020. SAS, THI and MML were all deteriorated after the EC alone treatment in 2020, while an improvement was seen in 2019. This suggests that EC alone could not counteract the stress by COVID-19 at all, and the stress, if not managed well, can significantly increase the severity of tinnitus and associated anxiety. Conclusions By using the EC subgroup in virtual control, we conclude that anxiety can serve as a promoting factor to tinnitus. We believe that this is the first study report that confirm the causative/promotive role of anxiety on tinnitus. [**note: well this certainly explains the ringing in my ears that has mysteriously increased since mid-March.**]
https://www.medrxiv.org/content/10.1101/2020.07.02.20145532v2

- To examine the association between antidepressant use and the risk of intubation or death in hospitalized patients with COVID-19. Design: Multicenter observational retrospective cohort study. Setting: Greater Paris University hospitals, France. Participants: 7,345 adults hospitalized with COVID-19 between 24 January and 1 April 2020, including 460 patients (6.3%) who received an antidepressant during the visit. Data source: Assistance Publique-Hopitaux de Paris Health Data Warehouse. Main outcome measures: The primary endpoint was a composite of intubation or death. We compared this endpoint between patients who received antidepressants and those who did not in time-to-event analyses adjusting for patient characteristics (such as age, sex, and comorbidities), disease severity and other psychotropic medications. The primary analyses were multivariable Cox models with inverse probability weighting. Results: Over a mean follow-up of 18.5 days (SD=27.1), 1,331 patients (18.1%) had a primary end-point event. Unadjusted hazard ratio estimates of the association between antidepressant use and the primary outcome stratified by age (i.e., 18-50, 51-70, 71-80, and 81+) were non-significant (all $p>0.072$), except in the group of patients aged 71-80 years (HR, 0.66; 95% CI, 0.45 to 0.98; $p=0.041$). Following adjustments, the primary analyses showed a significant association between use of any antidepressant (HR, 0.64; 95% CI, 0.51 to 0.80; $p<0.001$), SSRI (HR, 0.56; 95% CI, 0.42 to 0.75; $p<0.001$), and SNRI (HR, 0.57; 95% CI, 0.34 to 0.96; $p=0.034$), and reduced risk of intubation or death. Specifically, exposures to escitalopram, fluoxetine, and venlafaxine were significantly associated with lower risk of intubation or death (all $p<0.05$). These associations remain significant in multiple sensitivity analyses, except for the association between SNRI use and the outcome. Conclusions: SSRI use could be associated with lower risk of death or intubation in hospitalized patients with COVID-19. Double-blind controlled randomized clinical trials of these medications for COVID-19 are needed. [**note: this is a large Parisian cohort study looking at the effect of SSRIs on intubation and death from COVID-19. There seems to be a bit of protection but TIWWDCT. Let the clinical trials commence and level out our moods if this approach works.**] https://www.medrxiv.org/content/10.1101/2020.07.09.20143339v2

DRUG DEVELOPMENT

- Nothing new today

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing new today

DIAGNOSTIC DEVELOPMENT

- There is an ongoing need of developing sensitive and specific methods for the determination of SARS-CoV-2 seroconversion. For this purpose, we have developed a multiplexed flow cytometric bead array (C19BA) that allows the identification of IgG and IgM antibodies against three immunogenic proteins simultaneously: the spike receptor-binding domain (RBD), the spike protein subunit 1 (S1) and the nucleoprotein (N). Using different cohorts of samples collected before and after the pandemic, we show that this assay is more sensitive than ELISA. The combination of three viral antigens allows for the interrogation of full seroconversion,. Importantly, we have detected N-reactive antibodies in COVID-19-negative individuals. Here we present a novel immunoassay that is easily implemented and has superior potential to detect low antibody titers compared to current gold standard serology methods. [**note: from Spain,**

another useful multiplex serology approach.]
https://www.medrxiv.org/content/10.1101/2020.07.28.20162941v2