2020-09-14

Welcome to Week 26; Half a Year of Reporting on the Pandemic 😒

Let us stay with the piano and Beethoven! Here is the Sonata #23 "Appassionata" with the great Chilean pianist, Claudio Arrau. This is a live performance from Bonn, filmed in 1970 when Arrau was 67: https://www.youtube.com/watch?v=Tdg-DT8rTUQ and why not make this a twofer! Here is Arrau from Bonn in 1977 playing the "Waldstein" sonata: https://www.youtube.com/watch?v=dL0JLNt_3EE

In a New York Times column, Harvard Econ professor Greg Mankiw argues we should pay people to get a COVID-19 vaccine. Yes, this might help with the uptake. The proposal he discusses comes from Robert Litan at the Brookings Institution. Here is yet another article on the need for transparency from vaccine companies. I agree!

According to The Washington Post, Israel is about to enter a second big lockdown as the Jewish New Year approaches. SARS-CoV-2 doesn't understand religion or national boundaries.

Here is a STAT Q & A about various things COVID-19.

Nature discuss the Chinese military's role in vaccine development. Let us not forget the US military research efforts that have gone on at Walter Reed and other DOD sponsored institutions (Walter Reed has developed a prototype COVID-19 vaccine).

It is a short reading list today because of the weekend. I still want to see more clinical trial results!

MODELING

- As COVID-19 continues to spread across the world, it is increasingly important to understand the factors that influence its transmission. Seasonal variation driven by responses to changing environment has been shown to affect the transmission intensity of several coronaviruses. However, the impact of the environment on SARS-CoV-2 remains largely unknown, and thus seasonal variation remains a source of uncertainty in forecasts of SARS-CoV-2 transmission. Here we address this issue by assessing the association of temperature, humidity, UV radiation, and population density with estimates of transmission rate (R). Using data from the United States of America, we explore correlates of transmission across USA states using comparative regression and integrative epidemiological modelling. *We find that policy intervention (`lockdown') and reductions in individuals' mobility are the major predictors of SARS-CoV-2 transmission rates, but in their absence lower temperatures and higher population densities are correlated with increased SARS-CoV-2 transmission. Our results show that summer weather cannot be considered a substitute for mitigation policies, but that lower autumn and winter temperatures may lead to an increase in transmission intensity in the absence of policy interventions or behavioural changes. We outline how this information may improve the forecasting of SARS-CoV-2, its future seasonal dynamics, and inform intervention policies*. [**note: using data from the US, here is an environmental model for SARS-CoV-2 transmission. The clock keeps moving towards autumn and winter and we will see what the impacts of colder weather will be.**] https://www.medrxiv.org/content/10.1101/2020.09.12.20193250v1
- We estimate the basic reproductive number and case counts for 15 distinct SARS-CoV-2 outbreaks, distributed across 10 countries and one cruise ship, based solely on phylodynamic

analyses of genomic data. Our results indicate that, prior to significant public health interventions, the reproductive numbers for a majority (10) of these outbreaks are similar, with median posterior estimates ranging between 1.4 and 2.8. These estimates provide a view which is complementary to that provided by those based on traditional line listing data. The genomic-based view is arguably less susceptible to biases resulting from differences in testing protocols, testing intensity, and import of cases into the community of interest. In the analyses reported here, the genomic data primarily provides information regarding which samples belong to a particular outbreak. We observe that once these outbreaks are identified, the sampling dates carry the majority of the information regarding the reproductive number. *Finally, we provide genome-based estimates of the cumulative case counts for each outbreak, which allow us to speculate on the amount of unreported infections within the populations housing each outbreak. These results indicate that for the majority (7) of the populations studied, the number of recorded cases is much bigger than the estimated cumulative case counts, suggesting the presence of unsequenced pathogen diversity in these populations.* [**note: from ETH in Zurich, a phylogenetic examination of a number of COVID-19 outbreaks. The authors comments about the presence of unsequenced pathogen diversity is interesting.**]
https://www.medrxiv.org/content/10.1101/2020.09.12.20193284v1

- COVID-19 is a viral respiratory illness, caused by the SARS-CoV-2 virus with frequent symptoms of fever and shortness of breath. COVID-19 has a high mortality rate among elders. The virus has spread world-wide, leading to shut-down of many countries around the globe with the aim of stopping the spread of the disease. To date, there are uncertainties regarding the main factors in the disease spread, so sever social distancing measures and broad testing are required in order to protect the population at risk. With the increasing spread of the virus, there is growing fraction of the general population that may be immune to COVID-19, following infection. This immunised cohort can be uncovered via large-scale screening for the SARS-CoV-2 (Corona) virus and/or its antibodies. *We propose that this immune cohort be deployed as a buffer between the general population and the population most at risk from the disease. Here we show that under a broad range of realistic scenarios deploying such an immunized buffer between the general population and the population at risk may lead to a dramatic reduction in the number of deaths from the disease. This provides an impetus for: screening for the SARS-CoV-2 virus and/or its antibodies on the largest scale possible, and organizing at the family, community, national and international levels to protect vulnerable populations by deploying immunized buffers between them and the general population wherever possible*. [**note: from Israel's Weizmann Institute, a model for protecting the elderly based on immune-buffer. It's predicated on doing a lot of testing both viral and antibody.**]
https://www.medrxiv.org/content/10.1101/2020.09.12.20193094v1

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to assess the safety and efficacy of orally-administered dapansutrile capsules for the treatment of moderate COVID-19 symptoms and evidence of early cytokine release syndrome. [**note: sponsor is Olatec Therpeutics}** NCT04540120

CLINICAL TRIAL RESULTS

- Nothing New

DRUG DEVELOPMENT

- COVID-19 caused by the SARS-CoV-2 virus has become a global pandemic. 3CL protease is a virally encoded protein that is essential to the viral life cycle across a broad spectrum of coronaviruses with no close human analogs. The designed phosphate prodrug PF-07304814 is metabolized to PF-00835321 which is a potent inhibitor in vitro of the coronavirus family 3CL pro, with selectivity over human host protease targets. Furthermore, PF-00835231 exhibits potent in vitro antiviral activity against SARS-CoV-2 as a single agent and it is additive/synergistic in combination with remdesivir. We present the ADME, safety, and in vitro antiviral activity data to warrant clinical evaluation. [**note: here is more information on the Pfizer viral protease inhibitor. I hope they can get this in trials soon.**]
https://www.biorxiv.org/content/10.1101/2020.09.12.293498v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Angiotensin-converting enzyme 2 (ACE2) maintains cardiovascular and renal homeostasis but also serves as the entry receptor for the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2), the causal agent of novel coronavirus disease 2019 (COVID-19). COVID-19 disease severity is typically lower in pediatric patients than adults (particularly the elderly), but higher rates of hospitalizations requiring intensive care are observed in infants than in older children - the reasons for these differences are unknown. ACE2 is expressed in several adult tissues and cells, including alveolar type 2 cells of the distal lung epithelium, but expression at other ages is largely unexplored. Here we show that ACE2 transcripts are expressed in the lung and trachea shortly after birth, downregulated during childhood, and again expressed at high levels in late adulthood. Notably, the repertoire of cells expressing ACE2 protein in the mouse lung and airways shifts during key phases of lung maturation. In particular, podoplanin-positive cells, which are likely alveolar type I cells responsible for gas exchange, express ACE2 only in advanced age. Similar patterns of expression were evident in analysis of human lung tissue from over 100 donors, along with extreme inter- and intra-individual heterogeneity in ACE2 protein expression in epithelial cells. Furthermore, we find that apoptosis, which is a natural host defense system against viral infection, is dynamically regulated during lung maturation, resulting in periods of heightened apoptotic priming and dependence on pro-survival BCL-2 family proteins including MCL-1. Infection of human lung cells with SARS-CoV-2 triggers an unfolded protein stress response and upregulation of the endogenous MCL-1 inhibitor Noxa; in young individuals, MCL-1 inhibition is sufficient to trigger apoptosis in lung epithelial cells and may thus limit virion production and inflammatory signaling. Overall, we identify strong and distinct correlates of COVID-19 disease severity across lifespan and advance our understanding of the regulation of ACE2 and cell death programs in the mammalian lung. Furthermore, our work provides the framework for translation of apoptosis modulating drugs as novel treatments for COVID-19. [**note: from Harvard, a study age dependent regulation of viral entry genes and cell death that correleates with COVID-19 severity. Where is Ponce de Leon when we need him to get to the fountain of youth?**] https://www.biorxiv.org/content/10.1101/2020.09.13.276923v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is currently causing a global pandemic. The antigen specificity and kinetics of the antibody response mounted against this novel virus are not understood in detail. Here, we report that subjects with a more severe SARS-CoV-2 infection exhibit a larger antibody response against the spike and nucleocapsid protein

and epitope spreading to subdominant viral antigens, such as open reading frame 8 and non-structural proteins. Subjects with a greater antibody response mounted a larger memory B cell response against the spike, but not the nucleocapsid protein. Additionally, we revealed that antibodies against the spike are still capable of binding the D614G spike mutant and cross-react with the SARS-CoV-1 receptor binding domain. Together, this study reveals that subjects with a more severe SARS-CoV-2 infection exhibit a greater overall antibody response to the spike and nucleocapsid protein and a larger memory B cell response against the spike. [**note: this one is from Univ of Chicago that looks at antibody response in severe COVID-19 patients. It's interesting that there is a robust antibody response in severe cases but something else must be going on.**] https://www.biorxiv.org/content/10.1101/2020.09.12.294066v1

DIAGNOSTIC DEVELOPMENT

- Nothing New

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2020-09-15

There is no one 'best' pianists but rather a lot of 'best' pianists. Here is one of the 'best' in my opinion and I'm surprised that I've overlooked him in these musical preludes. Of course it is none other than Evgeny Kissin who made his concert debut at the age of 10 playing the Mozart piano concerto #20. Here he is in Moscow with a selection of eight Chopin etudes:
https://www.youtube.com/watch?v=5hwOveZlugk

US COVID-19 STATISTICS - **Infection Rate: 2.0; CFR: 2.9%** (IR up 0.1%; CFR down 0.1%; **note:** the CFR for this current outbreak drops below 1.5%; a good sign)

The Washington Post reports on a survey of local businesses that it might not be until next summer when the bulk of the region's office workers return. Large employers are being cautious partly as a result of uncertainty about public transit. Open air schools during a New England winter? Yes, it happened in the early 1900s when tuberculosis was a major infectious disease threat (check out the pictures!). Children of today are so coddled when we consider that we had to walk several miles to school each day. The Food Critic of the WaPo suddenly realizes that he has generated a mountain of trash from take out. What to do? Bars and restaurants may open for thee but not for me.

Pandemic marketing at its best!! According to The New York Times, PepsiCo is going to launch a new product Driftwell. The new drink contains 200 miligrams of L-theanine, an amino acid that studies suggest can improve sleep and reduce stress. The drink also contains 10 percent of the recommended daily value of magnesium, which can be used as a sleep aid.

Vaxart have announced FDA approval for Phase 1 studies of their oral adenovirus COVID-19 vaccine. I believe this is the first oral and room temperature stable vaccine to enter trials. Our current industrial policy, aka 'Operation Warp Speed' is NOT supporting this effort.

Eli Lilly issued a press release yesterday stating that baricitinib in combination with remdesivir reduces time to recovery in hospitalized COVID-19 patients. Study investigators noted an approximately one-day

reduction in median recovery time for the overall patient population treated with baricitinib in combination with remdesivir versus those treated with remdesivir. This finding was statistically significant. Recovery was defined as the participant being well enough for hospital discharge, meaning the participant either no longer required supplemental oxygen or ongoing medical care in the hospital, or was no longer hospitalized at Day 29. The study also met a key secondary endpoint comparing patient outcomes at Day 15 using an ordinal 8-point scale ranging from fully recovered to death. This came from the DSMB that is overseeing the trial.

Here is a story from my Yahoo news feed on a CDC report on child transmission from day care centers in Utah. Young children can be asymptomatic and still spread virus to adults and siblings.

ProPublica has a commentary on the approach of the 200,000th life lost to COVID-19. Yes, this country did miss major opportunities to squash the infection.

When will be able to stop wearing face masks?

STAT has an interview with philanthropist, Bill Gates.

Derek Lowe weighs in on vaccine transparency.

Some interesting papers today!

