2020-09-29

Welcome to Week 28 of the Newsletter!

To celebrate the conclusion to the Days of Awe the ended with Yom Kippur last night here are a couple of clips from Avi Schwartz, the cantor at the Park Avenue Synagogue in New York City. First we have the Hasidic Kaddish: https://www.youtube.com/watch?v=_e50J2VpW48 The Rabbi's comment at the end is priceless! The second clip is the closing segment of the Yom Kippur service: https://www.youtube.com/watch?v=6k_Orlkk9Ww Why not make it three clips? For the opera buffs, here is Cantor Schwartz singing Adon Olam to a variety of classic tenor arias: https://www.youtube.com/watch?v=pkxe8lxPTOA How many can you identify??

May all my readers have a happy and healthy New Year going forward and may you all be sealed in the Book of Life. Back to more secular things, namely COVID-19.

The Washington Post offers another story about problems at the CDC. This is too sad. For those readers who have children of Halloween age, here is a useful article on Trick or Treating.

The New York Times discusses 'long haulers' who have lingering COVID-19 symptoms. There are clusters of COVID-19 cases in Brooklyn and the northern suburbs. CDC have a new analysis showing that the virus is more common in teenagers than younger children. Here is an op-ed on what parents need to know about school coronavirus data. . CDC was pressured to change risk reporting of children returning to school. Despite all the debates about herd immunity the pandemic may be far from over.

INOVIO have reported a partial clinical hold on their Phase 2/3 COVID-19 vaccine. This one is a DNA based vaccine.

STAT have perhaps the most authoritative look at Operation Warp Speed. There is also a layperson's guide to how a COVID-19 vaccine could be approved. Here is a good opinion piece on the need for transparency at FDA.

The Lancet have an article on evaluating interest in off-label use of disinfectants for COVID-19. I don't know whether to laugh or cry. Here it is one more time, my advice for all my readers, "do not gargle or drink bleach!" Here are results of a very small Dutch trial of the mAb, IFX-1 (vilobelimab), in patients with severe COVID-19. In this small exploratory phase 2 part of the PANAMO trial, C5a inhibition with IFX-1 appears to be safe in patients with severe COVID-19. The secondary outcome results in favour of IFX-1 are preliminary because the study was not powered on these endpoints, but they support the investigation of C5a inhibition with IFX-1 in a phase 3 trial using 28-day mortality as the primary endpoint. Here is a commentary on this research.

Medscape cover a COVID-19 outbreak at the Brigham and Women's hospital apparently caused by one employee who thought his symptoms were a result of seasonal allergies. Here is a new look at safety of HCQ and COVID-19 treatment.

Kaiser Health News has a nice article on herd immunity; I like the title 'Corralling the Facts on Herd Immunity!'

Derek Lowe discusses one of the confounding and troubling issues associated with interferon and COVID-19. Here is the Science paper that Derek references. This may be one of the key features of male susceptibility to COVID-19.

MODELING

- Many complex mathematical and epidemiological methods have been used to model the Covid-19 pandemic. Among other results from these models has been the view that closing schools had little impact on infection rates in several countries1. We took a different approach. Making one assumption, we simply plotted cases, hospitalizations and deaths, on a log2 Y axis and a linear date-based X axis, and analyzed them using segmented regression, a powerful method that has largely been overlooked during this pandemic. Here we show that the data fit straight lines with correlation coefficients ranging from 92% - 99%, and that these lines broke at interesting intervals, revealing that school closings dropped infection rates in half, lockdowns dropped the rates 3 to 4 fold, and other actions (such as closing bars and mandating masks) brought the rates even further down. Hospitalizations and deaths paralleled cases, with lags of three to ten days. *The graphs, which are easy to read, reveal changes in infection rates that are not obvious using other graphing methods, and have several implications for modeling and policy development during this and future pandemics. Overall, other than full lockdowns, three interventions had the most impact: closing schools, closing bars and wearing masks: a message easily understood by the public*. [**note: this comes from Tony Fauci's institute at NIH. Yup, the message is easy but the implementation is hard.**]
  https://www.medrxiv.org/content/10.1101/2020.09.26.20202457v1
- When an outbreak starts spreading, policy makers have to make decisions that affects health of their citizens and the economic. Some might induce harsh measures, such as lockdown. Following a long harsh lockdown, economical declines force policy makers to rethink reopening. But what is the most effective reopening strategy? In order to provide an effective strategy, here we propose a control strategy model. Our model assesses the trade-off between social performance and limited medical resources by determining individuals' propensities. The proposed strategy also helps decision makers to find optimal lockdown and exit strategy for each region. Moreover, the financial loss is minimized. In addition, a study of a COVID-19 dataset for Los Angeles County is performed to validate our model and its results. [**note: here is a nice model for a Cornell Univ systems engineer.**]
  https://www.medrxiv.org/content/10.1101/2020.09.26.20202325v1
- We have studied the evolution of COVID-19 in 12 low and middle income countries in which reported cases have peaked and declined rapidly in the past 2-3 months. In most of these countries the declines happened while control measures were consistent or even relaxing, and without signs of significant increases in cases that might indicate second waves. For the 12 countries we studied, the hypothesis that these countries have reached herd immunity warrants serious consideration. The Reed-Frost model, perhaps the simplest description for the evolution of cases in an epidemic, with only a few constant parameters, fits the observed case data remarkably well, and yields parameter values that are reasonable. The best-fitting curves suggest that the effective basic reproduction number in these countries ranged between 1.5 and 2.0, indicating that the curve was flattened in some countries but not suppressed by pushing the reproduction number below 1. The results suggest that between 51 and 80% of the population

in these countries have been infected, and that between 0.05% and 2.50% of cases have been detected; values which are consistent with findings from serological and T-cell immunity studies. The infection rates, combined with data and estimates for deaths from COVID-19, allow us to estimate overall infection fatality rates for three of the countries. The values are lower than expected from reported infection fatality rates by age, based on data from several high-income countries, and the country population by age. COVID-19 may have a lower mortality risk in these three countries (to differing degrees in each country) than in high-income countries, due to differences in immune response, prior exposure to coronaviruses, disease characteristics or other factors. We find that the herd immunity hypothesis would not have fit the evolution of reported cases in several European countries, even just after the initial peaks; and subsequent resurgences of cases obviously prove that those countries have infection rates well below herd immunity levels. Our hypothesis that the 12 countries we studied have reached herd immunity should now be tested further, through serological and T cell immunity studies. [**note: this is an interesting model from three South African researchers. Maybe some of the countries covered here have reached herd immunity. Infection is thought to be between 51 and 80% of the population. The question is why they have lower mortality rates in some countries.**]
https://www.medrxiv.org/content/10.1101/2020.09.26.20201814v1

NEWLY REGISTERED CLINICAL TRIALS

- Yes, I did check and No there are not any new drugs or vaccine trials starting up.

CLINICAL TRIAL RESULTS

- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the agent of a major global outbreak of respiratory tract disease known as coronavirus disease-2019 (COVID-19). SARS-CoV-2 infects the lungs and may cause several immune-related complications such as lymphocytopenia and cytokine storm which are associated with the severity of the disease and predict mortality . The mechanism by which SARS-CoV-2 infection may result in immune system dysfunction is not fully understood. *Here we show that SARS-CoV-2 infects human CD4+ T helper cells, but not CD8+ T cells, and is present in blood and bronchoalveolar lavage T helper cells of severe COVID-19 patients. We demonstrated that SARS-CoV-2 spike glycoprotein (S) directly binds to the CD4 molecule, which in turn mediates the entry of SARS- CoV-2 in T helper cells in a mechanism that also requires ACE2 and TMPRSS2. Once inside T helper cells, SARS-CoV-2 assembles viral factories, impairs cell function and may cause cell death. SARS-CoV-2 infected T helper cells express higher amounts of IL-10, which is associated with viral persistence and disease severity. Thus, CD4-mediated SARS-CoV-2 infection of T helper cells may explain the poor adaptive immune response of many COVID- 19 patients*. [**note: this is from Brazil and shows another nefarious way the virus exacts a toll.**]
https://www.medrxiv.org/content/10.1101/2020.09.25.20200329v1
- A recent study by Wilk et al. of the transcriptome of peripheral blood mononuclear cells (PBMCs) in seven patients hospitalized with COVID-19 described a population of 'developing neutrophils' that were 'phenotypically related by dimensionality reduction' to plasmablasts, and that these two cell populations represent a 'linear continuum of cellular phenotype'. The authors suggest that, in the setting of acute respiratory distress syndrome (ARDS) secondary to severe COVID-19, a 'differentiation bridge from plasmablasts to developing neutrophils'

connected these distantly related cell types. This conclusion is controversial as it appears to violate several basic principles in cell biology relating to cell lineage identity and fidelity. Correctly classifying cells and their developmental history is an important issue in cell biology and we suggest that this conclusion is not supported by the data as we show here that: (1) regressing out covariates such as unique molecular identifiers (UMIs) can lead to overfitting; and (2) that UMAP embeddings may reflect the expression of similar genes but not necessarily direct cell lineage relationships. [**note: this is a contrary view to a paper I linked to a while back regarding one of the factors for severe COVID-19.**]
https://www.biorxiv.org/content/10.1101/2020.09.27.312538v1

DRUG DEVELOPMENT

- There is currently no approved vaccine to halt the spread of SARS-CoV-2 and only very few treatment options are available to manage COVID-19 patients. For development of preclinical countermeasures, reliable and well-characterized small animal disease models will be of paramount importance. Here we show that intranasal inoculation of SARS-CoV-2 into Syrian hamsters consistently caused moderate broncho-interstitial pneumonia, with high viral lung loads and extensive virus shedding, but animals only displayed transient mild disease. We determined the infectious dose 50 to be only five infectious particles, making the Syrian hamster a highly susceptible model for SARS-CoV-2 infection. Neither hamster age nor sex had any impact on the severity of disease or course of infection. Finally, prolonged viral persistence in interleukin 2 receptor gamma chain knockout hamsters revealed susceptibility of SARS-CoV-2 to adaptive immune control. In conclusion, the Syrian hamster is highly susceptible to SARS-CoV-2 making it a very suitable infection model for COVID-19 countermeasure development. [**note: the Syrian hamster appears to be a good animal model for SARS-CoV-2 infection.**]
https://www.biorxiv.org/content/10.1101/2020.09.25.314070v1
- The ongoing COVID-19 pandemic is responsible for worldwide economic damage and nearly one million deaths. Potent drugs for the treatment of severe SARS-CoV-2 infections are not yet available. To identify host factors that support coronavirus infection, we performed genome-wide functional genetic screens with SARS-CoV-2 and the common cold virus HCoV-229E in non-transgenic human cells. These screens identified PI3K type 3 as a potential drug target against multiple coronaviruses. We discovered that the lysosomal protein TMEM106B is an important host factor for SARS-CoV-2 infection. Furthermore, we show that TMEM106B is required for replication in multiple human cell lines derived from liver and lung and is expressed in relevant cell types in the human airways. Our results identify new coronavirus host factors that may potentially serve as drug targets against SARS-CoV-2 or to quickly combat future zoonotic coronavirus outbreaks. [**note: here is a lysosomal protein that may be another drug target for SARS-CoV-2. Do look at the link above as this is an interesting protein linked to some other medical conditions.**] https://www.biorxiv.org/content/10.1101/2020.09.28.316281v1
- SARS-CoV-2 neutralizing antibodies represent an important component of the ongoing search for effective treatment of and protection against COVID-19. We report here on the use of a naive phage display antibody library to identify a panel of fully human SARS-CoV-2 neutralizing antibodies. Following functional profiling in vitro against an early pandemic isolate as well as a recently emerged isolate bearing the D614G Spike mutation, the clinical candidate antibody, STI-1499, and the affinity-engineered variant, STI-2020, were evaluated for in vivo efficacy in the