MODELING

- Masks are a vital tool for limiting SARS-CoV-2 spread in the population. Here we utilize a mathematical model to assess the impact of masking on transmission within individual transmission pairs and at the population level. Our model quantitatively links mask efficacy to reductions in viral load and subsequent transmission risk. Our results reinforce that the use of masks by both a potential transmitter and exposed person substantially reduces the probability of successful transmission, even if masks only lower exposure viral load by ~50%. Slight increases in masking relative to current levels would reduce the reproductive number substantially below 1, particularly if implemented comprehensively in potential super-spreader environments. *Our model predicts that moderately efficacious masks that reduce transmission risk by 50% will lower exposure viral load 10-fold among people who do get infected, potentially limiting infection severity. Because peak viral load tends to occur pre-symptomatically, we also identify that antiviral therapy targeting symptomatic individuals is unlikely to impact transmission risk. Instead, antiviral therapy is only effective for this indication as post-exposure prophylaxis, specifically if given to ~50% of newly infected people within 3 days of an exposure. These results highlight the primacy of masking relative to other biomedical interventions under consideration for limiting the extent of the COVID-19 pandemic prior to widespread implementation of a vaccine*. [**note: from the Hutch in Seattle word to the wise, "PUT YOUR MASK ON!"** https://www.medrxiv.org/content/10.1101/2020.09.13.20193508v1
- Viral genome sequencing has guided our understanding of the spread and extent of genetic diversity of SARS-CoV-2 during the COVID-19 pandemic. SARS-CoV-2 viral genomes are usually sequenced from nasopharyngeal swabs of individual patients to track viral spread. Recently, RT-qPCR of municipal wastewater has been used to quantify the abundance of SARS-CoV-2 in

several regions globally. However, metatranscriptomic sequencing of wastewater can be used to profile the viral genetic diversity across infected communities. Here, we sequenced RNA directly from sewage collected by municipal utility districts in the San Francisco Bay Area to generate complete and near-complete SARS-CoV-2 genomes. The major consensus SARS-CoV-2 genotypes detected in the sewage were identical to clinical genomes from the region. Using a pipeline for single nucleotide variant (SNV) calling in a metagenomic context, we characterized minor SARS-CoV-2 alleles in the wastewater and detected viral genotypes which were also found within clinical genomes throughout California. Observed wastewater variants were more similar to local California patient-derived genotypes than they were to those from other regions within the US or globally. Additional variants detected in wastewater have only been identified in genomes from patients sampled outside of CA, indicating that wastewater sequencing can provide evidence for recent introductions of viral lineages before they are detected by local clinical sequencing. These results demonstrate that epidemiological surveillance through wastewater sequencing can aid in tracking exact viral strains in an epidemic context. [**note: another wastewater study, this time from Univ of California showing regional prevalent SARS-CoV-2 genomes can be identified}**
https://www.medrxiv.org/content/10.1101/2020.09.13.20193805v1

- Contact tracing is one of several strategies employed in many countries to curb the spread of SARS-CoV-2. Digital contact tracing (DCT) uses tools such as cell-phone applications to improve tracing speed and reach. We model the impact of DCT on the spread of the virus for a large epidemiological parameter space consistent with current literature on SARS-CoV-2. We also model DCT in combination with random testing (RT) and social distancing (SD). Modelling is done with two independently developed individual-based (stochastic) models that use the Monte Carlo technique, benchmarked against each other and against two types of deterministic models. For current best estimates of the number of asymptomatic SARS-CoV-2 carriers (approximately 40\%), their contagiousness (similar to that of symptomatic carriers), the reproductive number before interventions (R0 at least 3) we find that DCT must be combined with other interventions such as SD and/or RT to push the reproductive number below one. At least 60\% of the population would have to use the DCT system for its effect to become significant. *On its own, DCT cannot bring the reproductive number below 1 unless nearly the entire population uses the DCT system and follows quarantining and testing protocols strictly. For lower uptake of the DCT system, DCT still reduces the number of people that become infected. When DCT is deployed in a population with an ongoing outbreak where O(0.1\%) of the population have already been infected, the gains of the DCT intervention come at the cost of requiring up to 15% of the population to be quarantined (in response to being traced) on average each day for the duration of the epidemic, even when there is sufficient testing capability to test every traced person*. [**note: here is a German paper modeling the use of digital contact tracing. It's 'Debbie Downer' of a paper as there are other dependencies required to implement it and uptake of an app needs to be very high.]**
https://www.medrxiv.org/content/10.1101/2020.09.13.20192682v1

NEWLY REGISTERED CLINICAL TRIALS

- Did not look today.

CLINICAL TRIAL RESULTS

- Discussed the baricitinib results above.

DRUG DEVELOPMENT

- We present a comprehensive vaccine strategy for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by combining antigen optimization and nanoparticle display. We first developed a receptor binding domain (RBD)-specific antibody column for purification and displayed the RBD on self-assembling protein nanoparticles (SApNPs) using the SpyTag/SpyCatcher system. We then identified the heptad repeat 2 (HR2) stalk as a major cause of spike metastability, designed an HR2-deleted glycine-capped spike (S2GΔHR2), and displayed S2GΔHR2 on three SApNPs with high yield, purity, and antigenicity. Compared to the RBD, the RBD-ferritin SApNP elicited a more potent murine neutralizing antibody (NAb) response on par with the spike. S2GΔHR2 elicited two-fold-higher NAb titers than the proline-capped spike (S2P), while S2GΔHR2 SApNPs derived from multilayered E2p and I3-01v9 60-mers elicited up to 10-fold higher NAb titers. The S2GΔHR2-presenting I3-01v9 SApNP also induced critically needed T-cell immunity, thereby providing a next-generation vaccine candidate to battle the COVID-19 pandemic. [**note: this is from Scripps and Temple Univ, another strategy for vaccine. Some of the ideas in this paper have been used by others. A very solid immune response was seen in an animal model**] https://www.biorxiv.org/content/10.1101/2020.09.14.296715v1
- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), enters the host cells through two main pathways, both involving key interactions between viral envelope-anchored spike glycoprotein of the novel coronavirus and the host receptor, angiotensin-converting enzyme 2 (ACE2). To date, SARS-CoV-2 has infected up to 26 million people worldwide; yet, there is no clinically approved drug or vaccine available. Therefore, a rapid and coordinated effort to re-purpose clinically approved drugs that prevent or disrupt these critical entry pathways of SARS-CoV-2 spike glycoprotein interaction with human ACE2, could potentially accelerate the identification and clinical advancement of prophylactic and/or treatment options against COVID-19, thus providing possible countermeasures against viral entry, pathogenesis and survival. Herein, we discovered that Ambroxol hydrochloride (AMB), and its progenitor, Bromhexine hydrochloride (BHH), both clinically approved drugs are potent effective modulators of the key interaction between the receptor binding domain (RBD) of SARS-CoV-2 spike protein and human ACE2. We also found that both compounds inhibited SARS-CoV-2 infection-induced cytopathic effect at micromolar concentrations. Therefore, in addition to the known TMPRSS2 activity of BHH; we report for the first time that the BHH and AMB pharmacophore has the capacity to target and modulate yet another key protein-protein interaction essential for the two known SARS-CoV-2 entry pathways into host cells. Altogether, the potent efficacy, excellent safety and pharmacologic profile of both drugs along with their affordability and availability, makes them promising candidates for drug repurposing as possible prophylactic and/or treatment options against SARS-CoV-2 infection. [**note: both drugs are mucolytics. There are two foreign trials of bromheine, one in Mexico and the other in Slovenia.**]
https://www.biorxiv.org/content/10.1101/2020.09.13.295691v1
- The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes COVID-19, a pandemic that seriously threatens global health. SARS CoV-2 propagates by packaging its RNA genome into membrane enclosures in host cells. The packaging of the viral genome into the

nascent virion is mediated by the nucleocapsid (N) protein, but the underlying mechanism remains unclear. Here, we show that the N protein forms biomolecular condensates with viral RNA both in vitro and in mammalian cells. While the N protein forms spherical assemblies with unstructured RNA, it forms mesh like-structures with viral RNA strands that contain secondary structure elements. Cross-linking mass spectrometry identified an intrinsically-disordered region that forms interactions between N proteins in condensates, and truncation of this region disrupts phase separation. By screening 1,200 FDA approved drugs in vitro, we identified a kinase inhibitor nilotinib, which affects the morphology of N condensates in vitro and disrupts phase separation of the N protein in vivo. These results indicate that the N protein compartmentalizes viral RNA in infected cells through liquid-liquid phase separation, and this process can be disrupted by a possible drug candidate. [**note: this paper from Berkeley looks at viral assembly and mediation via the N protein. They find that the kinase inhibitor nilotinib disrupts this process and may be a potential drug of interest.**]
https://www.biorxiv.org/content/10.1101/2020.09.14.295824v2

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Viruses rely on the host translation machinery to synthesize their own proteins. Consequently, they have evolved varied mechanisms to co-opt host translation for their survival. SARS-CoV-2 relies on a non-structural protein, NSP1, for shutting down host translation. Despite this, it is currently unknown how viral proteins and host factors critical for viral replication can escape a global shutdown of host translation. Here, using a novel FACS-based assay called MeTAFlow, we report a dose-dependent reduction in both nascent protein synthesis and mRNA abundance in cells expressing NSP1. We perform RNA-Seq and matched ribosome profiling experiments to identify gene-specific changes both at the mRNA expression and translation level. We discover a functionally-coherent subset of human genes preferentially translated in the context of NSP1 expression. These genes include the translation machinery components, RNA binding proteins, and others important for viral pathogenicity. Importantly, we also uncover potential mechanisms of preferential translation through the presence of shared sites for specific RNA binding proteins and a remarkable enrichment for 5' terminal oligo-pyrimidine tracts. Collectively, the present study suggests fine tuning of host gene expression and translation by NSP1 despite its global repressive effect on host protein synthesis. [**note: more on how the virus coopts the mammalian protein synthesis machinery**]
https://www.biorxiv.org/content/10.1101/2020.09.13.295493v1

DIAGNOSTIC DEVELOPMENT

- Background: We assessed the performance, stability, and user acceptability of swab-independent self-collected saliva and saline mouth rinse/gargle sample types for the molecular detection of SARS-CoV-2 in adults and school-aged children. Methods: Outpatients who had recently been diagnosed with COVID-19 or were presenting with suspected COVID-19 were asked to have a nasopharyngeal swab collected and provide at least one self-collected sample type. A portion of participants were also asked about sample acceptability. Samples underwent molecular testing using multiple assays. Saline mouth rinse/gargle and saliva samples were tested daily at time zero, day one, and day 2 to assess nucleic acid stability at room temperature. *Results: 50 participants (aged 4 to 71 years) were included; of these, 40 had at*

least one positive sample and were included in the primary sample yield analysis. Saline mouth rinse/gargle samples had a sensitivity of 98% (39/40) while saliva samples had a sensitivity of 79% (26/33). Both saline mouth rinse/gargle and saliva samples showed stable viral RNA detection after 2 days of room temperature storage. Mouth rinse/gargle samples had the highest (mean 4.9) and HCW-collected NP swabs had the lowest acceptability scores (mean 3.1). Conclusion: Saline mouth rinse/gargle samples demonstrated the highest combined user acceptability ratings and analytical performance when compared with saliva and HCW collected NP swabs. This sample type is a promising swab-independent option, particularly for outpatient self-collection in adults and school aged children. [**note: good work from Vancouver on the usefulness of saline rinse/gargle for collecting viral samples for analysis.**] https://www.medrxiv.org/content/10.1101/2020.09.13.20188334v1

- Rapid diagnosis is critical for the treatment and prevention of diseases. In this research, we report sensing of antibodies specific to SARS-CoV-2 virus in seconds via an electrochemical platform consisting of gold micropillar array electrodes decorated with reduced graphene oxide and functionalized with recombinant viral antigens. The array electrodes are fabricated by Aerosol Jet (AJ) nanoparticle 3D printing, where gold nanoparticles (3-5nm) are assembled in 3D space, sintered, and integrated with a microfluidic device. The device is shown to detect antibodies to SARS-CoV-2 spike S1 protein and its receptor-binding-domain (RBD) at concentrations down to 1pM via electrochemical impedance spectroscopy and read by a smartphone-based user interface. In addition, the sensor can be regenerated within a minute by introducing a low-pH chemistry that elutes the antibodies from the antigens, allowing successive testing of multiple antibody samples using the same sensor. The detection time for the two antibodies tested in this work is 11.5 seconds. S1 protein sensing of its antibodies is specific, which cross-reacts neither with other antibodies nor with proteins such as Nucleocapsid antibody and Interleukin-6 protein. The proposed sensing platform is generic and can also be used for the rapid detection of biomarkers for other infectious agents such as Ebola, HIV, and Zika, which will benefit the public health. [**note: this is way cool work from Carnegie-Mellon! They use 3-D printing to create antigen coated electrodes to detect COVID-19 antibodies. You need to download the full paper and go to the end to see the neat pictures of how they designed this.**] https://www.medrxiv.org/content/10.1101/2020.09.13.20193722v1
- Rapid detection of pathogenic sequences or variants in DNA and RNA through a point-of-care diagnostic approach is valuable for accelerated clinical prognosis as has been witnessed during the recent COVID-19 outbreak. Traditional methods relying on qPCR or sequencing are difficult to implement in settings with limited resources necessitating the development of accurate alternative testing strategies that perform robustly. Here, we present FnCas9 Editor Linked Uniform Detection Assay (FELUDA) that employs a direct Cas9 based enzymatic readout for detecting nucleotide sequences and identifying nucleobase identity without the requirement of trans-cleavage activity of reporter molecules. We demonstrate that FELUDA is 100% accurate in detecting single nucleotide variants (SNVs) including heterozygous carriers of a mutation and present a simple design strategy in the form of a web-tool, JATAYU, for its implementation. *FELUDA is semi quantitative, can be adapted to multiple signal detection platforms and can be quickly designed and deployed for versatile applications such as infectious disease outbreaks like COVID-19. Using a lateral flow readout within 1h, FELUDA shows 100% sensitivity and 97% specificity across all range of viral loads in clinical samples. In combination with RT-RPA and a*

smartphone application True Outcome Predicted via Strip Evaluation (TOPSE), we present a prototype for FELUDA for CoV-2 detection at home. [**note: I guess today is a good day for new diagnostic stuff. Here is a paper from India with a new way to detect nucleotide sequences. It can even be adapted to home use.**
https://www.medrxiv.org/content/10.1101/2020.09.13.20193581v1

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2020-09-16

Here is an extra special treat for all you singers and fans of singers. Nicola Vaccai was an Italian opera composer and singing teacher. His book, "Practical Method of Italian Singing," may be one of the most widely used practice books of all time. It has been in print since its 1832 publication. I'm currently working my way through it. You can download a copy of the exercises at the IMSLP site if you wish. Last night, Will Crutchfield's Teatro Nuovo, premiered a YouTube recital of a group of opera singers who each took one of the exercises. It was great fun to watch and listen how professionals handle these: https://www.youtube.com/watch?v=vPyK1vjFaaE

The Washington Post covers a CDC report that notes 75% of children who die from COVID-19 are Hispanic, Black or American Indian. Here is an op-ed about why scientists cannot predict COVID-19's long-term trajectory. California's wineries are seeking new ways to sell wine to customers! More on the Maine wedding superspreader event that led to seven COVID-19 deaths. A Columbia Univ study projects that the New York City shutdown reduced the spread of COVID-19 by 70%. Here is an op-ed noting that some COVID-19 vaccine trials might fail to meet their endpoint. The Australian Prime Minister is taking a strong stand as he enforces lockdowns; perhaps a message for the US?

The New York Times covers how Europe is adjusting to COVID-19. This op-ed suggests pandemic precautions will need to be taken well into 2021.

STAT cover the firs announcement of results from Lilly's mAb trial. Patient size was 450 and there were some strange dose related findings. The trial measured reduction in SARS-CoV-2 levels.

The Lancet have a research article on the association of Type 1 and Type 2 diabetes with COVID-19 related mortality in England. The results of this nationwide analysis in England show that type 1 and type 2 diabetes were both independently associated with a significant increased odds of in-hospital death with COVID-19. Here is an accompanying editorial.

Here is a Medscape interview with Scripps Research leader Eric Topol that covers a number of good issues on data misuse. Restaurants may be a key component to COVID-19 spread as you cannot wear a mask while eating. We really don't need this! A new swine flu variant has appeared in China.

Annals of Internal Medicine have a useful opinion piece on developing guidelines before, during and after the COVID-19 pandemic.

Interesting drug discovery papers today.

MODELING

- The unprecedented widespread closing of buildings due to the COVID-19 pandemic has allowed water to stagnate in premise plumbing systems, creating conditions that may facilitate the growth of opportunistic pathogens. In this study, we flushed and collected samples from showers in buildings that had been unoccupied for approximately two months and quantified *Legionella pneumophila* using a commercial cultivation-based assay. In addition, all bacteria, *Legionella* spp., *L. pneumophila*, *L. pneumophila* serogroup 1, non-tuberculous mycobacteria (NTM), and *Mycobacterium avium* complex (MAC) were analyzed using quantitative PCR (qPCR). Despite low or negligible total chlorine in the stagnant pre-flush water samples, *L. pneumophila* were not detected by either method; *Legionella* spp., NTM, and MAC, however, were widespread. Using quantitative microbial risk assessment (QMRA), estimated risks of clinical illness from exposure to legionella and MAC via showering were generally low, but the risk of subclinical infection via *Legionella* spp. could exceed a 10^{-7} daily risk threshold if just a small fraction (≥0.1 %) of those legionellae detected by qPCR are highly infectious. Flushing cold and hot water lines rapidly restored a total chlorine (as chloramine) residual and decreased all bacterial gene targets to building inlet water levels within 30 min. Following flushing, the chlorine residual rapidly dissipated and bacterial gene targets rebounded, approaching pre-flush concentrations after 6 to 7 days of stagnation. These results suggest that stagnant water in premise plumbing may contain elevated levels of opportunistic pathogens; flushing, however, can rapidly improve water quality and reduce the health risk but the improvement will be short-lived if building disuse persists. [**note: this is an important issue with building having been shuttered for a considerable period of time. We don't need an outbreak of Legionnaire's Disease, that's for sure.}** https://www.medrxiv.org/content/10.1101/2020.09.14.20194407v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check for tomorrow's newsletter.

CLINICAL TRIAL RESULTS

- Mentioned the Lilly mAb trial above.

DRUG DEVELOPMENT

- Robust humoral anti-viral immune responses have the potential to generate a diverse set of neutralizing antibodies to eliminate viruses and protect against re-infection, transmission, and the evolution of mutations that escape targeted therapeutics. CD73 is present on the majority of human B cells and a subset of T cells where it plays a role in lymphocyte activation and migration. CD73 also functions as an ectoenzyme that converts AMP into adenosine, which can be immunosuppressive. Here we report on CPI-006, a humanized FcγR binding-deficient IgG1 anti-CD73 antibody that blocks CD73 enzymatic activity and directly activates CD73+ B cells, inducing differentiation into plasmablasts, immunoglobulin class switching, and antibody secretion independent of adenosine. Immunophenotypic analysis of peripheral blood from advanced cancer patients receiving CPI-006 revealed evidence of B cell activation, clonal expansion, and development of memory B cells. These immune effects suggested that CPI-006 may be effective at enhancing the magnitude, diversity, and duration of humoral and cellular responses to viruses such as SARS-CoV-2. We have therefore initiated a Phase 1, single-dose, dose-escalation trial in hospitalized patients with mild to moderate COVID-19. The objectives of

this trial are to evaluate the safety of CPI-006 in COVID-19 patients and to determine effects of CPI-006 on anti-SARS-CoV-2 antibody responses and the development of memory B cell and T cells. Ten patients have been enrolled in the trial receiving doses of 0.3 mg/kg or 1.0 mg/kg. All evaluable patients had low pre-treatment serum levels of anti-viral antibodies to the SARS-CoV-2 trimeric spike protein and its receptor binding domain, independent of the duration of their COVID-19 related symptoms prior to enrollment. Anti-viral antibody responses were induced 7 days after CPI-006 treatment and titers continued to rise past Day 56. Increases in the frequency of memory B cells and effector/memory T cells were observed 28 days after treatment. These preliminary results suggest that CPI-006 activates B cells and may enhance and prolong anti-SARS-CoV-2 antibody responses in patients with COVID-19. This approach may be useful for treating COVID-19 or as an adjuvant to enhance the efficacy of vaccines. [**note: from Corvus Pharmaceuticals, an immunotherapy approach to COVID-19 using a B Cell activating anti-CD73 antibody. This is interesting stuff!**]
https://www.medrxiv.org/content/10.1101/2020.09.10.20191486v1

- The recently discovered instrumental role of CK2 in the SARS-Cov2 infection has pointed out this protein kinase as a promising therapeutic target in Covid-19 disease. Accordingly, anti-SARS-Cov2 activity has been reported by CK2 inhibitors in vitro, however any anti-CK2 clinical approach has been assessed in Covid-19 patients so far. Here, we investigated the putative clinical benefit of CIGB-325, an anti-CK2 peptide previously used in cancer patients, which was added to the standard-of-care to treat Covid-19. Methods: A monocentric, randomized standard-of-care controlled trial of intravenous CIGB-325 in adults hospitalized with Covid-19. Twenty patients were randomly assigned to receive CIGB-300 (2.5 mg/kg/day during 5-consecutive days) plus standard-of-care (10 patients) or standard-of-care (10 patients). Primary outcomes were time to viral clearance defined as the time (in days) to have nasopharyngeal SARS-Cov2 negative PCR and clinical response. This trial is registered with https://rpcec.sld.cu/trials/RPCEC00000317-En (Code: IG/CIGB300I/CV/2001). Results: Most of the patients had initial positive by chest-computed tomography (CT). CIGB-325 treatment reduced both number of pulmonary lesions and lesion's extent compared to control group in seven days. Taking into account the Covid-19 chest-CT abnormalities, CIGB-325 was also superior to control as well as in terms of proportion of patients with such clinical benefit. Improvement of clinical status was experienced in 50% of patients in the CIGB-325 group and 25% in control. Accordingly, systemic levels of CPK, LDH and CRP were lowered at day 7 by treating with this anti-CK2 peptide. Both therapeutic regimens were similar respect to SARS-Cov2 clearance in nasopharynx swabs over the time. Conclusion: Our study revealed that consecutive-5 day regimen of intravenous CIGB-325 at 2.5 mg/kg quickly improved the chest-CT outcomes over standard-of-care. This is the first report describing signs of clinical benefit of an anti-CK2 approach in Covid-19. [**note: this is another clinical trial from Cuba and maybe there is something to this. However a randomized 20 patient clinical trial is way too few patients to provide much information.**]
https://www.medrxiv.org/content/10.1101/2020.09.03.20187112v1
- The main protease, Mpro, of SARSCoV2 is a key protein in the coronavirus life cycle and a major drug target. Based on crystal structures of SARSCoV2 Mpro complexed with peptidomimetic inhibitors, we recognized a structural motif shared with approved inhibitors of hepatitis C virus protease. Initial tests showed that several HCV protease inhibitors could indeed also inhibit

Mpro. Based on the identified molecular scaffolds we designed a new generation of ketoamide-based Mpro inhibitors with a preorganized backbone conformation. One of the designed inhibitors, ML1000, shows particularly high affinity towards Mpro and inhibits SARSCoV2 viral replication in human cells at sub-micromolar concentrations. Our findings identify ML1000 as a promising new scaffold for the development of anti-coronavirus drugs. [**note: good work from this Stanford group, designing a new class of protease inhibitors for possible treatment**] https://www.biorxiv.org/content/10.1101/2020.09.15.275891v1

- Many viruses including HIV, the common cold, SARS-CoV and SARS-CoV-2 use a unique mechanism known as -1 programmed ribosomal frameshifting (-1 PRF) to successfully replicate and infect cells in the human host. SARS-CoV (the coronavirus responsible for SARS) and SARS-CoV-2 possess a unique RNA structure, a three-stemmed pseudoknot, that stimulates -1 PRF. Recent experiments identified that small molecules can be introduced as antiviral agents to bind with the pseudoknot and disrupt its stimulation of -1 PRF. If successfully developed, small molecule therapy that targets -1 PRF in SARS-CoV-2 is an excellent strategy to improve patients' prognoses. Crucial to developing these successful therapies is modeling the structure of the SARS-CoV-2 -1 PRF pseudoknot. Following a structural alignment approach, we identify similarities in the -1 PRF pseudoknots of the novel coronavirus SARS-CoV-2, the original SARS-CoV, as well as a third coronavirus: MERS-CoV, the coronavirus responsible for Middle East Respiratory Syndrome (MERS). In addition, we provide a better understanding of the SARS-CoV-2 -1 PRF pseudoknot by comprehensively investigating the structural landscape using a hierarchical folding approach. Since understanding the impact of mutations is vital to long-term success of treatments that are based on predicted RNA functional structures, we provide insight on SARS-CoV-2 -1 PRF pseudoknot sequence mutations and their effect on the resulting structure and its function. [**note: from Australia, another possible target for small molecule inference with viral replication.**] https://www.biorxiv.org/content/10.1101/2020.09.15.298604v1
- We applied a novel computational approach to search among approved and clinically tested drugs from the DrugBank database. Candidates were selected based on Shannon entropy homology and predefined activity profiles of three small molecules with proven anti-SARS-CoV activity and a published data set. Antiviral activity of a predicted drug, azelastine, was tested in vitro in SARS-CoV-2 infection assays with Vero E6 monkey kidney epithelial cells and reconstituted human nasal tissue. The effect on viral replication was assessed by quantification of viral genomes by droplet digital PCR. Findings: The computational approach with four independent queries identified major drug families, most often and in overlapping fashion anti-infective, anti-inflammatory, anti-hypertensive, anti-histamine and neuroactive drugs. Azelastine, an histamine 1 receptor-blocker, was predicted in multiple screens, and based on its attractive safety profile and availability in nasal formulation, was selected for experimental testing. Azelastine significantly reduced cytopathic effect and SARS-CoV-2 infection of Vero E6 cells with an EC50 of ~6 µM both in a preventive and treatment setting. Furthermore, azelastine in a commercially available nasal spray tested at 5-fold dilution was highly potent in inhibiting viral propagation in SARS-CoV-2 infected reconstituted human nasal tissue. Interpretations: Azelastine, an anti-histamine, available in nasal sprays developed against allergic rhinitis may be considered as a topical prevention or treatment of nasal colonization with SARS-CoV-2. As such, it could be useful in reducing viral spread and prophylaxis of COVID-19. Ultimately, its potential

benefit should be proven in clinical studies. [**note: this one is from Hungary and finds that azelastine may be useful as a treatment. I was prescribed is a couple of years ago for some severe allergic rhinitis and found the nasal spray to be quite sedating.]**
https://www.biorxiv.org/content/10.1101/2020.09.15.296228v1