Syrian golden hamster model of COVID-19. Both antibodies demonstrated potent protection against the pathogenic effects of the disease and a dose-dependent reduction of virus load in the lungs, reaching undetectable levels following a single dose of 500 micrograms of STI-2020. These data support continued development of these antibodies as therapeutics against COVID-19 and future use of this approach to address novel emerging pandemic disease threats. [**note: this comes from the biotech company Sorrento Therapeutics and offers some additional mAbs for clinical study.**] https://www.biorxiv.org/content/10.1101/2020.09.27.316174v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS Cov2 is a newly emerged virus causing pandemic with fatality and co-morbidity. The greatest limitations emerged is the lack of effective treatment and vaccination due to frequent mutations and reassortment of the virus, leading to evolvement of different strains. We identified a wide variability in the whole genome sequences as well as spike protein variants (responsible for binding with ACE2 receptor) of SARS Cov2 identified globally. Structural variations of spike proteins identified from representative countries from all the continents, seven of them have revealed genetically similar, may be regarded as the dominant type. Novel non-synonymous mutations as S247R, R408I, G612D, A930V and deletion detected at amino acid position 144 . RMSD values ranging from 4.45 to 2.25 for the dominant variant spike1 with other spike proteins. This study is informative for future vaccine research and drug development with the dominant type. [**note: more genetic analysis of the Spike protein.**] https://www.biorxiv.org/content/10.1101/2020.09.26.314385v1
- Over the last two decades, there have been three deadly human outbreaks of Coronaviruses (CoVs) caused by emerging zoonotic CoVs: SARS-CoV, MERS-CoV, and the latest highly transmissible and deadly SARS-CoV-2, which has caused the current COVID-19 global pandemic. All three deadly CoVs originated from bats, the natural hosts, and transmitted to humans via various intermediate animal reservoirs. Because there is currently no universal pan-Coronavirus vaccine available, two worst-case scenarios remain highly possible: (1) SARS-CoV-2 mutates and transforms into a seasonal flu-like global pandemic; and/or (2) Other global COVID-like pandemics will emerge in the coming years, caused by yet another spillover of an unknown zoonotic bat-derived SARS-like Coronavirus (SL-CoV) into an unvaccinated human population. Determining the antigen and epitope landscapes that are conserved among human and animal Coronaviruses as well as the repertoire, phenotype and function of B cells and CD4+ and CD8+ T cells that correlate with resistance seen in asymptomatic COVID-19 patients should inform in the development of pan-Coronavirus vaccines. In the present study, using several immuno-informatics and sequence alignment approaches, we identified several human B-cell, CD4+ and CD8+ T cell epitopes that are highly conserved in: (i) greater than 81,000 SARS-CoV-2 human strains identified to date in 190 countries on six continents; (ii) six circulating CoVs that caused previous human outbreaks of the Common Cold; (iii) five SL-CoVs isolated from bats; (iv) five SL-CoV isolated from pangolins; (v) three SL-CoVs isolated from Civet Cats; and (vi) four MERS strains isolated from camels. *Furthermore, we identified cross-reactive asymptomatic epitopes that: (i) recalled B cell, CD4+ and CD8+ T cell responses from both asymptomatic COVID-19 patients and healthy individuals who were never exposed to SARS-CoV-2; and (ii) induced strong B cell and T cell responses in humanized Human Leukocyte Antigen (HLA)-DR/HLA-A*02:01 double transgenic mice. The findings herein pave the way to develop a pre-emptive multi-epitope*

pan-Coronavirus vaccine to protect against past, current, and potential future outbreaks. [**note: this is interesting work from Irvine. More work needs to be done on looking at cross viral epitopes.**] https://www.biorxiv.org/content/10.1101/2020.09.27.316018v1

DIAGNOSTIC DEVELOPMENT

- Nothing new today.

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2020-09-30

Today we have another one of the live Wigmore Hall concerts from London. Here is the Castalian String Quartet with Haydn and Beethoven quartets on the program. This group is quite fine: https://www.youtube.com/watch?v=Kq35oYPOJY4 I won't post any links to the pieces as the moderator has a very good commentary on the video. Both pieces are exceptionally played.

The Washington Post reports that the government's roll out of the rapid coronavirus antigen test is not going smoothly. At least CDC cannot be blamed for this mishap. The first major COVID-19 outbreak in the National Football League has now happened. Are COVID-19 rule-breakers narcissists? What happens if you are a chef and you get COVID-19 anosmia?

The New York Times has a good video on how America lost its way despite having a sound plan for dealing with a pandemic. Here is yet more on herd immunity. I'll go out on a short limb and state that herd immunity will one day appear either with a vaccine or without. The first route is preferable, though adherence to good public heath recommendations can help us through the second route. Times restaurant critic, Pete Wells, writes about whether one should be nervous about dining in doors as restaurants up there will be opening this week. These are a reasonable set of recommendations. Will Pfizer have enough data by the end of October to tell whether their mRNA vaccine is safe and effective? Here is op-ed columnist Farhad Manjoo asking the question 'why won't President Trump tell Americans to wear masks?' Excellent question!

STATE report that Regeneron have early data that their mAb resulted in a lowering of virus levels in infected non-hospitalized patients.

The Lancet have a letter from Italian researchers that notes transmission of SARS-CoV-2 via persistence on inanimate surfaces (fomites) is likely a low risk in real life conditions. The letter is in response to a study I linked to previously. I have previously noted that I also believe this route of transmission to be exaggerated; still it doesn't hurt to keep kitchen and bathroom surfaces sparkling clean with fine Clorox products (disclosure: I am a Clorox shareholder).

Medscape report that there may be an increased risk of severe COVID-19 for MS patients taking anti-B-cell monoclonal antibodies.

The New England Journal of Medicine presents the early results of the Moderna mRNA COVID-19 vaccine. "In this small study involving older adults, adverse events associated with the mRNA-1273 vaccine were mainly mild or moderate. The 100-µg dose induced higher binding- and neutralizing-

antibody titers than the 25-µg dose, which supports the use of the 100-µg dose in a phase 3 vaccine trial."

The Annals of Internal Medicine have a statement from the NIH COVID-19 Treatment Guidelines Panel on the use of convalescent plasma for treatment. "the Panel has determined that currently the data are insufficient to recommend for or against convalescent plasma for treating COVID-19. Prospective, well-controlled, and adequately powered RCTs are needed to determine whether convalescent plasma and other passive immunotherapies are effective and safe for COVID-19 treatment. Although providers have access to this therapy, the Panel cannot recommend it as a standard of care for treating COVID-19 at this time. This is consistent with the language of the convalescent plasma EUA Fact Sheet."

MODELING

- Objectives: To evaluate the effectiveness of widespread adoption of masks or face coverings to reduce community transmission of the SARS-CoV-2 virus that causes Covid-19. Methods: We employed an agent-based stochastic network simulation model, where Covid-19 progresses across census tracts according to a variant of SEIR. We considered a mask order that was initiated 3.5 months after the first confirmed Covid-19 case. We evaluated scenarios where wearing a mask reduces transmission and susceptibility by 50% or 80%; an individual wears a mask with a probability of 0%, 20%, 40%, 60%, 80%, or 100%. Results: If 60% of the population wears masks that are 50% effective, this decreases the cumulative infection attack rate (CAR) by 25%, the peak prevalence by 51%, and the population mortality by 25%. If 100% of people wear masks (or 60% wear masks that are 80% effective), this decreases the CAR by 38%, the peak prevalence by 67%, and the population mortality by 40%. Conclusions: After community transmission is present, masks can significantly reduce infections. [**note: masks work; but then you all knew that already**] https://www.medrxiv.org/content/10.1101/2020.09.27.20199737v1
- We analyzed 21,676 residual specimens from Ontario, Canada collected between March-August, 2020 to investigate the effect of antibody decline on SARS-CoV-2 seroprevalence estimates. Testing specimens orthogonally using the Abbott (anti-nucleocapsid) and then the Ortho (anti-spike) assays, seroprevalence estimates ranged from 0.4%-1.4%, despite ongoing disease activity. The geometric mean concentration (GMC) of antibody-positive specimens decreased over time (p=0.015), and the GMC of antibody-negative specimens increased over time (p=0.0018). The association between the two tests decreased each month (p<0.001), suggesting anti-N antibody decline. Lowering the Abbott index cut-off from 1.4 to 0.7 resulted in a 16% increase in positive specimens. [**note: here is a serology study from Ontario. More information on antibody decline but we still don't know what to make of it.**] https://www.medrxiv.org/content/10.1101/2020.09.28.20200915v1
- SARS-CoV-2 is a recently emerged, highly contagious virus and the cause of the current pandemic. It is a zoonotic virus, although its animal origin is not clear yet. Person-to-person transmission occurs by inhalation of infected droplets and aerosols, or by direct contact with contaminated fomites. Arthropods transmit numerous viral, parasitic, and bacterial diseases; however, the potential role of arthropods in SARS-CoV-2 transmission is not fully understood. Thus far, a few studies have demonstrated that SARS-CoV-2 replication is not supported in cells from certain insect species nor in certain species of mosquitoes after intrathoracic inoculation.

In this study, we expanded the work of SARS-CoV-2 susceptibility to biting insects after ingesting a SARS-CoV-2 infected blood meal. Species tested included *Culicoides sonorensis* biting midges, as well as *Culex tarsalis* and *Culex quinquefasciatus* mosquitoes, all known biological vectors for numerous RNA viruses. Arthropods were allowed to feed on SARS-CoV-2 spiked blood and at various time points post infection analyzed for the presence of viral RNA and infectious virus. Additionally, cell lines derived from *C. sonorensis* (W8a), *Ae. aegypti* (C6/36), *Cx. quinquefasciatus* (HSU), and *Cx. tarsalis* (CxTrR2) were tested for SARS-CoV-2 susceptibility. Our results indicate that none of the biting insects, nor the insect cell lines support SARS-CoV-2 replication. We conclude, that biting insect do not pose a risk for transmission of SARS-CoV-2 to humans or animals following a SARS-CoV-2 infected blood meal. [**note: this is a bit of good news in that some biting insects do not pose a transmission risk for SARS-CoV-2.**] https://www.biorxiv.org/content/10.1101/2020.09.29.317289v1

- Recently, respiratory aerosols with diameters smaller than 100 µm have been con- firmed as important vectors for the spread of SARS-CoV-2. While cloth masks afford some protection for larger ballistic droplets, they are typically inefficient at filtering these aerosols and require specialized fabrication devices to produce. We describe a fabrication technique that makes use of a folding procedure (origami) to transform a filtration material into a mask. These origami masks can be fabricated by non-experts at minimal cost and effort, provide adequate filtration efficiencies, and are easily scaled to different facial sizes. Using a mannequin fit test simulator, we demonstrate that these masks can provide optimal filtration efficiency and ease of breathing with minimal leak- age. Because this mask provides greater comfort compared to commercial alternatives, it is likely to promote greater mask wearing tolerance and acceptance. [**note: how cool is this??? Origami masks that require no sewing. This is a must paper to read for the DIY ingenuity.**] https://www.medrxiv.org/content/10.1101/2020.09.29.20204115v1

NEWLY REGISTERED CLINICAL TRIALS

- I'll check the database at some point this week.

CLINICAL TRIAL RESULTS

- The aim of this study was to evaluate the anti-inflammatory response to COVID-19, by assessing interleukin-10 (IL-10) and IL-10/lymphocyte count ratio and their association with patient outcomes. Methods: Adult patients presenting to the emergency department (ED) with laboratory-confirmed COVID-19 were recruited. The primary endpoint was peak COVID-19 severity within 30 days of index ED visit. Additional endpoints included COVID-19 severity at ED disposition, development of severe acute kidney injury (AKI) or secondary bacterial infections. Results: A total of 52 COVID-19 patients were enrolled. IL-10 and IL-10/lymphocyte count were significantly higher in patients with severe disease at both time points (all $p<0.05$), as well as in those who developed severe AKI and secondary bacterial infection (all $p\leq0.01$). In multivariable analysis, a one-unit increase in IL-10 was associated with 42% increased odds of severe COVID-19 ($p=0.031$), whilst a one-unit increase IL-10/lymphocyte ratio was also associated with 32% increase in odds of severe COVID-19 ($p=0.013$). Conclusions*: The hyperinflammatory response to COVID-19 is accompanied by a simultaneous anti-inflammatory response, which is associated with poor outcomes and may increase the risk of secondary bacterial infections. IL-10 and IL-10/lymphocyte ratio at ED presentation were independent predictors of COVID-19 severity*.

Functional immunoparalysis in COVID-19 requires further investigation to enable more precise immunomodulatory therapy against SARS-CoV-2. [**note: more information from Cincinnati on immune system dysfunction in severe COVID-19.**]
https://www.medrxiv.org/content/10.1101/2020.09.28.20203398v1

- A major issue in identification of protective T cell responses against SARS-CoV-2 lies in distinguishing people infected with SARS-CoV-2 from those with cross-reactive immunity generated by exposure to other coronaviruses. We characterised SARS-CoV-2 T cell immune responses in 168 PCR-confirmed SARS-CoV-2 infected subjects and 118 seronegative subjects without known SARS-CoV-2 exposure using a range of T cell assays that differentially capture immune cell function. Strong ex vivo ELISpot and proliferation responses to multiple antigens (including M, NP and ORF3) were found in those who had been infected by SARS-CoV-2 but were rare in pre-pandemic and unexposed seronegative subjects. However, seronegative doctors with high occupational exposure and recent COVID-19 compatible illness showed patterns of T cell responses characteristic of infection, indicating that these readouts are highly sensitive. By contrast, over 90% of convalescent or unexposed people showed proliferation and cellular lactate responses to spike subunits S1/S2, indicating pre-existing cross-reactive T cell populations. The detection of T cell responses to SARS-CoV-2 is therefore critically dependent on the choice of assay and antigen. Memory responses to specific non-spike proteins provides a method to distinguish recent infection from pre-existing immunity in exposed populations. [**note: T cell assays can differentiate clinical and subclinical SARS-CoV-2 infections from cross-reactive antiviral responses.**]
https://www.medrxiv.org/content/10.1101/2020.09.28.20202929v1

DRUG DEVELOPMENT

- Hydroxychloroquine (HCQ), which has been proposed as a therapeutic or prophylactic drug for SARS-COV-2, has been administered to thousands of individuals with varying efficacy; however, our understanding of its adverse effects is insufficient. It was reported that HCQ induced psychiatric symptoms in a few patients with autoimmune diseases, but it is still uncertain whether HCQ poses a risk to mental health. Therefore, in this study, we treated healthy mice with two different doses of HCQ that are comparable to clinically administered doses for 7 days. Psychiatric-like behaviors and the expression of related molecules in the brain were evaluated at two time points, i.e., 24 h and 10 days after drug administration. We found that HCQ increased anxiety behavior at both 24 h and 10 days and enhanced depressive behavior at 24 h. Furthermore, HCQ decreased the mRNA expression of interleukin-1beta and corticotropin-releasing hormone (Crh) in the hippocampus and decreased the mRNA expression of brain-derived neurotrophic factor (Bdnf) in both the hippocampus and amygdala. Most of these behavioral and molecular changes were sustained beyond 10 days after drug administration, and some of them were dose-dependent. Although this animal study does not prove that HCQ has a similar effect in humans, it indicates that HCQ poses a significant risk to mental health and suggests that further clinical investigation is essential. According to our data, we recommend that HCQ be carefully used as a prophylactic drug in people who are susceptible to mental disorders. [**note: this study from China though an animal study may provide another good reason to refuse HCQ treatment. Of course the best reason is that it doesn't work.**]
https://www.biorxiv.org/content/10.1101/2020.09.27.316158v1

- COVID-19 vaccines are being developed urgently worldwide, among which single-shot adenovirus vectored vaccines represent a major approach. Here, we constructed two novel adenovirus vectored COVID-19 vaccine candidates on simian adenovirus serotype 23 (Sad23L) and human adenovirus serotype 49 vectors (Ad49L) carrying the full-length gene of SARS-CoV-2 spike protein (S), designated Sad23L-nCoV-S and Ad49L-nCoV-S vaccines, respectively. The immunogenicity elicited by these two vaccine strains was individually evaluated in mice. Specific humoral and cellular immune responses were proportionally observed in a dose-dependent manner, and stronger responses were obtained by boosting. Furthermore, five rhesus macaques were intramuscularly injected with a dose of 5X10^9 PFU Sad23L-nCoV-S for prime vaccination, followed by boosting with 5X10^9 PFU of Ad49L-nCoV-S vaccine at a 4-week interval. Three macaques were injected with Sad23L-GFP and Ad49L-GFP vector viruses as negative controls. Both mice and macaques tolerated well the vaccine inoculation without detectable clinical and pathologic changes. In macaques, prime-boost vaccination regimen induced high titers of 10^3.16 S binding antibody (S-BAb), 10^2.75 RBD-BAb and 10^2.38 neutralizing antibody (NAb to pVNT) a week after boosting injection, followed by sustained high levels. Robust IFN-γ; secreting T-cell response (712.6 SFCs/106 cells), IL-2 secreting T-cell response (334 SFCs/106 cells) and intracellular IFN-γ; expressing CD4+/CD8+ T cell response (0.39%/0.55%) to S peptides were detected in the vaccinated macaques. The study concluded that prime-boost immunization with Sad23L-nCoV-S and Ad49L-nCoV-S vaccines can safely elicit strong immunity in animals in preparation of clinical phase 1/2 trials. [**note: from China, animal studies on two adenovirus COVID-19 vaccine constructs.**]
  https://www.biorxiv.org/content/10.1101/2020.09.28.311480v1
- The beta-coronavirus SARS-CoV-2 has caused a global pandemic. Affinity reagents targeting the SARS-CoV-2 spike protein, the most exposed surface structure of the virus, are of interest for the development of therapeutics and diagnostics. We used affinity selection-mass spectrometry for the rapid discovery of synthetic high affinity peptide binders for the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. From library screening with 800 million synthetic peptides, we identified three sequences with nanomolar affinities (dissociation constants of 80 to 970 nM) for RBD and selectivity over human serum proteins. Picomolar RBD concentrations in biological matrix could be detected using the biotinylated lead peptide in ELISA format. These peptides might associate with the SARS-CoV-2-spike-RBD at a site unrelated to ACE2 binding, making them potential orthogonal reagents for sandwich immunoassays. We envision our discovery as a robust starting point for the development of SARS-CoV-2 diagnostics or conjugates for virus directed delivery of therapeutics. [**note: here is some good research on some novel peptides that bind to the Spike protein. These can be used for both diagnostic and therapeutic use.**] https://www.biorxiv.org/content/10.1101/2020.09.29.317131v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- High quality recombinant virus proteins are required for research related to the development of vaccines and improved assays, and to the general understanding of virus action. The receptor-binding domain (RBD) of the 2019-nCoV spike (S) protein contains disulfide bonds and N-linked glycosylations, therefore, it is typically produced by secretion. Here, we describe a construct and protocol for the expression and purification of yellow fluorescent protein (YFP) labeled 2019-nCoV spike RBD. The fusion protein, in the vector pcDNA 4/TO, comprises an N-terminal

interferon alpha 2 (IFNα2) signal peptide, an eYFP, a FLAG-tag, a human rhinovirus 3C protease cleavage site, the RBD of the 2019-nCoV spike protein and a C-terminal 8x His-tag. We stably transfected HEK 293 cells. Following expansion of the cells, the fusion protein was secreted from adherent cells into serum-free medium. Ni-NTA IMAC purification resulted in very high protein purity, based on analysis by SDS-PAGE. The fusion protein was soluble and monodisperse, as confirmed by size-exclusion chromatography (SEC) and negative staining electron microscopy. Deglycosylation experiments confirmed the presence of N-linked glycosylations in the secreted protein. Complex formation with the peptidase domain of human angiotensin-converting enzyme 2 (ACE2), the receptor for the 2019-nCoV spike RBD, was confirmed by SEC, both for the YFP-fused spike RBD and for spike RBD alone, after removal of YFP by proteolytic cleavage. Possible applications for the fusion protein include binding studies on cells or in vitro, fluorescent labeling of potential virus-binding sites on cells, the use as an antigen for immunization studies or as a tool for the development of novel virus- or antibody-detection assays. [**note: this is some cool research on creating a tagged version of the SARS-CoV-2 Spike protein. It has a yellow fluorescent label that allows for visualization.**] https://www.biorxiv.org/content/10.1101/2020.09.29.318196v1