- There are few therapeutic options and no approved vaccines. Here we examine the properties of highly potent human monoclonal antibodies (hu-mAbs) in a mouse adapted model of SARS-CoV-2 infection (SARS-CoV-2 MA). In vitro antibody neutralization potency did not uniformly correlate with in vivo activity, and some hu-mAbs were more potent in combination in vivo. Analysis of antibody Fc regions revealed that binding to activating Fc receptors is essential for optimal protection against SARS-CoV-2 MA. The data indicate that hu-mAb protective activity is dependent on intact effector function and that in vivo testing is required to establish optimal hu-mAb combinations for COVID-19 prevention. [**note: some good work from Rockefeller on mAb treatment. It just might be that a combination therapy is optimally required.**]
https://www.biorxiv.org/content/10.1101/2020.09.15.298067v1
- Using a high-throughput screening system to investigate the interaction between spike receptor binding domain (S-RBD) and ACE2 extracellular domain, we screened 3581 FDA-approved drugs and natural small molecules and identified ceftazidime as a potent compound to inhibit S-RBD-ACE2 interaction by binding to S-RBD. In addition to significantly inhibit S-RBD binding to HPAEpiC cells, ceftazidime efficiently prevented SARS-CoV-2 pseudovirus to infect ACE2-expressing 293T cells. The inhibitory concentration (IC50) was 113.2 μM, which is far below the blood concentration (over 300 μM) of ceftazidime in patients when clinically treated with recommended dose. Notably, ceftazidime is a drug clinically used for the treatment of pneumonia with minimal side effects compared with other antiviral drugs. Thus, ceftazidime has both anti-bacterial and anti-SARS-CoV-2 effects, which should be the first-line antibiotics used for the clinical treatment of COVID-19. [**note: remember the old Wrigley's commercial for Doublemint Gum? "Double your pleasure, double your fun" Here is a potential drug that both goes after bacterial pneumonia and COVID-19. It appears to inhibit viral binding.**]
Pleashttps://www.biorxiv.org/content/10.1101/2020.09.14.295956v1
- Adoptive cell therapy with viral-specific T cells has been successfully used to treat life-threatening viral infections, supporting the application of this approach against COVID-19. We expanded SARS-CoV-2 T-cells from the peripheral blood of COVID-19-recovered donors and non-exposed controls using different culture conditions. We observed that the choice of cytokines modulates the expansion, phenotype and hierarchy of antigenic recognition by SARS-CoV-2 T-cells. Culture with IL-2/4/7 but not other cytokine-driven conditions resulted in >1000 fold expansion in SARS-CoV-2 T-cells with a retained phenotype, function and hierarchy of antigenic recognition when compared to baseline (pre-expansion) samples. Expanded CTLs were directed against structural SARS-CoV-2 proteins, including the receptor-binding domain of Spike. SARS-CoV-2 T-cells could not be efficiently expanded from the peripheral blood of non-exposed controls. Since corticosteroids are used for the management of severe COVID-19, we developed an efficient strategy to inactivate the glucocorticoid receptor gene (NR3C1) in SARS-CoV-2 CTLs using CRISPR-Cas9 gene editing. [**note: good paper from MD Anderson on creation of glucocorticoid resistant T-cells for adoptive cell therapy]**
https://www.biorxiv.org/content/10.1101/2020.09.15.298547v1

- Nothing today.

DIAGNOSTIC DEVELOPMENT

- We assessed the performances of a new ELISA microplate assay quantifying SARS-CoV-2 nucleocapsid antigen (N-antigen) in serum or plasma. Methods. The specificity of the assay, determined on 63 non-COVID patients, was 98.4% (95% confidence interval [CI], 85.3 to 100). Performances were determined on 227 serum samples from 165 patients with RT-PCR confirmed SARS-CoV-2 infection included in the French COVID and CoV-CONTACT cohorts. Findings. Sensitivity was 132/142, 93.0% (95% CI, 84.7 to 100), within the first two weeks after symptoms onset. A subset of 73 COVID-19 patients had a serum collected within 24 hours following or preceding a positive nasopharyngeal swab. Among patients with high nasopharyngeal viral loads, Ct value below 30 and 33, only 1/50 and 4/67 tested negative for N-antigenemia, respectively. Among patients with a negative nasopharyngeal RT-PCR, 8/12 presented positive N-antigenemia. The lower respiratory tract was explored for 6/8 patients, showing positive PCR in 5 cases. Interpretation. This is the first demonstration of the N-antigen antigenemia during COVID-19. Its detection presented a robust sensitivity, especially within the first 14 days after symptoms onset and high nasopharyngeal viral loads. These findings have to be confirmed with higher representation of outpatients. This approach could provide a valuable new option for COVID-19 diagnosis, only requiring a blood draw and easily scalable in all clinical laboratories. [**note: from France a new approach to testing for COVID-19 using a test for the Nucleocapsid protein.**] https://www.medrxiv.org/content/10.1101/2020.09.14.20191759v1

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2020-09-17

I received an email from a loyal reader regarding 'best' pianist. I noted that there were a lot of 'best' pianists but the reader noted that there is one best pianist, Arthur Rubenstein, and one second best, van Cliburn. I already posted on van Cliburn and today will address my reader's wish to have a Rubenstein piece or two. Here is Beethoven's Emperor Concerto from Jerusalem in 1975: https://www.youtube.com/watch?v=K25fZRwbeGM and a live recital from Warsaw in 1966 featuring the Schubert B flat sonata D. 960 and the Schumann Carnival Op. 9: https://www.youtube.com/watch?v=qLTM-Y3dXs4&t=139s Alas, I cannot find any evidence that Robenstein played the fiendish 'Warsaw Concerto' by Richard Addinsell which should have been warmly embraced by the pianist who was born in Poland. We must remedy this! Here is Bulgarian pianist Ani Aramova and the Harmony Orchestra 'Prinses Irene Huizen' - Joop Boerstoel conductor - at the 'Oude Kerk' (church) of Huizen, Netherlands on 28 May 2010: https://www.youtube.com/watch?v=P4Is4RB8se8

The Washington Posts covers the Big 10 (really 14) decision to day to have a football season this fall overturning the previous postponement. I guess the $$$s were just too much to give up to the other conferences that decided to move ahead, It also looks like the last of the Power Five conferences, the Pac-12 is also going to start up in October. COVID-19 be damned. Of course the football players will be treated differently from other students as they will be getting tested for COVID-19 on a daily basis. Money quote from Big 10 Commissioner Kevin Warren, "Our goal has always been to return to competition so all student-athletes can realize their dream of competing in the sports they love." In

addition to the daily testing, any football player testing positive will undergo comprehensive cardiac testing and must have a clearance from a cardiologist before he can return to practice. The league will also establish a cardiac registry (the only good common-sense thing about this whole proposal!). I think the term "student-athletes" needs to be changed to "athletes and incidental students." The Big 10 will be using a rapid antigen test daily. I don't know what the specificity and sensitivity of this test is. It is not only possible, but quite likely that there will be a number of false negatives and positives. WaPo sports columnists, Barry Svirluga and John Feinstein expose the hypocrisy of this decision. Enough on sports! CDC director Robert Redfield testified at a Senate hearing yesterday noting that most Americans would not receive a COVID vaccine until 2021. States may need as much as $6 billion to distribute the vaccine particularly investing in cold chain storage.

The New York Times in the COVID-19 update section discuss the release by Moderna of the company's details (unfortunately the link to the Moderna protocol is dead) on how it is running it's late stage vaccine trial. The document suggests that the first analysis of the trial data may not be conducted until late December, and that there may not be enough information then to determine whether the vaccine works. I don't want to say anything more until the protocol is available to read. SUNY Oneonta will provide the case study on how NOT to reopen a college campus! Finally, a Times sports columnist weighs in on the Big 10 decision to play football this fall.

Before leaving the Big 10 altogether, here is an update on Mitch Daniels's Purdue University. According to their COVID-19 dashboard another 177 positive tests were logged during the past week. The overall positivity rate took a slight uptick to 3.17%.

The Boston Globe has an article on the Moderna vaccine progress. CEO Stepahne Bancel indicated the company will probably know in November whether its vaccine is safe and effective and could deliver 100 million doses to the US government in early 2021.

STAT have a good piece by Luciana Borio and former CBER director Jesse Goodman on the roll out of a COVID-19 vaccine. There is a very good section here on safety monitoring.

The New England Journal of Medicine has some interesting COVID-19 items this week. The MIT group has a correspondence on their SHERLOCK one pot test for SARS-CoV-2. "Here, we describe a simple test for detection of SARS-CoV-2. The sensitivity of this test is similar to that of reverse-transcription–quantitative polymerase-chain-reaction (RT-qPCR) assays. STOP (SHERLOCK testing in one pot) is a streamlined assay that combines simplified extraction of viral RNA with isothermal amplification and CRISPR-mediated detection. This test can be performed at a single temperature in less than an hour and with minimal equipment." There is a useful perspective on Operation Warp Speed. Unfortunately, I see no signs this is working in the therapeutics area other than mAb development. The only NMEs that I see right now are the Merck and Pfizer drugs. Even a lot of papers covering repurposed drugs there seems to be little going on clinically. I classify this as a disappointment. Avorn and Kesselheim discuss the recent pharma industry vaccine paper and Federal acceleration of a COVID-19 vaccine approval. This is an interesting read and I did not know that the Prevnar vaccine was the largest selling Pfizer product in 2019.

The Lancet have correspondence from Israeli doctors who may have observed a case of developing Parkinson's Disease in a patient who contracted SARS-CoV-2. Clearly more data on this is warranted.

Kaiser Health News discuss the lack of antigen testing in the US.

Derek Lowe on the Lilly mAb data which was a mixed bag.

MODELING

- Nothing Today

NEWLY REGISTERED CLINICAL TRIALS

- Trial of a Multi-peptide vaccine to prevent COVID-19 infection in adults. [**note: I don't know much more about this vaccine. The sponsor is the University Hospital in Tuegingen which I guess is where this was developed. It is a three part trial with 12 people in each part.**] NCT04546841
- The purpose of this research study is to look at high dose zinc versus multivitamin micronutrient supplementation to support immune health in the setting of the COVID-19 pandemic. This is a two-cohort prospective randomized study intended to test the role of Zinc versus multivitamin supplementation in supporting immune health in the setting of the COVID-19 pandemic. Individuals over 50 years old or primary health care professionals over the age of 18 who have had no evidence of prior COVID-19 infection and who have been asymptomatic for 7 days prior to enrollment will be randomized at the individual level to take either PreserVision AREDS formulation with 69.6mg/day Zinc supplementation or to receive a Centrum Adult (under 50) multivitamin supplement. [**note: I'm interested in following this Mayo Clinic trial. I have been taking AREDS-2 supplement for the past three years. This is the updated version without beta-carotene and most retina doctors suggest this one rather than the original formulation. Maybe I need to up my zinc intake though the supplement already has 80mg**] NCT04551339
- The objective of this project is to investigate the impacts of the COVID-19 pandemic on frontline healthcare workers, and determine if a virtual music therapy can improve mood and emotional state in this population. The purpose of this study is to investigate (a) the self-described impact that the COVID-19 pandemic has had on frontline healthcare workers, and (b) the use of music therapy to improve mental healththis population. Built on top of our successful neuroscience and neuroimaging research, we aim to use behavioural assessments combined with portable EEG technology to monitor how metrics of brain function and mental health change over time in frontline healthcare workers undergoing Music Therapy. [**note: this is from Simon Fraser in Vancouver**] NCT04551274

CLINICAL TRIAL RESULTS

- Mutations in desmosomal Plakophilin-2 (PKP2) are the most prevalent drivers of arrhythmogenic-cardiomyopathy (ACM) and a common cause of sudden death in young athletes. However, partner proteins that elucidate PKP2 cellular mechanism behind cardiac dysfunction in ACM are mostly unknown. Here we identify the actin-based motor proteins Myh9 and Myh10 as key PKP2 interactors and demonstrate that expression of the ACM-related PKP2 mutant R735X alters actin fiber organization and cell mechanical stiffness. We also show that SARS-CoV-2 Nsp1 protein acts similarly to this known pathogenic R735X mutant, altering the actomyosin component distribution on cardiac cells. Our data reveal that Nsp1 hijacks PKP2 into the cytoplasm and mimics the effect of delocalized R735X mutant. These results demonstrate

that cytoplasmic PKP2 drives actomyosin deregulation and structural collapse, validating a critical role of PKP2 localization in the regulation of actomyosin architecture. *The fact that Nsp1 and R735X share similar phenotypes also suggests that direct SARS-CoV-2 heart infection could induce a transient ACM-like disease in COVID-19 patients, which may contribute to right ventricle dysfunction, observed in patients with poor prognosis*. [**note: this is from Spain and may explain some of the cardiac impacts of SARS-CoV-2. This obviously warrants further research as relates to the observed cardiomyopathy in some COVID-19 patients.**]
https://www.biorxiv.org/content/10.1101/2020.09.14.296178v1