- The D614G substitution in the S protein is most prevalent SARS-CoV-2 strain circulating globally, but its effects in viral pathogenesis and transmission remain unclear. We engineered SARS-CoV-2 variants harboring the D614G substitution with or without nanoluciferase. The D614G variant replicates more efficiency in primary human proximal airway epithelial cells and is more fit than wildtype (WT) virus in competition studies. With similar morphology to the WT virion, the D614G virus is also more sensitive to SARS-CoV-2 neutralizing antibodies. Infection of human ACE2 transgenic mice and Syrian hamsters with the WT or D614G viruses produced similar titers in respiratory tissue and pulmonary disease. However, the D614G variant exhibited significantly faster droplet transmission between hamsters than the WT virus, early after infection. Our study demonstrated the SARS-CoV2 D614G substitution enhances infectivity, replication fitness, and early transmission. [**note: more information on the current D614G variant showing enhanced infectivity.**] https://www.biorxiv.org/content/10.1101/2020.09.28.317685v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-10-01

Welcome to October!

West Side Story is one of the iconic Broadway musicals of all time. It's as fresh today as when it premiered back in 1957. Here is the Frankfurt Radio Big Band live in a recent concert with an instrumental only version: https://www.youtube.com/watch?v=Izl4Si-6Kds

The Washington Post notes that while Gilead's remdesivir may not cure COVID-19 it will make the company a lot of money. As colleges reopened in late August, COVID-19 cases among those 18-22 years of age increased. This is not a surprise. Here is a good FAQ about COVID-19 masks. A group of former FDA Commissioners opine on political interference with Agency decisions. Here is a very good lay article

on monoclonal antibodies for COVID-19. Cornell Univ shows how to open a major research university safely in the COVID-19 era (disclosure: I did my post-doc at Cornell in the late 1970s and still have several friends on the faculty). As you know, I have been tracking Purdue University's efforts in reopening. In the past week, the overall positivity rate took a 1% uptick in those tested with 216 infections. It's still low for a large research university but another uptick will be worrisome to President Mitch Daniels.

The New York Times discusses how winter skiing in Austria earlier this year helped to seed COVID-19 infections in more than 40 countries in five continents, a true superspreader event. Laissez les bon temps rouler!! A CDC proposal to keep cruise ships from sailing is overturned by the Administration. If you do book a cruise, make sure to take lots of Clorox wipes with you! (disclosure: I own stock in Clorox) Here is a summary of a large study of COVID-19 patients in India. One of the takeaways, "The contact tracing study also found that children of all ages can become infected with the coronavirus and spread it to others — offering compelling evidence on one of the most divisive questions about the virus." The full study is HERE.

STAT have an opinion piece on the need for a national COVID-19 testing strategy. Loyal readers will know that this has been my call too.

JAMA have a paper on the Univ of Pennsylvania HCQ prophylaxis trial in health care workers. "In this randomized clinical trial, although limited by early termination, there was no clinical benefit of hydroxychloroquine administered daily for 8 weeks as pre-exposure prophylaxis in hospital-based HCWs exposed to patients with COVID-19." Here is a CDC viewpoint on preventing and responding to COVID-19 on college campuses. According to CDC, young children in childcare centers can spread COVID-19.

MODELING

- The rapid spread of SARS-CoV-2 has gravely impacted societies around the world. Outbreaks in different parts of the globe are shaped by repeated introductions of new lineages and subsequent local transmission of those lineages. Here, we sequenced 3940 SARS-CoV-2 viral genomes from Washington State to characterize how the spread of SARS-CoV-2 in Washington State (USA) was shaped by differences in timing of mitigation strategies across counties, as well as by repeated introductions of viral lineages into the state. Additionally, we show that the increase in frequency of a potentially more transmissible viral variant (614G) over time can potentially be explained by regional mobility differences and multiple introductions of 614G, but not the other variant (614D) into the state. At an individual level, we see evidence of higher viral loads in patients infected with the 614G variant. *However, using clinical records data, we do not find any evidence that the 614G variant impacts clinical severity or patient outcomes. Overall, this suggests that at least to date, the behavior of individuals has been more important in shaping the course of the pandemic than changes in the virus*. [**note: here is some good genomic data for Washington State from the Univ of Washington and the Hutch. The finding that the 614G variant seems not to impact clinical severity or patient outcomes is important and I would like to see data from other regions as well.**]
  https://www.medrxiv.org/content/10.1101/2020.09.30.20204230v1
- Here we look into the spread of aerosols indoors that may potentially carry viruses. Many viruses, including the novel Covid-19, are known to spread via airborne and air-dust pathways.

From the literature data and our own research on the propagation of fine aerosols, we simulate herein the carryover of viral aerosols in indoor air. We demonstrate that a great deal of fine droplets released from an infected person coughing, sneezing or talking propagate very fast and to large distances indoors, as well as bend around obstacles, lift up and down over staircases, and so on. This study suggests equations to evaluate the concentration of those droplets, depending on time and distance from the source of infection. Estimates are given for the safe distance to the source of infection, and available methods for neutralizing viral aerosols indoors are considered. [**note: the bioaersol study comes from Russia.**]
https://www.medrxiv.org/content/10.1101/2020.09.30.20199943v1

NEWLY REGISTERED CLINICAL TRIALS

- Yes, I know I have been lax at not doing daily updates on this topic. I'll get to it this week!

CLINICAL TRIAL RESULTS

- Coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a serious threat to global public health. Hydroxychloroquine (HCQ) and the antibiotic azithromycin (AZ) are still being used by thousands and numerous hospitals to treat COVID-19. In a related context, immunotherapy using checkpoint blockade (ICB) with antibodies such as anti-PD-1 has revolutionised cancer therapy. Given that cancer patients on ICB continue to be infected with SARS-CoV-2, an understanding of the effects of HCQ and AZ on the elimination of tumors by anti-PD-1 ICB is urgently needed. In this study, we report that HCQ alone, or in combination with AZ, at doses used to treat COVID-19 patients, reverses the therapeutic benefit of anti-PD-1 in controlling B16 melanoma tumor growth in mice. No deleterious effect was seen on untreated tumors, or in using AZ alone in anti-PD-1 immunotherapy. Mechanistically, HCQ and HCQ/AZ inhibited PD-L1 expression on tumor cells, while specifically targeting the anti-PD-1 induced increase in progenitor CD8+CD44+PD-1+TCF1+ tumor-infiltrating T-cells (TILs) and the generation of CD8+CD44+PD-1+ effectors. Surprisingly, it also blocked the appearance of a subset of terminally exhausted CD8+ TILs. No effect was seen on the presence of CD4+ T-cells, FoxP3+ Tregs, thymic subsets, B-cells, antibody production, myeloid cells, or the vasculature of mice. Lastly, we identified TCF-1 expression in peripheral CD8+ T-cells from cancer or non-cancer human patients infected with SARs CoV2 as a marker for the effects of COVID-19 and HCQ on the immune system. *This study indicates for the first time that HCQ and HCQ/AZ negatively impact the ability of anti-PD-1 checkpoint blockade to promote tumor rejection*. [**note: here is a really good reason to avoid HCQ therapy if you are be treating with a cancer immunotherapy drug!]**
https://www.medrxiv.org/content/10.1101/2020.09.29.20193110v1
- Background: The role of combination immunomodulatory therapy with systemic corticosteroids and tocilizumab (TCZ) for aged patients with COVID19 associated cytokine release syndrome remains unclear. Methods: We conducted a retrospective single center study including consecutive patients older than 65 years that developed severe COVID19 between March 3 and May 1, 2020 and were treated with corticosteroids at various doses (methylprednisolone [0.5 mg/Kg/12 hours to 250 mg/24 hours]), either alone (CS group) or associated to intravenous tocilizumab (400 to 600 mg, one to three doses) (CS/TCZ group). Primary outcome was all cause mortality by day +14, whereas secondary outcomes included mortality by day +28 and clinical

improvement (discharge and/or a 2 point decrease on a six point ordinal scale) by day +14. Propensity score (PS)based adjustment and inverse probability of treatment weights (IPTW) were applied. Results: Overall, 181 and 80 patients were included in the CS and CS TCZ groups. All cause 14 day mortality was lower in the CS/TCZ group, both in the PS adjusted (hazard ratio [HR]: 0.34; 95% confidence interval [CI]: 0.17 to 0.68; P value = 0.002) and IPTW weighted models (odds ratio [OR]: 0.38; 95% CI: 0.21 to 0.68; P value = 0.001). This protective effect was also observed for 28 day mortality (PS adjusted HR: 0.38; 95% CI: 0.21 to 0.72; P value = 0.003). Clinical improvement by day +14 was higher in the CS/TCZ group in the IPTW analysis only (OR: 2.26; 95% CI: 1.49 to 3.41; P value <0.001). The occurrence of secondary infection was similar between both groups. Conclusions: The combination of corticosteroids and TCZ was associated with better outcomes among patients older than 65 years with severe COVID-19. [**note: here is a single site Spanish study showing that corticosteroid + tocilizumab leads to better clinical outcomes than just the steroid therapy.**]
https://www.medrxiv.org/content/10.1101/2020.09.26.20202283v1

DRUG DEVELOPMENT

- While the COVID-19 pandemic is causing important loss of life, knowledge of the effects of the causative SARS-CoV-2 virus on human cells is currently limited. Investigating protein-protein interactions (PPIs) between viral and host proteins can provide a better understanding of the mechanisms exploited by the virus and enable the identification of potential drug targets. We therefore performed an in-depth computational analysis of the interactome of SARS-CoV-2 and human proteins in infected HEK293 cells published by Gordon et al. to reveal processes that are potentially affected by the virus and putative protein binding sites. Specifically, we performed a set of network-based functional and sequence motif enrichment analyses on SARS-CoV-2-interacting human proteins and on a PPI network generated by supplementing viral-host PPIs with known interactions. *Using a novel implementation of our GoNet algorithm, we identified 329 Gene Ontology terms for which the SARS-CoV-2-interacting human proteins are significantly clustered in the network. Furthermore, we present a novel protein sequence motif discovery approach, LESMoN-Pro, that identified 9 amino acid motifs for which the associated proteins are clustered in the network. Together, these results provide insights into the processes and sequence motifs that are putatively implicated in SARS-CoV-2 infection and could lead to potential therapeutic targets*. [**note: this builds upon a large study done by UCSF researchers several months ago.**] https://www.biorxiv.org/content/10.1101/2020.09.29.318931v1
- Effective vaccine against SARS-CoV-2 is the utmost importance in the current world. More than 1 million deaths are accounted for relevant pandemic disease COVID-19. Recent data showed that D614G genotype of the virus is highly infectious and responsible for almost all infection for 2nd wave. Despite of multiple vaccine development initiatives, there are currently no report that has addressed this critical variant D614G as vaccine candidate. Here we report the development of an mRNA-LNP vaccine considering the D614G variant and characterization of the vaccine in preclinical trial. The surface plasmon resonance (SPR) data with spike protein as probe and competitive neutralization with RBD and S2 domain revealed that immunization generated specific antibody pools against the whole extracellular domain (RBD and S2) of the spike protein. The anti-sera and purified IgGs from immunized mice on day 7 and 14 neutralized SARS-CoV-2 pseudovirus in ACE2-expressing HEK293 cells in a dose dependent manner.

Importantly, immunization protected mice lungs from pseudovirus entry and cytopathy. The immunologic responses have been implicated by a balanced and stable population of CD4+ cells with a Th1 bias. The IgG2a to IgG1 and (IgG2a+IgG2b) to (IgG1+IgG3) ratios were found 0.8-1.2 and 1.14-1.34, respectively. These values are comparatively higher than relevant values for other published SARS-CoV-2 vaccine in development,1,2 and suggesting higher viral clearance capacity for our vaccine. The data suggested great promise for immediate translation of the technology to the clinic. [**note: Bangladesh company, Globe Biotech have developed an mRNA COVID-19 vaccine based on the D614G variant. Good work!!!**] https://www.biorxiv.org/content/10.1101/2020.09.29.319061v1

- Development of innovative direct-acting antiviral agents is sorely needed to address this virus. Peptide-conjugated morpholino oligomers (PPMO) are antisense agents composed of a phosphordiamidate morpholino oligomer covalently conjugated to a cell-penetrating peptide. PPMO require no delivery assistance to enter cells and are able to reduce expression of targeted RNA through sequence-specific steric blocking. Objectives and Methods: Five PPMO designed against sequences of genomic RNA in the SARS-CoV-2 5'-untranslated region and a negative control PPMO of random sequence were synthesized. Each PPMO was evaluated for its effect on the viability of uninfected cells and its inhibitory effect on the replication of SARS-CoV-2 in Vero-E6 cell cultures. Cell viability was evaluated with an ATP-based method and viral growth was measured with quantitative RT-PCR and TCID50 infectivity assays. Results: PPMO designed to base-pair with sequence in the 5'-terminal region or the leader transcription regulatory sequence-region of SARS-CoV-2 genomic RNA were highly efficacious, reducing viral titers by up to 4-6 log10 in cell cultures at 48-72 hours post-infection, in a non-toxic and dose-responsive manner. Conclusion: The data indicate that PPMO have the ability to potently and specifically suppress SARS-CoV-2 growth and are promising candidates for further pre-clinical development. [**note: this is a pretty cool technology that can directly block the RNA from replicated.**] https://www.biorxiv.org/content/10.1101/2020.09.29.319731v1
- SARS-CoV-2 has caused a global outbreak of severe respiratory disease (COVID-19), leading to an unprecedented public health crisis. To date, there has been over 33 million diagnosed infections, and over one million deaths. No vaccine or targeted therapeutics are currently available. We previously identified a human monoclonal antibody, 47D11, capable of cross-neutralising SARS-CoV-2 and the related 2002/2003 SARS-CoV. Here we present the structural basis of its neutralization mechanism. We describe cryo-EM structures of trimeric SARS-CoV and SARS-CoV-2 spike ectodomains in complex with the 47D11 Fab. These data reveal that 47D11 binds specifically to the closed conformation of the receptor binding domain, distal to the ACE2 binding site. The CDRL3 stabilises the N343 glycan in an upright conformation, exposing a conserved and mutationally constrained hydrophobic pocket, into which the CDRH3 loop inserts two aromatic residues. Interestingly, 47D11 preferentially selects for the partially open conformation of the SARS-CoV-2 spike, suggesting that it could be used effectively in combination with other antibodies that target the exposed receptor-binding motif. Taken together, these results expose a cryptic site of vulnerability on the SARS-CoV-2 RBD and provide a structural roadmap for the development of 47D11 as a prophylactic or post-exposure therapy for COVID-19. [**note: these Dutch researchers show how a neutralizing monoclonal antibody works on SARS-CoV-2.**] https://www.biorxiv.org/content/10.1101/2020.09.30.318261v1

- A deficient interferon response to SARS-CoV-2 infection has been implicated as a determinant of severe COVID-19. To identify the molecular effectors that govern interferon control of SARS-CoV-2 infection, we conducted a large-scale gain-of-function analysis that evaluated the impact of human interferon stimulated genes (ISGs) on viral replication. A limited subset of ISGs were found to control viral infection, including endosomal factors that inhibited viral entry, nucleic acid binding proteins that suppressed viral RNA synthesis, and a highly enriched cluster of ER and Golgi-resident ISGs that inhibited viral translation and egress. These included the type II integral membrane protein BST2/tetherin, which was found to impede viral release, and is targeted for immune evasion by SARS-CoV-2 Orf7a protein. Overall, these data define the molecular basis of early innate immune control of viral infection, which will facilitate the understanding of host determinants that impact disease severity and offer potential therapeutic strategies for COVID-19. [**note: here is more on the impact of the immune system and which factors are important in controlling viral replication. We are getting more information on this which is useful.**] https://www.biorxiv.org/content/10.1101/2020.09.29.319566v1
- SARS-CoV-2 enters cells via its spike glycoprotein which must be cleaved sequentially at the S1/S2, then the S2' cleavage sites (CS) to mediate membrane fusion. SARS-CoV-2 has a unique polybasic insertion at the S1/S2 CS, which we demonstrate can be cleaved by furin. Using lentiviral pseudotypes and a cell-culture adapted SARS-CoV-2 virus with a S1/S2 deletion, we show that the polybasic insertion is selected for in lung cells and primary human airway epithelial cultures but selected against in Vero E6, a cell line used for passaging SARS-CoV-2. We find this selective advantage depends on expression of the cell surface protease, TMPRSS2, that allows virus entry independent of endosomes thus avoiding antiviral IFITM proteins. SARS-CoV-2 virus lacking the S1/S2 furin CS was shed to lower titres from infected ferrets and was not transmitted to cohoused sentinel animals. Thus, the polybasic CS is a key determinant for efficient SARS-CoV-2 transmission. [**note: more confirmation of the furin cleavage site as a key determinant for transmission of the virus.**]
  https://www.biorxiv.org/content/10.1101/2020.09.30.318311v1
- Coronaviruses infect many different species including humans. The last two decades have seen three zoonotic coronaviruses with SARS-CoV-2 causing a pandemic in 2020. Coronaviral non-structural proteins (nsp) built up the replication-transcription complex (RTC). Nsp7 and nsp8 interact with and regulate the RNA-dependent RNA-polymerase and other enzymes in the RTC. However, the structural plasticity of nsp7+8 complex has been under debate. Here, we present the framework of nsp7+8 complex stoichiometry and topology based on a native mass spectrometry and complementary biophysical techniques of nsp7+8 complexes from seven coronaviruses in the genera Alpha- and Betacoronavirus including SARS-CoV-2. Their complexes cluster into three groups, which systematically form either heterotrimers or heterotetramers or both, exhibiting distinct topologies. Moreover, even at high protein concentrations mainly heterotetramers are observed for SARS-CoV-2 nsp7+8. From these results, the different assembly paths can be pinpointed to specific residues and an assembly model is proposed. [**note: here is a look at a couple of the viral nonstructural proteins.**]
  https://www.biorxiv.org/content/10.1101/2020.09.30.320762v1

DIAGNOSTIC DEVELOPMENT

- The December 2019 outbreak of a novel respiratory virus, SARS-CoV-2, has become an ongoing global pandemic due in part to the challenge of identifying symptomatic, asymptomatic and pre-symptomatic carriers of the virus. CRISPR-based diagnostics that utilize RNA and DNA-targeting enzymes can augment gold-standard PCR-based testing if they can be made rapid, portable and accurate. Here we report the development of an amplification-free CRISPR-Cas13a-based mobile phone assay for direct detection of SARS-CoV-2 from nasal swab RNA extracts. The assay achieved ~100 copies/µL sensitivity in under 30 minutes and accurately detected a set of positive clinical samples in under 5 minutes. We combined crRNAs targeting SARS-CoV-2 RNA to improve sensitivity and specificity, and we directly quantified viral load using enzyme kinetics. Combined with mobile phone-based quantification, this assay can provide rapid, low-cost, point-of-care screening to aid in the control of SARS-CoV-2. [**note: how cool is this? From UC Berkeley and UCSR, a CRISPR-based mobile phone approach to point of care screening. The paper has a good diagram of the methodology.**] https://www.medrxiv.org/content/10.1101/2020.09.28.20201947v1
- Managing the pandemic caused by SARS-CoV-2 requires new capabilities in testing, including the possibility of identifying, in minutes, infected individuals as they enter spaces where they must congregate in a functioning society, including workspaces, schools, points of entry, and commercial business establishments. Here, the only useful tests (a) require no sample transport, (b) require minimal sample manipulation, (c) can be performed by unlicensed individuals, (d) return results on the spot in much less than one hour, and (e) cost no more than a few dollars. The sensitivity need not be as high as normally required by the FDA for screening asymptomatic carriers (as few as 10 virions per sample), as these viral loads are almost certainly not high enough for an individual to present a risk for forward infection. This allows tests specifically useful for this pandemic to trade-off unneeded sensitivity for necessary speed, simplicity, and frugality. In some studies, it was shown that viral load that creates forward-infection risk may exceed 105 virions per milliliter, easily within the sensitivity of an RNA amplification architecture, but unattainable by antibody-based architectures that simply target viral antigens. Here, we describe such a test based on a displaceable probe loop amplification architecture. [**note: this is a useful diagnostics paper as it posits that you don't need ultrasensitive diagnostic tests to do rapid screening. Persons with very load viral loads may not be generators of infection. I tend to agree with this point and of course it fits into the massive testing paradigm.**] https://www.medrxiv.org/content/10.1101/2020.09.29.20204131v1

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2020-10-02

Lawrence Brownlee is one of my favorite singers. I've seen him in both opera and recital and am always happy when I leave the theater. Here he is singing from the living room of his Florida home in mid-August with remote accompaniment from Myra Wang who is in New York. Some good songs and arias in this recital: https://www.youtube.com/watch?v=AgNTHoPuSsM **NOTE:** skip ahead to the 7 minute mark of the this video. The camera was running while Brownlee was moving in and out of the room.