DRUG DEVELOPMENT

- Both viral and cellular proteases play a crucial role in SARS-CoV-2 replication, and inhibitors targeting proteases have already shown success at inhibiting SARS-CoV-2 in cell culture models. Here, we study proteolytic cleavage of viral and cellular proteins in two cell line models of SARS-CoV-2 replication using mass spectrometry to identify protein neo-N-termini generated through protease activity. *We identify multiple previously unknown cleavage sites in multiple viral proteins, including major antigenic proteins S and N, which are the main targets for vaccine and antibody testing efforts. We discovered significant increases in cellular cleavage events consistent with cleavage by SARS-CoV-2 main protease, and identify 14 potential high-confidence substrates of the main and papain-like proteases. We showed that siRNA depletion of these cellular proteins inhibits SARS-CoV-2 replication, and that drugs targeting two of these proteins: the tyrosine kinase SRC and the Ser/Thr kinase MYLK/MLCK, showed a dose-dependent reduction in SARS-CoV-2 titres. Overall, our study provides a powerful resource to understand proteolysis in the context of viral infection, and to inform the development of targeted strategies to inhibit SARS-CoV-2 and treat COVID-19 disease*. [**note: more information on the proteases important for SARS-CoV-2 replication.]**
https://www.biorxiv.org/content/10.1101/2020.09.16.297945v1
- The SARS-CoV-2 spike protein binds to the human angiotensin-converting enzyme 2 (ACE2) receptor via receptor binding domain (RBD) to enter into the cell. Inhibiting this interaction is a main approach to block SARS-CoV-2 infection and it is required to have high affinity to RBD independently of viral mutation for effective protection. To this end, we engineered ACE2 to enhance the affinity with directed evolution in human cells. Three cycles of random mutation and cell sorting achieved more than 100-fold higher affinity to RBD than wild-type ACE2. The extracellular domain of modified ACE2 fused to the Fc region of the human immunoglobulin IgG1 had stable structure and neutralized SARS-CoV-2 pseudotyped lentivirus and authentic virus with more than 100-fold lower concentration than wild-type. Engineering ACE2 decoy receptors with directed evolution is a promising approach to develop a SARS-CoV-2 neutralizing drug that has affinity comparable to monoclonal antibodies yet displaying resistance to escape mutations of virus. [**note: these Japanese research create a high affinity modified ACE2 receptor that can act like a decoy and bind SARS-CoV-2**]
https://www.biorxiv.org/content/10.1101/2020.09.16.299891v1
- Lopinavir was identified as an inhibitor of the HIV protease, and a lopinavir-ritonavir combination therapy was reported to be beneficial for the treatment of SARS and MERS. However, recent clinical tests could not prove that lopinavir-ritonavir therapy was an effective treatment for COVID-19. In this report, we examined the effect of lopinavir and ritonavir to the

activity of the purified main protease (Mpro) protein of SARS-CoV-2, the causative virus of COVID-19. Unexpectedly, lopinavir and ritonavir did not inhibit Mpro activity. These results will aid the drug candidate selection for ongoing and future COVID-19 clinical trials. [**note: this study looks at the purified Mpro enzyme and shows no inhibition. There have been other negative studies as well. There are some ongoing clinical trials that should provide conclusive evidence if they are ever completed.**]
https://www.biorxiv.org/content/10.1101/2020.09.16.299800v1

- Encouraging progress has been made in developing antivirals targeting SARS-CoV-2, the etiological agent of COVID-19. Among the drug targets being investigated, the viral main protease (Mpro) is one of the most extensively studied drug targets. Mpro is a cysteine protease that hydrolyzes the viral polyprotein at more than 11 sites and it is highly conserved among coronaviruses. In addition, Mpro has a unique substrate preference for glutamine in the P1 position. Taken together, it appears that Mpro inhibitors can achieve both broad-spectrum antiviral activity and a high selectivity index. Structurally diverse compounds have been reported as Mpro inhibitors, with several of which also showed antiviral activity in cell culture. In this study, we investigated the mechanism of action of six previously reported Mpro inhibitors, ebselen, disulfiram, tideglusib, carmofur, shikonin, and PX-12 using a consortium of techniques including FRET-based enzymatic assay, thermal shift assay, native mass spectrometry, cellular antiviral assays, and molecular dynamics simulations. Collectively, the results showed that the inhibition of Mpro by these six compounds is non-specific and the inhibition is abolished or greatly reduced with the addition of reducing reagent DTT. In the absence of DTT, these six compounds not only inhibit Mpro, but also a panel of viral cysteine proteases including SARS-CoV-2 papain-like protease, the 2Apro and 3Cpro from enterovirus A71 (EV-A71) and EV-D68. However, none of the compounds inhibits the viral replication of EV-A71 or EV-D68, suggesting that the enzymatic inhibition potency IC50 values obtained in the absence of DTT cannot be used to faithfully predict their cellular antiviral activity. Overall, we provide compelling evidence suggesting that ebselen, disulfiram, tideglusib, carmofur, shikonin, and PX-12 are non-specific SARS-CoV-2 Mpro inhibitors, and urge the scientific community to be stringent with hit validation. [**note: more on putative Mpro inhibitors from a Univ of Arizona group pointing to the need for demonstration of specific inhibition of the enzyme.**]
https://www.biorxiv.org/content/10.1101/2020.09.15.299164v1
- Prior reports used cell lines to demonstrate the antiviral potential of nafamostat against coronaviral infections and determined its mechanism of action through inhibition of transmembrane protease serine 2 (TMPRSS2). We selected a biologically relevant pre-clinical experimental model of SARS-CoV-2 lung infection using a 3D human reconstituted airway epithelial model of nasal origin to characterize the effects of nafamostat on tissue-level cellular ultrastructure and viral infection kinetics. Our results confirm the not only the relevance of this model for the preclinical evaluation of safety and efficacy of antiviral candidates, but also the highly potent nature of nafamostat SARS-CoV-2 antiviral activity. The studies described herein provided evidence demonstrating the therapeutic potential of nafamostat against COVID-19, as well as its safety upon exposure to lung airway cellular. [**note: here is a model study showing that nafamostat may be active against COVID-19. There are ongoing clinical trials of this compound**] https://www.biorxiv.org/content/10.1101/2020.09.16.300483v1

- Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The initial interaction between Transmembrane Serine Protease 2 (TMPRSS2) primed SARS-CoV-2 spike (S) protein and host cell receptor angiotensin-converting enzyme 2 (ACE-2) is a pre-requisite step for this novel coronavirus pathogenesis. Here, we expressed a GFP-tagged SARS-CoV-2 S-Ectodomain in Tni insect cells. That contained sialic acid-enriched N- and O-glycans. Surface resonance plasmon (SPR) and Luminex assay showed that the purified S-Ectodomain binding to human ACE-2 and immunoreactivity with COVID-19 positive samples. We demonstrate that bromelain (isolated from pineapple stem and used as a dietary supplement) treatment diminishes the expression of ACE-2 and TMPRSS2 in VeroE6 cells and dramatically lowers the expression of S-Ectodomain. Importantly, bromelain treatment reduced the interaction between S-Ectodomain and VeroE6 cells. Most importantly, bromelain treatment significantly diminished the SARS-CoV-2 infection in VeroE6 cells. Altogether, our results suggest that bromelain or bromelain rich pineapple stem may be used as an antiviral against COVID-19. [**note: this is the second report on this. As I noted earlier, you need to have a way of getting this enzyme into the body so it can work.**]
  https://www.biorxiv.org/content/10.1101/2020.09.16.297366v1
- Baricitinib, a clinically approved JAK1/2 inhibitor with potent anti-inflammatory properties is currently being investigated in COVID-19 human clinical trials. Recent reports suggest that baricitinib may also have antiviral activity in limiting viral endocytosis. Here, we investigated the immunologic and virologic efficacy of baricitinib in a rhesus macaque model of SARS-CoV-2 infection. Viral shedding measured from nasal and throat swabs, bronchoalveolar lavages and tissues was not reduced with baricitinib. Type I IFN antiviral responses and SARS-CoV-2 specific T cell responses remained similar between the two groups. Importantly, however, animals treated with baricitinib showed reduced immune activation, decreased infiltration of neutrophils into the lung, reduced NETosis activity, and more limited lung pathology. Moreover, baricitinib treated animals had a rapid and remarkably potent suppression of alveolar macrophage derived production of cytokines and chemokines responsible for inflammation and neutrophil recruitment. These data support a beneficial role for, and elucidate the immunological mechanisms underlying, the use of baricitinib as a frontline treatment for severe inflammation induced by SARS-CoV-2 infection. {**note: here is animal data on baricitinib showing it may be useful in treating SARS-CoV-2 induced inflammation. The compound is in clinical trials.**]
  https://www.biorxiv.org/content/10.1101/2020.09.16.300277v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing Today

DIAGNOSTIC DEVELOPMENT

- Nothing Today

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2020-09-18

I love the NPR Tiny Desk concerts. Most of the time they are a tiny desk concert but not in the case of Chick Corea and Gary Burton. NPR had a Yamaha Grand piano hauled up to the fourth floor and fitted in the rented vibes. Enjoy this great jazz duo: https://www.youtube.com/watch?v=15IHNYq6stw

NOTE: there will not be a newsletter tomorrow as I will be observing the Jewish New Year. I wish my readers of all faiths a happy, healthy, and prosperous time going forth. We will get through this!

The Washington Post reports that health care workers make up one in seven globally recorded COVID-19 cases. Both Pfizer and Moderna have now released information on their vaccine trials. SARS-CoV-2 does not believe in God. Apparently, some of us are not in this fight together; these parents sent their COVID-19 infected student to school. Here is how you can safety enjoy all your favorite fall activities!

The New York Times discusses possible vaccine availability. More on the CDC testing guidelines flap. Maybe wearing glasses is a good idea.

STAT discusses what went right vs wrong with respect to COVID-19 communications. Three lessons on COVID-19 accelerating biopharma innovation.

The Lancet has an article on a novel point of care test for SARS-CoV-2. The CovidNudge platform was a sensitive, specific, and rapid point of care test for the presence of SARS-CoV-2 without laboratory handling or sample pre-processing. The device, which has been implemented in UK hospitals since May, 2020, could enable rapid decisions for clinical care and testing programmes. These French researchers looked at hospitalizations for acute myocardial infarctions during the pandemic lockdown. A marked decrease in hospital admissions was observed following the lockdown, irrespective of patient characteristics and regional prevalence of COVID-19. Health authorities should be aware of these findings, in order to adapt their message if the COVID-19 pandemic persists or recurs, or in case of future major epidemics. Lancet editors learn a valuable lesson from the retraction of a large HCQ study. Here is a nice history of Herd Immunity (I'm still looking for the history of Herd Mentality!)

JAMA have a quick perspective of COVID-19 and blood type association. The Science of Persuasion and lessons for COVID-19 prevention; this is a nice interview. Here is a deserved critique of the US response to the COVID-19 pandemic by Johns Hopkins investigators. More on eyeglass wearing and potential protection against COVID-19 (I need to order a pair of everyday non-prescription eyeglasses for added protection when I am out and about!) and here is an invited commentary on this noting that correlation is not always causation. Does Contact Tracing work? Yes, sometimes it does and the National Academies of Sciences, Engineering and Medicine outline practices that will help. Here is a short research letter on cardiovascular magnetic resonance imaging of competitive athletes who recovered from COVID-19 symptoms. The sample size is small but there are a number of college football programs that have experienced viral outbreaks. These players should be studied.

MODELING

- Nothing

NEWLY REGISTERED CLINICAL TRIALS

- Did not check today.

CLINICAL TRIAL RESULTS

- Risk stratification of COVID-19 patients upon hospital admission is key for their successful treatment and efficient utilization of hospital resources. Objective: To evaluate the risk factors associated with ventilation need and mortality. Design, setting and participants: We established a retrospective cohort of COVID-19 patients from Mass General Brigham hospitals. Demographic, clinical, and admission laboratory data were obtained from electronic medical records of patients admitted to hospital with laboratory-confirmed COVID-19 before May 19th, 2020. Using patients admitted to Massachusetts General Hospital (MGH, derivation cohort), multivariable logistic regression analyses were used to construct the Ventilation in COVID Estimator (VICE) and Death in COVID Estimator (DICE) risk scores. Measurements: The primary outcomes were ventilation status and death. Results: The entire cohort included 1042 patients (median age, 64 years; 56.8% male). The derivation and validation cohorts for the risk scores included 578 and 464 patients, respectively. We found seven factors to be independently predictive for ventilation requirement (diabetes mellitus, dyspnea, alanine aminotransferase, troponin, C-reactive protein, neutrophil-lymphocyte ratio, and lactate dehydrogenase), and 10 factors to be predictors of in-hospital mortality (age, sex, diabetes mellitus, chronic statin use, albumin, C-reactive protein, neutrophil-lymphocyte ratio, mean corpuscular volume, platelet count, and procalcitonin). Using these factors, we constructed the VICE and DICE risk scores, which performed with C-statistics of at least 0.8 in our cohorts. *Importantly, the chronic use of a statin was associated with protection against death due to COVID-19*. The VICE and DICE score calculators have been placed on an interactive website freely available to the public (https://covid-calculator.com/). Limitations: One potential limitation is the modest sample sizes in both our derivation and validation cohorts. Conclusion: The risk scores developed in this study may help clinicians more appropriately determine which COVID-19 patients will need to be managed with greater intensity. [**note: here is a useful paper on risk stratification from the Mass General Brigham hospitals. Note the finding about statins!!!!!**
https://www.medrxiv.org/content/10.1101/2020.09.14.20194670v1
- The pathophysiology of the disease relies on aberrant activation of immune system and lymphopenia that has been recognized as a prognosis marker. We wondered if the myeloid compartment was affected in Covid-19 and if monocytes and macrophages could be infected by SARS-CoV-2. We show here that SARS-CoV-2 efficiently infects monocytes and macrophages without any cytopathic effect. Infection was associated with the secretion of immunoregulatory cytokines (IL-6, IL-10, TGF-b) and the induction of a macrophagic specific transcriptional program characterized by the upregulation of M2-type molecules. In addition, we found that in vitro macrophage polarization did not account for the permissivity to SARS-CoV-2, since M1- and M2-type macrophages were similarly infected. Finally, in a cohort of 76 Covid-19 patients ranging from mild to severe clinical expression, all circulating monocyte subsets were decreased, likely related to massive emigration into tissues. Monocytes from Covid-19 patients exhibited decreased expression of HLA-DR and increased expression of CD163, irrespective of the clinical status. Hence, SARS-CoV-2 drives circulating monocytes and macrophages inducing immunoparalysis of the host for the benefit of Covid-19 disease progression. [**note: more on how the immune system may go out of whack as COVID-19 disease progresses.**]
https://www.biorxiv.org/content/10.1101/2020.09.17.300996v1