SARS-CoV-2 doesn't care what your status is in life and is an equal opportunity infector; world leaders react to the news of President Trump's positive COVID-19 test. [**NOTE;** this is all I am going to post on this news. It is easy for you to find lots of stories in the press and I will only conclude by noting the

simple rules in my signature line. Schadenfreude at this time is a totally inappropriate behavior!! I try to keep this newsletter as apolitical as I possibly can.]

The New York Times has a short story on Tony Fauci's view on wearing masks; no surprise he is for it! Who is the largest drive of the "infordemic?" Some colleges are successfully handling COVID-19 by extensive testing.

The Washington Post discusses how Twitter has moved to remote work, a project that was two years in the making. Will other companies follow this lead? COVID-19 'Long Haulers' are setting up their own support groups on line. There is a wariness between the students and citizens of college towns resulting from COVID-19. Amazon notes that 20K employees have contracted COVID-19. While a large number, the infection rate is about 1.44%.

The Lancet have a good perspective on completing clinical trials during a pandemic such as COVID-19. Money quote from Martin Landary, one of those involved in the UK RECOVERY trial effort, "We have ended up with an overly complicated trial system which has lost sight of the one key question: if I give this drug to my patient will he or she do better or worse than if I do not give them the drug?" Editor Richard Horton opines on 'science and the breakdown of trust.' There are a number of letters on a remdesivir trial; here is the author's reply and you can find links to the letters within.

Medscape have a nice article on how split pool testing ups the efficiency for SARS-CoV-2 detection.

MODELING

- Nothing today.

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to evaluate if using nasal irrigation, also known as nasal lavage, for 14 days after a positive test can reduce the severity of symptoms associated with COVID-19. Nasal lavage consists of running salt water in one nostril and out the other to get rid of germs. Nasal irrigation will be done with either Betadine or baking soda to determine if adding an antimicrobial or changing the pH of the mucous helps. **[note: this may be the Occam's Razor clinical trial. I've been holding off on my nasal irrigation for fear it might increase susceptibility to SARS-CoV-2. I'm anxiously awaiting the results of this trial!**] NCT04559035
- A randomized, double blind, placebo-controlled, phase 2 clinical trial to investigate the efficacy and safety of 2 doses of NuSepin® intravenous infusion in COVID-19 pneumonia patients [**note: I don't know much about the compound. The company is Shaperon and more information is at the link with some nice diagrams.**] NCT04565379
- Prospective, Randomized, Double-Blind, Placebo-Controlled Phase II Trial of Intravenous L-Citrulline to Delay and Potentially Prevent the Need for Invasive Mechanical Ventilation for Acute Hypoxemic Respiratory Failure in Patients with COVID-19 (SARS-CoV2) Illness. To evaluate safety and efficacy of a bolus loading dose and continuous intravenous infusion of L-Citrulline compared to placebo in patients hospitalized with COVID-19 infection (SARS-CoV-2). Intravenous L-citrulline administration will safely restore the homeostasis of nitric oxide synthase by increasing both plasma citrulline and arginine levels. Investigators also reason that restoration of citrulline/arginine balance through citrulline administration will safely re-establish

homeostasis of NOS, lower oxidative stress, and reduce inflammation, thereby delaying and potentially preventing the need for invasive mechanical ventilation in participants hospitalized with COVID-19 infection (SARS-CoV-2). The body lives in a delicate balance of homeostasis. The urea/NO cycle plays a critical role in maintaining redox homeostasis and as such, also plays a role in regulating inflammation. The biochemical relationships are complex and depend on inter-organ transfer, membrane transport, and intracellular compartmentation. However, data above demonstrate that citrulline, arginine, and NO are critical in maintaining this homeostasis through their regulation of NOS. Inflammation, especially from infection, results in decreased activity of CPS1 and increased activity of arginase, which decreases levels of both citrulline and arginine. These decreased levels result in dysregulated and uncoupled NOS, which drives both overexuberant NO production and formation of ROS. Both the NO production and ROS further exacerbate the inflammatory cascade, resulting in other organ dysfunctions, including acute lung injury. Both inflammation and oxidative stress have been shown to be driving forces for the development of ALI and regulated NOS function is vital to reducing both. Both plasma citrulline and arginine are deficient in sepsis and levels are inversely associated with development of ALI. Furthermore, citrulline replacement safely increases plasma levels of both citrulline and arginine in healthy volunteers, BMT patients, adults with sepsis, children with sickle cell disease, and children after congenital heart surgery. It seems highly likely that citrulline therapy in the setting of COVID-19 (SARS-CoV2) induced acute hypoxemic respiratory illness will safely increase citrulline and arginine levels and help re-establish NOS homeostasis, resulting in NO production in compartments that are more homeostatically appropriate so as to reduce pulmonary vascular resistance and enhance coupling of NOS to minimize superoxide production thus reducing free radical mediated ALI. [**note: don't know if this is just a shot in the dark. Sponsor is Asklepion Pharmaceuticals.**] NCT04570384

CLINICAL TRIAL RESULTS

- Nothing today.

DRUG DEVELOPMENT

- Enveloped viruses utilize the host cell secretory pathway to synthesize viral glycoproteins and direct them to sites of assembly. Using an image-based screen, we identified two thiopurines, 6-thioguanine (6-TG) and 6-thioguanosine (6-TGo), that selectively disrupted the processing and accumulation of influenza A virus glycoproteins hemagglutinin (HA) and neuraminidase (NA). Selective disruption of IAV glycoprotein processing and accumulation by 6-TG and 6-TGo correlated with unfolded protein response (UPR) activation. 6-TG and 6-TGo also inhibited replication of the human coronavirus OC43 (HCoV-OC43), which correlated with UPR/ISR activation and diminished accumulation of ORF1ab and nucleocapsid (N) mRNAs, which suggests broader disruption of coronavirus gene expression in ER-derived cytoplasmic compartments. The chemically similar thiopurine 6-mercaptopurine (6-MP) had little effect on the UPR and did not affect IAV or HCoV-OC43 replication. Consistent with reports on other CoV Spike (S) proteins, ectopic expression of SARS-CoV-2 S protein caused UPR activation. 6-TG inhibited accumulation of full length S0 or furin-cleaved S2 fusion proteins, but spared the S1 ectodomain. DBeQ, which inhibits the p97 AAA-ATPase required for retrotranslocation of ubiquitinated misfolded proteins during ER-associated degradation (ERAD) restored accumulation of S0 and S2

proteins in the presence of 6-TG, suggesting that 6-TG induced UPR accelerates ERAD-mediated turnover of membrane-anchored S0 and S2 glycoproteins. *Taken together, these data indicate that 6-TG and 6-TGo are effective host-targeted antivirals that trigger the UPR and disrupt accumulation of viral glycoproteins. Importantly, our data demonstrate for the first time the efficacy of these thiopurines in limiting IAV and HCoV-OC43 replication in cell culture models*. [**note: it's curious that these old drugs have not come up before to my knowledge. I'm unsure about their utility because of nasty side effects.**]
https://www.biorxiv.org/content/10.1101/2020.09.30.319863v1

- SARS-CoV-2 infection causes an inflammatory cytokine storm and acute lung injury. Currently there are no effective antiviral and/or anti-inflammatory therapies. Here we demonstrate that 2019 SARS-CoV-2 spike protein subunit 1 (CoV2-S1) induces high levels of NF-κB activations, production of pro-inflammatory cytokines and mild epithelial damage, in human bronchial epithelial cells. *CoV2-S1-induced NF-κB activation requires S1 interaction with human ACE2 receptor and early activation of endoplasmic reticulum (ER) stress, and associated unfolded protein response (UPR), and MAP kinase signalling pathways. We developed an antagonistic peptide that inhibits S1-ACE2 interaction and CoV2-S1-induced productions of pro-inflammatory cytokines.* The existing FDA-approved ER stress inhibitor, acid 4-phenylburic (4-PBA), and MAP kinase inhibitors, trametinib and ulixertinib, ameliorated CoV2-S1-induced inflammation and epithelial damage. These novel data highlight the potentials of peptide-based antivirals for novel ACE2-utilising CoVs, while repurposing existing drugs may be used as treatments to dampen elevated inflammation and lung injury mediated by SARS-CoV-2. [**note: this Australian group found an antagonistic peptide that might prove clinically useful in controlling the viral induced inflammatory response. Three drugs were also looked at as noted.**]
https://www.biorxiv.org/content/10.1101/2020.09.30.317818v1
- SARS-CoV-2 poses a public health threat for which therapeutic agents are urgently needed. Herein, we report that high-throughput microfluidic screening of antigen-specific B-cells led to the identification of LY-CoV555, a potent anti-spike neutralizing antibody from a convalescent COVID-19 patient. Biochemical, structural, and functional characterization revealed high-affinity binding to the receptor-binding domain, ACE2 binding inhibition, and potent neutralizing activity. In a rhesus macaque challenge model, prophylaxis doses as low as 2.5 mg/kg reduced viral replication in the upper and lower respiratory tract. These data demonstrate that high-throughput screening can lead to the identification of a potent antiviral antibody that protects against SARS-CoV-2 infection. [**note: here is animal data for the AbCellera/Lilly monoclonal antibody that is currently in clinical trials.**]
https://www.biorxiv.org/content/10.1101/2020.09.30.318972v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Background The SARS-CoV-2 pandemic necessitated rapid and global responses across all areas of healthcare, including an unprecedented interest in serological immunoassays to detect antibodies to the virus. The dynamics of the immune response to SARS-CoV-2 is still not well understood. Methods We measure SARS-CoV-2 antibody levels in plasma samples from 880 people in Northern Ireland by Roche Elecsys Anti-SARS-CoV-2 IgG/IgA/IgM, Abbott SARS-CoV-2 IgG and EuroImmun IgG SARS-CoV-2 ELISA immunoassays to analyse immune dynamics over time. Using these results, we develop a "pseudo gold standard" reference cohort against which

to assess immunoassay performance. We report performance metrics for the UK-RTC AbC-19 rapid lateral flow immunoassay (LFIA) against a characterised panel of 304 positives established using the "pseudo gold standard" system and 350 negative samples. Results We detect persistence of SARS-CoV-2 IgG up to 140 days (20 weeks) post infection, across all three antibody immunoassays, at levels up to 4.4 times the cut-off for a positive result by Roche measurement. Using our "pseudo gold standard" cohort (n=348 positive, n=510 negative) we determine the sensitivity and specificity of the three commercial immunoassays used (EuroImmun; Sens. 98.9% [97.7-99.7%]; Spec. 99.2% [98.4-99.8%]; Roche; Sens. 99.4% [98.6-100%]; Spec. (96.7% [95.1-98.2%]; Abbott; Sens. 86.8% [83.1-90.2%]; Spec. (99.2% [98.4-99.8%]). The UK-RTC AbC-19 lateral flow immunoassay using shows a sensitivity of 97.70% (95.72%-99.34%) and specificity of 100% (100.00-100.00%). Conclusions *Through comprehensive analysis of a large cohort of pre-pandemic and pandemic individuals, we show detectable levels of IgG antibodies, lasting up to 140 days, providing insight to immunity levels at later time points. We propose an alternative to RT-PCR positive status as a standard for assessing SARS-CoV-2 antibody assays and show strong performance metrics for the AbC-19 rapid test*. [**note: here is a serology test of 880 people in Northern Ireland that shows persistence of IgG antibodies 140 days out from the start of the test. This is good to see and the researchers also compare some different types of tests.**]
https://www.medrxiv.org/content/10.1101/2020.09.29.20201509v1

- Worldwide COVID-19 epidemiology data indicate clear differences in disease incidence among sex and age groups. Specifically, male patients are at a higher death risk than females. However, whether this difference is the consequence of a pre-existing sex-bias in immune genes or a differential response to the virus has not been studied yet. *We created DeCovid, an R shiny app that combines gene expression data of different human tissue from the Genotype-Tissue Expression (GTEx) project and the COVID-19 Disease Map gene collection to explore basal gene expression differences across healthy demographic groups. We used this app to study differential gene expression between men and women for COVID-19 associated genes. We identified that healthy women present higher levels in the expression of interferon genes and the JAK-STAT pathway leading to cell survival*. [**note: from Univ of Florida, an approach to studying the sex differences that may be important for cell survival. There is still so much we don't know.**]
https://www.biorxiv.org/content/10.1101/2020.09.30.321059v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-10-03

I'm always a sucker for a well sung Flower Duet from the Delibes opera, Lakmé. What could be better than an outdoor performance right next to the Eiffel Tower in Paris? It's really cool how the tower lights up during the aria. Here are Elina Garanča and Olga Peretyatko:
https://www.youtube.com/watch?v=borTi9_i0YU

Good news for music fans. Rolling Stone have updated the top 500 albums of all time. My hero Isaac Hayes is there and you all need to listen to his cover of 'By the Time I get to Phoenix' (prepare yourself

for 17 wonderful minutes!). This is how soul is done. What puzzles me is that Freddie Mercury and Queen don't even merit a mention.

President Trump was hospitalized late yesterday at Walter Reed for treatment of his SARS-CoV-2 infection. According to reports he was given a single dose of Regeneron's paired monoclonal antibody, placed on a remdesivir drip and administered zinc, vitamin D, melatonin, famotidine, and a daily aspirin. Other than the aspirin, all the other compounds are being studied in randomized controlled clinical trials. Again, I refer you all to the numerous Internet news sites for further information as there are way too many links to post here. However, this STAT piece has a useful discussion of how COVID-19 infection progresses and what clinicians look out for in hospitalized patients.

Given the number of people who have tested positive after attending the White House gathering to introduce Supreme Court nominee, Amy Coney Barrett, there has been discussion of whether this might have been a superspreading event. Only further contact tracing and testing will show if this in fact was the case. Related to this, The Atlantic has a provocative article on the overlooked variable driving the pandemic. The focus ought to be on the cause of superspreading persons/events and this requires further backwards tracing than is normally done. I think this article is on target with my own theory of stochastic environments as relates to viral spread. Here is the concluding money quote, "Could we get back to a much more normal life by focusing on limiting the conditions for super-spreading events, aggressively engaging in cluster-busting, and deploying cheap, rapid mass tests—that is, once we get our case numbers down to low enough numbers to carry out such a strategy? (Many places with low community transmission could start immediately.) Once we look for and see the forest, it becomes easier to find our way out." I recommend all my readers take a look at this article as it may be useful in making sense of what just happened at the White House gathering.

The Washington Post covers the COVID-19 outbreak at the NFL Tennessee Titans. Their next game against Pittsburg has been postponed and an additional two players have come down with the virus.

On the economic front, The New York Times notes that while employment has bounced back since the shutdown last March, it has not been fully robust. "We've entered a longer, slower grind that puts the economy at risk for the indefinite future."

Medscape provide a good summary of the National Academy of Sciences, Engineering and Medicine recommendations on administration of an approved COVID-19 vaccine. The full 'framework' is at THIS LINK and can be downloaded at no cost. Healthcare workers should be in the first group to be vaccinated. Here is a summary of how a single point mutation may help explain different national COVID-19 death rates.

Kaiser Health News cover a COVID-19 outbreak on Utah mink farms. This phenomenon was first noted in The Netherlands several months ago at the beginning of the pandemic in that country. It appears that this transmission is unidirectional. I thought mink farming was a thing of the past but I guess not. Here is a story done in conjunction with National Public Radio on the topic of mask wearing. Remember to mask up when you go out!

Speaking of masks, The New England Journal of Medicine has a nice first person commentary from Brigham and Women's physician.

Week ends tend to be short on reading material.

MODELING

- Background: SARS-CoV-2, the virus causing the Covid-19 pandemic emerged in December 2019 in China and raised fears that it could overwhelm healthcare systems worldwide. In June 2020, all African countries registered human infections with SARS-CoV-2. The virus is mutating steadily and this is monitored by a well curated database of viral nucleotide sequences from samples taken from infected individual thus enabling phylogenetic analysis and phenotypic associations. Methods: We downloaded from the GISAID database, SARS-CoV-2 sequences established from four West African countries Ghana, Gambia, Senegal and Nigeria and then performed phylogenetic analysis employing the nextstrain pipeline. Based on mutations found within the sequences we calculated and visualized statistics characterizing clades according to the GISAID nomenclature. Results: We found country-specific patterns of viral clades: the later Europe-associated G-clades predominantly in Senegal and Gambia, and combinations of the earlier (L, S, V) and later clades in Ghana and Nigeria. Contrary to our expectations, the later Europe-associated G-clades emerged before the earlier clades. Detailed analysis of distinct samples showed that some of the earlier clades might have circulated latently and some reflect migration routes via Mali and Tunisia. Conclusions: The distinct patterns of viral clades in the West African countries point at its emergence from Europe and China via Asia and Europe. The observation that the later clades emerged before the earlier clades could be simply due to founder effects or due to latent circulation of the earlier clades. Only a marginal correlation of the G-clades associated with the D614G mutation could be identified with the relatively low case fatality (0.6-3.2). [**note: here is a good phylogenetic analysis of the spread of SARS-CoV-2 from China to Europe and on to West Africa.**]
  https://www.biorxiv.org/content/10.1101/2020.10.02.323519v1
- The novel coronavirus (SARS-CoV-2), which started in Hubei, China in December 2019, has caused an ongoing pandemic. Due to pauci-symptomatic cases, the virus may spread invisibly in a community. Healthcare systems have repeatedly been challenged with a rapid onset of patients. In the absence of vaccination, non-pharmaceutical interventions (NPIs) like interpersonal distancing were implemented in several countries and have been key to effectively reduce viral spreading. In Germany after an exponential growth of cases numbers in March 2020, NPIs were able to effectively control the pandemic and sufficiently reduced the daily reported new infections allowing for partial release of NPIs. Since these measures were unable to remove the virus entirely from the population, responsible behavior and interpersonal distancing are still required. Methods are needed which can rapidly identify SARS-CoV-2 outbreaks. To this end, we developed a novel statistical method evaluating the earliest possible events of infections, the contacts between individuals, which is essential for virus transmission. We derived the contact index, an index for the contact intensity of the population from spatial proximity between individuals as proxy for physical interaction based on complex network science. In an application, we estimated the contact index from GPS mobile phone data of about 1 million users in Germany, and investigated its association with infection rates in Germany. The results show that the contact index is able to model the time evolution of new infections of SARS-CoV-2. It shows a strong association with the effective reproduction number of the virus about seven days later in all observed phases in Germany (Pearson correlation r=0.88): 1) the early phase of the first wave with the highest reproduction rate, 2) phase of strict NPIs (lockdown) with the lowest reproduction, 3) release of NPIs accompanied with an increase of

reproduction. This work presents the foundation to create a geographic information system that can display daily updated human contact data, which we plan to further extend to a full early warning system for SARS-CoV-2. [**note: here is some interesting work from German on how GPS data from mobile phones can be used to look at an association rate of COVID-19 infection.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20188136v1

NEWLY REGISTERED CLINICAL TRIALS

- Hey, I checked yesterday!!!