DRUG DEVELOPMENT

- The yeast *Pichia pastoris* is a cost-effective and easily scalable system for recombinant protein production. In this work we compared the conformation of the receptor binding domain (RBD) from SARS-CoV-2 Spike protein expressed in *P. pastoris* and in the well established HEK-293T mammalian cell system. RBD obtained from both yeast and mammalian cells was properly folded, as indicated by UV-absorption, circular dichroism and tryptophan fluorescence. They also had similar stability, as indicated by temperature-induced unfolding (observed Tm were 50 °C and 52 °C for RBD produced in *P. pastoris* and HEK-293T cells, respectively). Moreover, the stability of both variants was similarly reduced when the ionic strength was increased, in agreement with a computational analysis predicting that a set of ionic interactions may stabilize RBD structure. Further characterization by HPLC, size-exclusion chromatography and mass spectrometry revealed a higher heterogeneity of RBD expressed in *P. pastoris* relative to that produced in HEK-293T cells, which disappeared after enzymatic removal of glycans. The production of RBD in *P. pastoris* was scaled-up in a bioreactor, with yields above 45 mg/L of 90% pure protein, thus potentially allowing large scale immunizations to produce neutralizing antibodies, as well as the large scale production of serological tests for SARS-CoV-2. [**note: good work from these Argentinian researchers. This yeast strain has been used for a long time to produce recombinant proteins and the technology is well established. I recall having read another paper on this some time ago but don't know whether this is being pursued as a vaccine approach.**] https://www.biorxiv.org/content/10.1101/2020.09.17.300335v1
- A novel coronavirus, SARS-CoV-2, has been identified as the causative agent of the current COVID-19 pandemic. Animal models, and in particular non-human primates, are essential to understand the pathogenesis of emerging diseases and to the safety and efficacy of novel vaccines and therapeutics. Here, we show that SARS-CoV-2 replicates in the upper and lower respiratory tract and causes pulmonary lesions in both rhesus and cynomolgus macaques, resembling the mild clinical cases of COVID-19 in humans. Immune responses against SARS-CoV-2 were also similar in both species and equivalent to those reported in milder infections and convalescent human patients. Importantly, we have devised a new method for lung histopathology scoring that will provide a metric to enable clearer decision making for this key endpoint. In contrast to prior publications, in which rhesus are accepted to be the optimal study species, we provide convincing evidence that both macaque species authentically represent mild to moderate forms of COVID-19 observed in the majority of the human population and both species should be used to evaluate the safety and efficacy of novel and repurposed interventions against SARS-CoV-2. Accessing cynomolgus macaques will greatly alleviate the pressures on current rhesus stocks. [**note: these English researchers find that cynomolgus macaques can also serve as a good animal model for SARS-CoV-2 infection.**] https://www.biorxiv.org/content/10.1101/2020.09.17.301093v1
- Combination therapies have become a standard for the treatment for HIV and HCV infections. They are advantageous over monotherapies due to better efficacy and reduced toxicity, as well as the ability to prevent the development of resistant viral strains and to treat viral co-infections. Here, we identify several new synergistic combinations against emerging and re-emerging viral infections in vitro. We observed synergistic activity of nelfinavir with investigational drug EIDD-2801 and convalescent serum against SARS-CoV-2 infection in human lung epithelial Calu-3 cells. We also demonstrated synergistic activity of vemurafenib combination with emetine, homoharringtonine, gemcitabine, or obatoclax against echovirus 1

infection in human lung epithelial A549 cells. We also found that combinations of sofosbuvir with brequinar and niclosamide were synergistic against HCV infection in hepatocyte derived Huh-7.5 cells, whereas combinations of monensin with lamivudine and tenofovir were synergistic against HIV-1 infection in human cervical TZM-bl cells. Finally, we present an online resource that summarizes novel and known antiviral drug combinations and their developmental status. Overall, the development of combinational therapies could have a global impact improving the preparedness and protection of the general population from emerging and re-emerging viral threats. [**note: this is a useful reference for antiviral drug combinations against a number of viruses**] https://www.biorxiv.org/content/10.1101/2020.09.17.299933v1

- Soluble forms of ACE2 have recently been shown to inhibit SARS-CoV-2 infection. *We report on an improved soluble form of ACE2, termed a "microbody" in which the ACE2 ectodomain is fused to Fc domain 3 of the immunoglobulin heavy chain. The protein is smaller than previously described ACE2-Ig Fc fusion proteins and contains an H345A mutation in the catalytic active site that inactivates the enzyme without reducing its affinity for the SARS-CoV-2 spike*. The disulfide-bonded ACE2 microbody inhibited entry of SARS-CoV-2 spike protein pseudotyped virus and live SARS-CoV-2 with a potency 10-fold higher than unmodified soluble ACE2 and retained activity even after the virus had bound to the cell. The ACE2 microbody inhibited entry of ACE2-utilizing β coronaviruses and entry of viruses with the high infectivity variant D614G spike. The ACE2 microbody may be a valuable therapeutic for COVID-19 that is active against SARS-CoV-2 variants and against coronaviruses that may arise in the future. [**note: from NYU a nice approach to therapy by manipulating the ACE2 protein and fusing it to a single iG Fc domain**] https://www.biorxiv.org/content/10.1101/2020.09.16.300319v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The emergence of a novel coronavirus (SARS-CoV-2) associated with severe acute respiratory disease (COVID-19) has prompted efforts to understand the genetic basis for its unique characteristics and its jump from non-primate hosts to humans. Tests for positive selection can identify apparently nonrandom patterns of mutation accumulation within genomes, highlighting regions where molecular function may have changed during the origin of a species. Several recent studies of the SARS-CoV-2 genome have identified signals of conservation and positive selection within the gene encoding Spike protein based on the ratio of synonymous to nonsynonymous substitution. Such tests cannot, however, detect changes in the function of RNA molecules. Methods: Here we apply a test for branch-specific oversubstitution of mutations within narrow windows of the genome without reference to the genetic code. Results: We recapitulate the finding that the gene encoding Spike protein has been a target of both purifying and positive selection. In addition, we find other likely targets of positive selection within the genome of SARS-CoV-2, specifically within the genes encoding Nsp4 and Nsp16. Homology-directed modeling indicates no change in either Nsp4 or Nsp16 protein structure relative to the most recent common ancestor. Thermodynamic modeling of RNA stability and structure, however, indicates that RNA secondary structure within both genes in the SARS-CoV-2 genome differs from those of RaTG13, the reconstructed common ancestor, and Pan-CoV-GD (Guangdong). These SARS-CoV-2-specific mutations may affect molecular processes mediated by the positive or negative RNA molecules, including transcription, translation, RNA stability, and evasion of the host innate immune system. Our results highlight the importance of considering

mutations in viral genomes not only from the perspective of their impact on protein structure, but also how they may impact other molecular processes critical to the viral life cycle. [**note: more on the RNA gene structure and the possible impact of mutations.**] https://www.biorxiv.org/content/10.1101/2020.09.16.300038v1

- SARS-CoV-2, the virus responsible for the current COVID-19 pandemic, displays a corona-shaped layer of spikes which play fundamental role in the infection process. Recent structural data suggest that the spikes possess orientational freedom and the ribonucleoproteins segregate into basketlike structures. How these structural features regulate the dynamic and mechanical behavior of the native virion, however, remain unknown. By imaging and mechanically manipulating individual, native SARS-CoV-2 virions with atomic force microscopy, here we show that their surface displays a dynamic brush owing to the flexibility and rapid motion of the spikes. The virions are highly compliant and able to recover from drastic mechanical perturbations. Their global structure is remarkably temperature resistant, but the virion surface becomes progressively denuded of spikes upon thermal exposure. Thus, both the infectivity and thermal sensitivity of SARS-CoV-2 rely on the dynamics and the mechanics of the virus. [**note: this is an interesting study of thermal effects on the virus**] https://www.biorxiv.org/content/10.1101/2020.09.17.302380v1
- Understanding how human ACE2 genetic variants differ in their recognition by SARS-CoV-2 can have a major impact in leveraging ACE2 as an axis for treating and preventing COVID-19. In this work, we experimentally interrogate thousands of ACE2 mutants to identify over one hundred human single-nucleotide variants (SNVs) that are likely to have altered recognition by the virus, and make the complementary discovery that ACE2 residues distant from the spike interface can have a strong influence upon the ACE2-spike interaction. These findings illuminate new links between ACE2 sequence and spike recognition, and will find wide-ranging utility in SARS-CoV-2 fundamental research, epidemiological analyses, and clinical trial design. [**note: this paper covers the discovery of ACE2 mutants that are likely to have altered recognition by the virus.**] https://www.biorxiv.org/content/10.1101/2020.09.17.301861v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-09-20

For reflection Sunday (and there is an awful lot to reflect on today) there is no composer to turn to better than Johan Sebastian Bach. Yes, he wrote for the St. Thomas Church in Leipzig for most of his composing career, but the music transcends the religious to the spiritual that can force all of us to meditate on creative glory. There is no 'best' Bach piece as something original can be found in every composition (I well remember laboring over Bach chorales when I took music theory). The Mass in B Minor was one of his final compositions. Here is John Elliot Gardiner, the English Baroque Soloists and the Monteverdi Choir: https://www.youtube.com/watch?v=CT6vRpmyiW0 Nothing more need be said, just listen and think!

According to The Washington Post, the popcorn industry is undergoing a bit of an upheaval. The virus doesn't care what country it is in as Spain, France and Israel are all seeing spikes in infection. The WaPo

also offers us a COVID-19 etiquette guide. China and Russia are pushing ahead with COVID-19 vaccine development. These WaPo writers reflect on the deaths from COVID-19 as the toll approaches 200K; this is a sobering story. The elderly are often able to handle the pandemic well (prime example – writing a daily newsletter!). This doc discusses COVID-19 'long haulers' from the perspective of one who contracted and recovered from Ebola! Here is a salute to the college newspapers who are documenting their campus's battle with COVID-19. The CDC believes that 11,000 have been exposed on airline flights.

The New York Times discusses the politics of FDA and HHS senior management. Nothing good will come of this. The logistics of shipping a COVID-19 vaccine at -80C (brrrrrrrrrrrrrrrrrrrr, that is really cold) are complex. Let us look at the pandemic from the view of SARS-CoV-2! This op-ed looks at what is in store for the pandemic fall and winter. This San Diego area school district is making a brave attempt to hold in class instruction. Let us hope they succeed and provide a road map for others. Here is a very good piece on how to control the spread of SARS-CoV-2; build a better fence.

The British Medical Journal has a provocative editorial on pre-existing immunity to SARS-CoV-2. There are a number of small studies that have found antibody response to SARS-CoV-2 in sera samples but there also have been demonstrations that these are not strongly neutralizing. Still this is an interesting area for further research. Drew Altman of the Kaiser Family Foundation discusses the US failure on coronavirus.

The Atlantic's medical writer, James Hamblin, notes that it could be tough winter with SARS-CoV-2 still around. However, there is some good advice here so give it a read!

The New Yorker has a good article on research into protein folding. You can participate by letting your home computer run software when you are not using it. Be part of the solution!!!!

Perhaps it is time again to consider traveling by train.

MODELING

- Understanding transmission and impact of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in school children is critical to implement appropriate mitigation measures. Objective: To determine the variation in SARS-CoV-2 seroprevalence in school children across districts, schools, grades, and classes, and the relationship of SARS-CoV-2 seroprevalence with self-reported symptoms. Design: Cross-sectional analysis of baseline measurements of a longitudinal cohort study (Ciao Corona) from June-July 2020. Setting: 55 randomly selected schools and classes stratified by district in the canton of Zurich, Switzerland (1.5 million inhabitants). Participants: Children, aged 6-16 years old, attending grades 1-2, 4-5 and 7-8. Exposure: Exposure to circulating SARS-CoV-2 between February and June 2020 including public lock-down and school closure (March 16-May 10, 2020). Main Outcomes and Measures: Variation in seroprevalence of SARS-CoV-2 in children across 12 cantonal districts, schools, and grades using a Luminex-based antibody test with four targets for each of IgG, IgA and IgM. Clustering of cases within classes. Analysis of associations of seropositivity and symptoms. Comparison of seroprevalence with a randomly selected adult population, based on Luminex-based IgG and IgA antibody test of Corona Immunitas. Results: In total, 55 schools and 2585 children were recruited (1337 girls, median age 11, age range 6-16 years). Overall seroprevalence was 2.8 % (95% CI 1.6-4.1%), ranging from 1.0% to 4.5% across districts.