CLINICAL TRIAL RESULTS

- An outbreak of the novel coronavirus SARS-CoV-2, the causative agent of Coronavirus Disease-2019 (COVID-19), a respiratory disease, has infected over 34,000,000 people since the end of 2019, killed over 1,000,000, and caused worldwide social and economic disruption. Due to the mechanisms of SARS-CoV-2 infection to host cells and its pathogenesis remain largely unclear, there are currently no antiviral drugs with proven efficacy nor are there vaccines for its prevention. Besides severe respiratory and systematic symptoms, several comorbidities may also increase risk of fatal disease outcome. Therefore, it is required to investigate the impacts of COVID-19 on pre-existing diseases of patients, such as cancer and other infectious diseases. In the current study, we have reported that SARS-CoV-2 encoded proteins and some anti-COVID-19 drugs currently used are able to induce lytic reactivation of Kaposi sarcoma-associated herpesvirus (KSHV), one of major human oncogenic viruses through manipulation of intracellular signaling pathways. Our data indicate that those KSHV+ patients especially in endemic areas exposure to COVID-19 or undergoing the treatment may have increased risks to develop virus-associated cancers, even after they have fully recovered from COVID-19. [**note: this is a troubling co-morbidity of SARS-CoV-2 infection that needs to be followed. There could be others.😠]** https://www.biorxiv.org/content/10.1101/2020.10.02.324228v1

DRUG DEVELOPMENT

- The large SARS-CoV-2 spike (S) protein is the main target of current COVID-19 vaccine candidates but can induce non-neutralizing antibodies, which may cause vaccination-induced complications or enhancement of COVID-19 disease. Besides, encoding of a functional S in replication-competent virus vector vaccines may result in the emergence of viruses with altered or expanded tropism. Here, we have developed a safe single round rhabdovirus replicon vaccine platform for enhanced presentation of the S receptor-binding domain (RBD). Structure-guided design was employed to build a chimeric minispike comprising the globular RBD linked to a transmembrane stem-anchor sequence derived from rabies virus (RABV) glycoprotein (G). Vesicular stomatitis virus (VSV) and RABV replicons encoding the minispike not only allowed expression of the antigen at the cell surface but also incorporation into the envelope of secreted non-infectious particles, thus combining classic vector-driven antigen expression and particulate virus-like particle (VLP) presentation. A single dose of a prototype replicon vaccine, VSVΔG-minispike-eGFP (G), stimulated high titers of SARS-CoV-2 neutralizing antibodies in mice, equivalent to those found in COVID-19 patients. Boost immunization with the identical replicon further enhanced neutralizing activity. These results demonstrate that rhabdovirus minispike replicons represent effective and safe alternatives to vaccination approaches using replication-

competent viruses and/or the entire S antigen. [**note: after a week without a new vaccine paper, here is one from Germany that is interesting. It uses only part of the Spike protein and link to the stem-anchor of the rabies virus. It generates potent immune response in mice. They argue this might be a safer alternative to whole Spike protein vaccnes.**]
https://www.biorxiv.org/content/10.1101/2020.10.02.324046v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- T cell-mediated immunity may play a critical role in controlling and establishing protective immunity against SARS-CoV-2 infection; yet the repertoire of viral epitopes responsible for T cell response activation remains mostly unknown. Identification of viral peptides presented on class I human leukocyte antigen (HLA-I) can reveal epitopes for recognition by cytotoxic T cells and potential incorporation into vaccines. Here, we report the first HLA-I immunopeptidome of SARS-CoV-2 in two human cell lines at different times post-infection using mass spectrometry. We found HLA-I peptides derived not only from canonical ORFs, but also from internal out-of-frame ORFs in Spike and Nucleoprotein not captured by current vaccines. Proteomics analyses of infected cells revealed that SARS-CoV-2 may interfere with antigen processing and immune signaling pathways. Based on the endogenously processed and presented viral peptides that we identified, we estimate that a pool of 24 peptides would provide one or more peptides for presentation by at least one HLA allele in 99% of the human population. These biological insights and the list of naturally presented SARS-CoV-2 peptides will facilitate data-driven selection of peptides for immune monitoring and vaccine development. [**note: this is interesting in that it further looks into immune system interference.**]
https://www.biorxiv.org/content/10.1101/2020.10.02.324145v1

DIAGNOSTIC DEVELOPMENT

- Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has developed into a global pandemic since its first outbreak in the winter of 2019. An extensive investigation of SARS-CoV-2 is critical for disease control. Various recombinant monoclonal antibodies of human origin that neutralize SARS-CoV-2 infection have been isolated from convalescent patients and will be applied as therapies and prophylaxis. However, the need for dedicated monoclonal antibodies in molecular pathology research is not fully addressed. Here, we produced mouse anti-SARS-CoV-2 spike monoclonal antibodies that exhibit not only robust performance in immunoassays including western blotting, ELISA, immunofluorescence, and immunoprecipitation, but also neutralizing activity against SARS-CoV-2 infection in vitro. Our monoclonal antibodies are of mouse origin, making them compatible with the experimental immunoassay setups commonly used in basic molecular biology research laboratories, and large-scale production and easy distribution are guaranteed by conventional mouse hybridoma technology. [**note: these Japanese researchers create neutralizing mouse monoclonal antibodies that will be very useful in immunoassays and research.]**
https://www.biorxiv.org/content/10.1101/2020.10.01.323220v1

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2020-10-04

For this reflection Sunday, here is a live concert with the Vienna Philharmonic from the Schönbrunn Palace. What a wonderful site for this performance. Valery Gregiev is the conductor with about as small a baton as I have ever seen. German tenor, Jonas Kaufmann is the featured soloist. Enjoy this one: https://www.youtube.com/watch?v=hf6XSkDgAYs

Obviously, the major news centers on President Trump's condition. I will refer everyone to major news outlets for updates. There are very few preprints today as is usual for Sunday.

The Washington Post discusses some troubling increases in COVID-19 cases in certain states. Here is a story on contact tracing or lack thereof following some of the recent Presidential events.

The New York Times has a story on the failure of contact tracing in many western countries. Here is a useful guide for distinguishing symptoms between influenza and COVID-19. Get your flue shot; it's time! I wonder if all the masking up, social distancing, and reduced air travel will limit this year's influenza numbers. Pope Francis weighs in.

Here is Derek Lowe on the J&J vaccine data.

MODELING

- Nothing today.

NEWLY REGISTERED CLINICAL TRIALS

- I do a weekly check and today is not the day of the week for this.

CLINICAL TRIAL RESULTS

- Objectives: To assess the influence of corticosteroid pulses on 60-days mortality in hospitalized patients with severe COVID-19, intensive care admission, and hospital stay. Methods: We designed a multicenter retrospective cohort study in three teaching hospitals of Castilla y Leon, Spain (865.096 people). We selected patients with confirmed COVID-19 and lung involvement with a pO2/FiO2 < 300, excluding those exposed to immunosuppressors before or during hospitalization, patients terminally ill at admission, or died the first 24 hours. We performed a propensity score matching (PSM) adjusting covariates that modify the probability of being treated. Then we used a Cox regression model in the PSM group to consider factors affecting mortality. Results: From 2933 patients, 257 fulfilled the inclusion and exclusion criteria. 124 patients were on corticosteroid pulses, and 133 were not. 30,3% (37/122) of patients died in the corticosteroid pulses group and 42,9% (57/133) in the non-exposed cohort. These differences (12,6%) were statically significant (log-rank 4.72, p=0,03). We performed PSM using the exact method. Mortality differences remained in the PSM group (log-rank 5.31, p=0,021) and were still significant after a Cox regression model (HR for corticosteroid pulses 0,561, p= 0,039). There were no significant differences in intensive care admission rate (p=0,173). The hospital stay was longer in the corticosteroid group (p<0,001). Conclusions: *This study provides evidence about treatment with corticosteroid pulses in severe COVID-19 that might significantly reduce mortality. Strict inclusion and exclusion criteria with that selection process set a reliable frame to compare mortality in both exposed and non-exposed groups*. [**note: here is a retrospective study from Spain looking at pulsed corticosteroid therapy in hospitalized patients with COVID-19.]** https://www.medrxiv.org/content/10.1101/2020.09.30.20204719v1

- Particular host and environmental factors influence susceptibility to severe COVID-19. We analyzed RNA-sequencing data from bronchial epithelial brushings - a relevant tissue for SARS-CoV-2 infection - obtained from three cohorts of uninfected individuals, and investigated how non-genetic and genetic factors affect the regulation of host genes implicated in COVID-19. We found that ACE2 expression was higher in relation to active smoking, obesity, and hypertension that are known risk factors of COVID-19 severity, while an association with interferon-related inflammation was driven by the truncated, non-binding ACE2 isoform. *We discovered that expression patterns of a suppressed airway immune response to early SARS-CoV-2 infection, compared to other viruses, are similar to patterns associated with obesity, hypertension, and cardiovascular disease, which may thus contribute to a COVID-19-susceptible airway environment. eQTL mapping identified regulatory variants for genes implicated in COVID-19, some of which had pheWAS evidence for their potential role in respiratory infections. These data provide evidence that clinically relevant variation in the expression of COVID-19-related genes is associated with host factors, environmental exposures, and likely host genetic variation*. [**note: this comes from a large group of US researchers, mainly pulmonologists. It shows further factors that may lead to susceptibility to COVID-19.**]
  https://www.medrxiv.org/content/10.1101/2020.10.01.20202820v1
- Background: Lymphopenia due to a plummeting T-cell count is a major feature of severe COVID-19. T-cell proliferation is telomere length (TL)-dependent and TL shortens with age. Older persons are disproportionally affected by severe COVID-19, and we hypothesized that those with short TL have less capacity to mount an adequate T-cell proliferative response to SARS-CoV-2. This hypothesis predicts that among older patients with COVID-19, shorter telomeres of peripheral blood mononuclear cells (PBMCs) will be associated with a lower lymphocyte count. Methods: Our sample comprised 17 COVID-19 and 21 non-COVID-19 patients, aged 87(8) (mean(SD)) and 87 (9) years, respectively. We measured TL by the Telomere Shortest Length Assay, a novel method that measures and tallies the short telomeres directly relevant to telomere-mediated biological processes. The primary analysis quantified TL as the proportion of telomeres shorter than 2 kilobases. For comparison, we also quantified TL by Southern blotting, which measures the mean length of telomeres. Results: Lymphocyte count (109/L) was 0.91 (0.42) in COVID-19 patients and 1.50(0.50) in non-COVID-19 patients ($P < 0.001$). In COVID-19 patients, but not in non-COVID-19 patients, lymphocyte count was inversely correlated with the proportion of telomeres shorter than 2 kilobases ($P = 0.005$) and positively correlated with the mean of telomeres measured by TeSLA ($P = 0.03$). Lymphocyte counts showed no statistically significant correlations with Southern blotting results in COVID-19 or non-COVID-19 patients. Conclusions: These results support the hypothesis that a compromised TL-dependent T-cell proliferative response contributes to lymphopenia and the resulting disproportionate severity of COVID-19 among old adults. We infer that infection with SARS-CoV-2 uncovers the limits of the TL reserves of older persons. [**note: another reason why it sucks to be old. Here is evidence that telomere length may be associated with severity of COVID-19 in older patients.]**
  https://www.medrxiv.org/content/10.1101/2020.10.01.20205393v1

DRUG DEVELOPMENT

- Nothing new.

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing new.

DIAGNOSTIC DEVELOPMENT

- Nothing new.