Seroprevalence was 3.8% (1.9-6.1%) in grades 1-2, 2.5% (1.1-4.2%) in grades 4-5, and 1.5% (0.5-3.0%) in grades 7-8. At least one case was present in 36/55 tested schools and in 43/128 classes with ≥50% participation rate and ≥5 children tested. 73% of children reported COVID-19 compatible symptoms since January 2020, but none were reported more frequently in seropositive compared to seronegative children. Seroprevalence of children was very similar to seroprevalence of randomly selected adults in the same region in June-July 2020, measured with the same Corona Immunitas test, combining IgG and IgA (3.1%, 95% CI 1.4-5.4%, versus 3.3%, 95% CI 1.4-5.5%). Conclusions and Relevance*: Seroprevalence was inversely related to age and revealed a dark figure of around 90 when compared to 0.03% confirmed PCR+ cases in children in the same area by end of June. We did not find clustering of SARS-CoV-2 seropositive cases in schools so far, but the follow-up of this school-based study will shed more light on transmission within and outside schools*. [**note: this is a large school age serological study in Zurich.**] https://www.medrxiv.org/content/10.1101/2020.09.18.20191254v1

- Georgia timely implemented effective response measures, with testing, contact tracing and isolation being the main pillar of the national response, achieving the lowest cumulative incidence of SARS-CoV-2 in the European region. Methods: We conducted a survey to estimate SARS-CoV-2 IgG antibody seroprevalence among adult residents of capital city of Tbilisi (adult population: 859,328). Participants were recruited through respondent driven sampling during May 18-27, 2020. Blood specimens were tested for SARS-CoV-2 IgG antibodies using commercially available lateral flow immunoassay (COVID-19 IgG/IgM Rapid Test Cassette, Zhejiang Orient Gene Biotech). Crude seroprevalence was weighted by population characteristics (age, sex, district of Tbilisi) and further adjusted for test accuracy. Results: Among 1,068 adults recruited 963 (90.2%) were between 18 and 64 years-old, 682 (63.9%) women. 176 (16.5%) reported symptoms indicative of SARS-CoV-2 infection occurring in previous three months. Nine persons tested positive for IgG: crude seroprevalence: 0.84%, (95% CI: 0.33%-1.59%), weighted seroprevalence: 0.94% (95% CI: 0.37%-1.95%), weighted and adjusted for test accuracy: 1.02% (95% CI: 0.38%-2.18%). *The seroprevalence estimates translate into 7,200 to 8,800 infections among adult residents of Tbilisi, which is at least 20 times higher than the number of confirmed cases. Conclusions: Low seroprevalence confirms that Georgia successfully contained spread of SARS-CoV-2 during the first wave of pandemic. Findings also suggest that undocumented cases due to asymptomatic or very mild disease account for majority of infections. Given that asymptomatic persons can potentially spread the virus, test and isolate approach should be further expanded to control the epidemic*. [**note: here is a serological study of Tblisi in Geogia (the country not the state** 😉]
  https://www.medrxiv.org/content/10.1101/2020.09.18.20195024v1
- Background To identify blood donors eligible to donate Coronavirus Disease-2019 (COVID-19) Convalescent Plasma (CCP), a large blood center began testing for antibodies to SARS-CoV-2, the etiologic agent of COVID-19. We report the seroprevalence of total immunoglobulin directed against the S1 spike protein of SARS-CoV-2 in US blood donors. Methods Unique non-CCP donor sera from June 1-July 31, 2020 were tested with the Ortho VITROS Anti-SARS-CoV-2 total immunoglobulin assay (positive: signal-to-cutoff (S/C) =>1). Donor age, sex, race/ethnicity, ABO/RhD, education, and experience were compared to June and July 2019. Multivariate regressions were conducted to identify demographics associated with the presence of antibodies and with S/C values. Results Unique donors (n=252,882) showed an overall

seroprevalence of 1.83% in June (1.37%) and July (2.26%), with the highest prevalence in northern New Jersey (7.3%). In a subset of donors with demographic information (n=189,565), higher odds of antibody reactivity were associated with non-Hispanic Native American/Alaskan (NH-NAA/A) and Black (NH-B), and Hispanic (H) race/ethnicity, age 18-64, middle school or lesser education, blood Group A, and never or non-recent donor status. In positive donors (n=2,831), antibody signal was associated with male sex, race/ethnicity (NH-NAA/A, NH-B and H) and geographic location. Conclusions *Seroprevalence remains low in US blood donors but varies significantly by region. Temporal trends in reactivity may be used to gauge the effectiveness of public health measures. Before generalizing these data from healthy donors to the general population however, rates must be corrected for false positive test results among low prevalence test subjects and adjusted to match the wider demography*. [**note: this is a large seroprevalence study of US blood donors. It remains low in US blood donors and as the authors note needs to be corrected.**] https://www.medrxiv.org/content/10.1101/2020.09.17.20195131v1

- Los Angeles (LA) County has sustained a large outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To learn about the transmission history of SARS-CoV-2 in LA County, we sequenced 142 viral genomes from unique patients seeking care at UCLA Health System. 86 of these genomes are from samples collected before April 19, 2020. We found that the early outbreak in LA, as in other international air travel hubs, was seeded by multiple introductions of strains from Asia and Europe. We identified a US-specific strain, B.1.43, which has been found predominantly in California and Washington State. While samples from LA County carry the ancestral B.1.43 genome, viral genomes from neighbouring counties in California and from counties in Washington State carry additional mutations, suggesting a potential origin of B.1.43 in Southern California. We quantified the transmission rate of SARS-CoV-2 over time, and found evidence that the public health measures put in place in LA County to control the virus were effective at preventing transmission, but may have been undermined by the many introductions of SARS-CoV-2 into the region. Our work demonstrates that genome sequencing can be a powerful tool for investigating outbreaks and informing the public health response. Our results reinforce the critical need for the U.S. to have coordinated inter-state responses to the pandemic. [**note: from UCLA a phylogenetic analysis of the Los Angeles outbreak.**] https://www.medrxiv.org/content/10.1101/2020.09.15.20194712v1

NEWLY REGISTERED CLINICAL TRIALS

- Up to 1/3 of all patients infected with COVID-19 can develop complications that require hospitalization. Severe pneumonia associated with acute respiratory distress syndrome (ARDS) is the most threatening and feared complication of COVID-19 infection, with mortality rates close to 50% in some groups. Autopsies between these severe cases reveal severe capillary involvement, with signs of intense inflammatory changes, microvascular thrombosis, endothelial injury and abnormal tissue repair. The available evidence suggests that abnormal activation or imbalance in the counter-regulation of the kallikrein-kinin system may play a central role in a positive feedback cycle, leading to consequent diffuse microangiopathy. Blockade of the kallikrein-kinin system can therefore prevent deterioration of lung function by reducing inflammation, edema and microthrombosis. The objective of this phase IIb study is to assess the preliminary effects on the oxygenation parameters of an antisense oligonucleotide that inhibits pre-kallikrein synthesis in patients with moderate to severe COVID-19. [**note: this is an anti-**

sense oligonucleotide from Ionis Pharmaceuticals who have developed and received approval for an anti-sense drug some years ago.] NCT04549922

- Since ARDS is a major complication of COVID - 19 with subsequent formation of non-cardiogenic pulmonary edema , worsening the oxygenation of the patients and foamy and even bloody sputum formation, so the idea is to use alcohol inhalation as it reduce surface tension on the alveoli and markedly decrease sputum formation with improvement on oxygenation beside its cytolethal effect on virus lipid bilayer. A lot of researches and publications proved the role of alcohol inhalation in treatment of pulmonary edema. Alcohol inhalation may has inflammatory effect and dangerous effect on patients but this can be controlled by the actual concentration used and the way we use it according to general condition of the patient and with the help of anti - inflammatory action of Asprin . [**note: this trial comes from Mansora Univ in Egypt. I don't think this is a DIY treatment despite the ingredients being generally available.]** NCT04554433
- Omega-3, Nigella Sativa, Indian Costus, Quinine, Anise Seed, Deglycyrrhizinated Licorice, Artemisinin, Febrifugine on Immunity of Patients With (COVID-19) [**note: this one is from Saudi Arabia. I don't know how they arrived at this combination of compounds.**] NCT04553705
- The primary purpose of this study is to evaluate if adding rhC1-INH to standard of care (SOC) in patients admitted for stage II COVID-19 infection may reduce the risk of disease progression, i.e. ALI requiring mechanical ventilation, or increase the chance of a faster clinical improvement compared to SOC alone. [**note: sponsor is Pharming Technologies and information on this drug is at the link. Seems like a long shot to me.**] NCT04530136

CLINICAL TRIAL RESULTS

- Coronavirus disease 2019 (COVID-19) is driven by dysregulated immune responses yet the role of immunometabolism in COVID-19 pathogenesis remains unclear. By investigating 47 patients with confirmed SARS-CoV-2 infection and 16 uninfected controls, we found an immunometabolic dysregulation specific for patients with progressed disease that was reversible in the recovery phase. Specifically, T cells and monocytes exhibited increased mitochondrial mass, accumulated intracellular ROS and these changes were accompanied by disrupted mitochondrial architecture. Basigin (CD147), but not established markers of T cell activation, was up-regulated on T cells from progressed COVID-19 patients and correlated with ROS accumulation, reflected in the transcriptome. *During recovery, basigin and ROS decreased to match the uninfected controls. In vitro analyses confirmed the correlation and showed a down-regulation of ROS by dexamethasone treatment. Our findings provide evidence of a basigin-related and reversible immunometabolic dysregulation in COVID-19*. [**note: from Germany more clinical information on immune system recovery.]** https://www.medrxiv.org/content/10.1101/2020.09.18.20194175v2
- SARS-CoV-2 infection induces a wide spectrum of neurologic dysfunction. Here we show that a particularly vulnerable population with neurologic manifestations of COVID-19 harbor an influx of inflammatory cytokines within the cerebrospinal fluid in the absence of viral neuro-invasion. The majority of these inflammatory mediators are driven by type 2 interferon and are known to induce neuronal injury in other disease models. Levels of matrix metalloproteinase-10 within the spinal fluid correlate with the degree of neurologic dysfunction. Furthermore, this neuroinflammatory process persists weeks following convalescence from the acute respiratory infection. These prolonged neurologic sequelae following a systemic cytokine release syndrome

lead to long-term neurocognitive dysfunction with a wide range of phenotypes. [**note: this is from Sloan Kettering and looks at 18 cancer patients with confirmed SARS-CoV-2 respiratory infection who developed moderate to severe neurologic symptoms. I don't know if anyone has looked at the overall frequency of this in all hospitalized patients to find out how frequent it is.**] https://www.medrxiv.org/content/10.1101/2020.09.15.20195511v1

- **Background**: Convalescent plasma (CP), despite limited evidence on its efficacy, is being widely used as a compassionate therapy for hospitalized patients with COVID-19. We aimed to evaluate the efficacy and safety of early CP therapy in COVID-19 progression. **Methods**: Open-label, single-center, randomized clinical trial performed in an academic center in Santiago, Chile from May 10, 2020, to July 18, 2020, with final follow-up August 17, 2020. The trial included patients hospitalized within the first 7 days of COVID-19 symptoms onset, presenting risk factors for illness progression and not on mechanical ventilation. The intervention consisted in immediate CP (early plasma group) versus no CP unless developing pre-specified criteria of deterioration (deferred plasma group). Additional standard treatment was allowed in both arms. The primary outcome was a composite of mechanical ventilation, hospitalization for >14 days or death. Key secondary outcomes included: time to respiratory failure, days of mechanical ventilation, hospital length-of-stay, mortality at 30 days, and SARS-CoV-2 RT-PCR clearance rate. **Results**: Of 58 randomized patients (mean age, 65.8 years, 50% male), 57 (98.3%) completed the trial. A total of 13 (43.3%) participants from the deferred group received plasma based on clinical aggravation. We found no benefit in the primary outcome (32.1% vs 33.3%, OR 0.95, 95% CI 0.32-2.84, p>0.99) in the early versus deferred CP group. In-hospital mortality rate was 17.9% vs 6.7% (OR 3.04, 95% CI 0.54-17.2, p=0.25), mechanical ventilation 17.9% vs 6.7% (OR 3.04, 95% CI 0.54-17.2, p=0.25), and prolonged hospitalization 21.4% vs 30% (OR 0.64, 95%CI, 0.19-2.1, p=0.55) in early versus deferred CP group, respectively. Viral clearance rate on day 3 (26% vs 8%, p=0.20) and day 7 (38% vs 19%, p=0.37) did not differ between groups. Two patients experienced serious adverse events within 6 or less hours after plasma transfusion. **Conclusion**: *Immediate addition of CP therapy in early stages of COVID-19 -compared to its use only in case of patient deterioration- did not confer benefits in mortality, length of hospitalization or mechanical ventilation requirement*. [**note: from Chile a study of the use of convalescent plasma. The numbers are small and don't show any benefit. This is why we do clinical trials.**] https://www.medrxiv.org/content/10.1101/2020.09.17.20196212v1
- Objective To assess the effectiveness of corticosteroids on outcomes of patients with mild COVID-19 pneumonia. Methods We used routine care data from 51 hospitals in France and Luxembourg to assess the effectiveness of corticosteroids at 0.8 mg/kg/day eq. prednisone (CTC group) vs standard of care (no-CTC group) among patients ≤ 80 years old with COVID-19 pneumonia requiring oxygen without mechanical ventilation. The primary outcome was intubation or death at Day 28. Baseline characteristics of patients were balanced using propensity score inverse probability of treatment weighting. Results Among the 891 patients included in the analysis, 203 were assigned to the CTC group. At day 28, corticosteroids did not reduce the rate of the primary outcome (wHR 0.92, 95% CI 0.61 to 1.39) nor the cumulative death rate (wHR 1.03, 95% CI 0.54 to 1.98). Corticosteroids significantly reduced the rate of the primary outcome for patients requiring oxygen ≥ at 3L/min (wHR 0.50, 95% CI 0.30 to 0.85) or C-Reactive Protein (CRP) ≥ 100mg/L (wHR 0.44, 95%CI 0.23 to 0.85). We found a higher number of hyperglycaemia events among patients who received corticosteroids, but number of infections

were similar across the two groups. Conclusions *We found no association between the use of corticosteroids and intubation or death in the broad population of patients ≤80 years old with COVID-19 hospitalized in non-ICU settings. However, the treatment was beneficial for patients with ≥ 3L/min oxygen or CRP ≥ 100mg/L at baseline. These data support the need to confirm the right timing of corticosteroids for patients with mild COVID*. [**note: this is a large observational trial from France/Luxembourg on the use of corticosteroids. Somewhat confounding results**] https://www.medrxiv.org/content/10.1101/2020.09.16.20195750v1

- Background: Substantial COVID-19 research investment has been allocated to randomized clinical trials (RCTs) on hydroxychloroquine/chloroquine, which currently face recruitment challenges or early discontinuation. We aimed to estimate the effects of hydroxychloroquine and chloroquine on survival in COVID-19 from all currently available RCT evidence, published and unpublished. Methods: Rapid meta-analysis of ongoing, completed, or discontinued RCTs on hydroxychloroquine or chloroquine treatment for any COVID-19 patients (protocol: https://osf.io/QESV4/). We systematically identified published and unpublished RCTs by September 14, 2020 (ClinicalTrials.gov, WHO International Clinical Trials Registry Platform, PubMed, Cochrane COVID-19 registry). All-cause mortality was extracted (publications/preprints) or requested from investigators and combined in random-effects meta-analyses, calculating odds ratios (ORs) with 95% confidence intervals (CIs), separately for hydroxychloroquine/chloroquine. Prespecified subgroup analyses included patient setting, diagnostic confirmation, control type, and publication status. Results: Sixty-two trials were potentially eligible. We included 16 unpublished trials (1596 patients) and 10 publications/preprints (6317 patients). The combined summary OR on all-cause mortality for hydroxychloroquine was 1.08 (95%CI: 0.99, 1.18; I-square=0%; 24 trials; 7659 patients) and for chloroquine 1.77 (95%CI: 0.15, 21.13, I-square=0%; 4 trials; 307 patients). We identified no subgroup effects. Conclusions: *We found no benefit of hydroxychloroquine or chloroquine on the survival of COVID-19 patients. For hydroxychloroquine, the confidence interval is compatible with increased mortality (OR 1.18) or negligibly reduced mortality (OR 0.99). Findings have unclear generalizability to outpatients, children, pregnant women, and people with comorbidities*. [**note: let's hope this puts the final stake into HCQ. This is a large collaborative meta-analysis of all such trials. No benefit was observed.**] https://www.medrxiv.org/content/10.1101/2020.09.16.20194571v1
- Objectives: A plethora of medicines have been repurposed or used as adjunctive therapies for COVID-19. We characterized the utilization of medicines as prescribed in routine practice amongst patients hospitalized for COVID-19 in South Korea, China, Spain, and the USA. Design: International network cohort Setting: Hospital electronic health records from Columbia University Irving Medical Centre (NYC, USA), Stanford (CA, USA), Tufts (MA, USA), Premier (USA), Optum EHR (USA), department of veterans affairs (USA), NFHCRD (Honghu, China) and HM Hospitals (Spain); and nationwide claims from HIRA (South Korea) Participants: patients hospitalized for COVID-19 from January to June 2020 Main outcome measures: Prescription/dispensation of any medicine on or 30 days after hospital admission date Analyses: Number and percentage of users overall and over time Results: 71,921 people were included: 304 from China, 2,089 from Spain, 7,599 from South Korea, and 61,929 from the USA. A total of 3,455 medicines were identified. Common repurposed medicines included hydroxychloroquine (<2% in NFHCRD to 85.4% in HM), azithromycin (4.9% in NFHCRD to 56.5% in HM),

lopinavir/ritonavir (<3% in all US but 34.9% in HIRA and 56.5% in HM), and umifenovir (0% in all except 78.3% in NFHCRD). Adjunctive medicines were used with great variability, with the ten most used treatments being (in descending order): bemiparin, enoxaparin, heparin, ceftriaxone, aspirin, vitamin D, famotidine, vitamin C, dexamethasone, and metformin. Hydroxychloroquine and azithromycin increased rapidly in use in March-April but declined steeply in May-June. Conclusions: Multiple medicines were used in the first months of COVID-19 pandemic, with substantial geographic and temporal variation. Hydroxychloroquine, azithromycin, lopinavir-ritonavir, and umifenovir (in China only) were the most prescribed repurposed medicines. Antithrombotics, antibiotics, H2 receptor antagonists and corticosteroids were often used as adjunctive treatments. Research is needed on the comparative risk and benefit of these treatments in the management of COVID-19. [**note: this is a large observational study by the OHDSI group on the various medicines used to treat COVID-19 in several international regions from January to June 2020.**]
https://www.medrxiv.org/content/10.1101/2020.09.15.20195545v1

DRUG DEVELOPMENT

- Effective intervention strategies are urgently needed to control the COVID-19 pandemic. Human angiotensin-converting enzyme 2 (ACE2) is a carboxypeptidase that forms a dimer and serves as the cellular receptor for SARS-CoV-2. It is also a key negative regulator of the renin-angiotensin system (RAS), conserved in mammals, which modulates vascular functions. We report here the properties of a trimeric ACE2 variant, created by a structure-based approach, with binding affinity of ~60 pM for the spike (S) protein of SARS-CoV-2, while preserving the wildtype peptidase activity as well as the ability to block activation of angiotensin II receptor type 1 in the RAS. Moreover, the engineered ACE2 potently inhibits infection of SARS-CoV-2 in cell culture. These results suggest that engineered, trimeric ACE2 may be a promising anti-SARS-CoV-2 agent for treating COVID-19. [**note: another group has developed a decoy ACE2 that may be of use for treating COVID-19.**] https://www.biorxiv.org/content/10.1101/2020.09.18.301952v1
- The SARS coronavirus 2 (SARS-CoV-2) has caused an ongoing global pandemic with currently 29 million confirmed cases and close to a million deaths. At this time, there are no FDA-approved vaccines or therapeutics for COVID-19, but Emergency Use Authorization has been granted for remdesivir, a broad-spectrum antiviral nucleoside analog. However, remdesivir is only moderately efficacious against SARS-CoV-2 in the clinic, and improved treatment strategies are urgently needed. To accomplish this goal, we devised a strategy to identify compounds that act synergistically with remdesivir in preventing SARS-CoV-2 replication. We conducted combinatorial high-throughput screening in the presence of submaximal remdesivir concentrations, using a human lung epithelial cell line infected with a clinical isolate of SARS-CoV-2. We identified 20 approved drugs that act synergistically with remdesivir, many with favorable pharmacokinetic and safety profiles. Strongest effects were observed with established antivirals, Hepatitis C virus nonstructural protein 5 A (HCV NS5A) inhibitors velpatasvir and elbasvir. Combination with their partner drugs sofosbuvir and grazoprevir further increased efficacy, increasing remdesivir's apparent potency 25-fold. *We therefore suggest that the FDA-approved Hepatitis C therapeutics Epclusa (velpatasvir/sofosbuvir) and Zepatier (elbasvir/grazoprevir) should be fast-tracked for clinical evaluation in combination with remdesivir to improve treatment of acute SARS-CoV-2 infections*. [**note: this UC Berkeley group**

has identified some drugs that might act synergistically with remdesivir. Some are in trials right now but maybe only on is being looked at with remdesivir. Good luck getting these combo drugs into the clinic!] https://www.biorxiv.org/content/10.1101/2020.09.18.302398v1

- Convalescent plasma has emerged as a promising COVID-19 treatment. However, the humoral factors that contribute to efficacy are poorly understood. This study functionally and phenotypically profiled plasma from eligible convalescent donors. In addition to viral neutralization, convalescent plasma contained antibodies capable of mediating such Fc-dependent functions as complement activation, phagocytosis and antibody-dependent cellular cytotoxicity against SARS-CoV-2. These activities expand the antiviral functions associated with convalescent plasma and together with neutralization efficacy, could be accurately and robustly from antibody phenotypes. These results suggest that high-throughput profiling could be used to screen donors and plasma may provide benefits beyond neutralization. [**note: this paper discusses the utility of antibody signatures in convalescent plasma.**] https://www.medrxiv.org/content/10.1101/2020.09.16.20196154v1
- The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been identified as the prime target for vaccine development. The spike protein mediates both binding to host cells and membrane fusion and is also so far the only known viral target of neutralizing antibodies. Coronavirus spike proteins are large trimers that are relatively instable, a feature that might be enhanced by the presence of a polybasic cleavage site in the SARS-CoV-2 spike. Exchange of K986 and V987 to prolines has been shown to stabilize the trimers of SARS-CoV-1 and the Middle Eastern respiratory syndrome coronavirus spikes. Here, we test multiple versions of a soluble spike protein for their immunogenicity and protective effect against SARS-CoV-2 challenge in a mouse model that transiently expresses human angiotensin converting enzyme 2 via adenovirus transduction. Variants tested include spike protein with a deleted polybasic cleavage site, the proline mutations, a combination thereof, as well as the wild type protein. While all versions of the protein were able to induce neutralizing antibodies, only the antigen with both a deleted cleavage site and the PP mutations completely protected from challenge in this mouse model. **Importance** A vaccine for SARS-CoV-2 is urgently needed. A better understanding of antigen design and attributes that vaccine candidates need to have to induce protective immunity is of high importance. The data presented here validates the choice of antigens that contain the PP mutation and suggests that deletion of the polybasic cleavage site could lead to a further optimized design. [**note: from Mt. Sinai, perhaps a better antigen for vaccination.**] https://www.biorxiv.org/content/10.1101/2020.09.16.300970v1
- COVID-19 vaccines are being rapidly developed and human trials are underway. Almost all of these vaccines have been designed to induce antibodies targeting spike protein of SARS-CoV-2 in expectation of neutralizing activities. However, non-neutralizing antibodies are at risk of causing antibody-dependent enhancement. Further, the longevity of SARS-CoV-2-specific antibodies is very short. Therefore, in addition to antibody-induced vaccines, novel vaccines on the basis of SARS-CoV-2-specific cytotoxic T lymphocytes (CTLs) should be considered in the vaccine development. Here, we attempted to identify HLA-A*02:01-restricted CTL epitopes derived from the non-structural polyprotein 1a of SARS-CoV-2. Eighty-two peptides were firstly predicted as epitope candidates on bioinformatics. Fifty-four in 82 peptides showed high or medium binding affinities to HLA-A*02:01. HLA-A*02:01 transgenic mice were then immunized with each of the 54 peptides encapsulated into liposomes. The intracellular cytokine staining assay revealed that 18 out of 54 peptides were CTL epitopes because of the induction of IFN-g-producing $CD8^+$ T cells. In the 18 peptides, 10 peptides were chosen for the following analyses because of their high responses. To identify dominant CTL epitopes, mice were immunized with

liposomes containing the mixture of the 10 peptides. Some peptides were shown to be statistically predominant over the other peptides. Surprisingly, all mice immunized with the liposomal 10 peptide mixture did not show the same reaction pattern to the 10 peptides. There were three pattern types that varied sequentially, suggesting the existence of an immunodominance hierarchy, which may provide us more variations in the epitope selection for designing CTL-based COVID-19 vaccines. [**note: these two Japanese researchers say novel vaccines on the basis of SARS-CoV-2-specific cytotoxic T lymphocytes (CTLs) should be considered in the vaccine development.**]
https://www.biorxiv.org/content/10.1101/2020.09.18.304493v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Coronavirus disease 2019 (COVID-19) displays high clinical variability but the parameters that determine disease severity are still unclear. Pre-existing T cell memory has been hypothesized as a protective mechanism but conclusive evidence is lacking. *Here we demonstrate that all unexposed individuals harbor SARS-CoV-2-specific memory T cells with marginal cross-reactivity to common cold corona and other unrelated viruses. They display low functional avidity and broad protein target specificities and their frequencies correlate with the overall size of the CD4+ memory compartment reflecting the immunological age of an individual. COVID-19 patients have strongly increased SARS-CoV-2-specific inflammatory T cell responses that are correlated with severity.* Strikingly however, patients with severe COVID-19 displayed lower TCR functional avidity and less clonal expansion. Our data suggest that a low avidity pre-existing T cell memory negatively impacts on the T cell response quality against neoantigens such as SARS-CoV-2, which may predispose to develop inappropriate immune reactions especially in the elderly. *We propose the immunological age as an independent risk factor to develop severe COVID-19.* [**note: from Germany, an examination of pre-existing T cell memory.**
https://www.medrxiv.org/content/10.1101/2020.09.15.20188896v1

DIAGNOSTIC DEVELOPMENT

- Nothing of interest.