2020-10-12

Welcome to Week 30 or 'Where has All the Time Gone?'

The mention of the name Van Dyke Parks sends people immediately to Wikipedia with the question, who in the heck is he? Parks is a wonderful singer/songwriter and arranger who has worked with some of the biggest names around (including Frank Sinatra!!). I remember back in 1967 hearing his first album, 'Song Cycle' and being amazed at the creativity of it. Do read the Wikipedia entry and you will see that he had a recurring role on 'The Honeymooners' with Jackie Gleason! Here is Parks with Laraine Newman singing the mariachi inspired 'Cheek to Cheek': https://www.youtube.com/watch?v=Lo7iJxEbdm8 Here is Parks with the Lovin' Spoonful's John Sebastian with a couple of blues numbers: https://www.youtube.com/watch?v=t8VKqupaDuY Here is one of his original compositions 'Tokyo Rose': https://www.youtube.com/watch?v=2KdHVN37P0E and finally a short documentary on Parks and Jimmy Webb who helped out on Carly Simon's album, 'Film Noir': https://www.youtube.com/watch?v=6TKQ25nhoRw Seriously, what other COVID-19 newsletter provides you with this wonderful stuff!!!

Here is an unpleasant report from The New York Times on lingering symptoms some COVID-19 patients experience following recovery. The FDA Emergency Use Authorization procedure has been used frequently during COVID-19 (mostly for diagnostic tests) but is little understood in times of it's breadth. This article is a good primer. The Regeneron monoclonal antibody duo may need to be rationed as supplies are limited.

The Washington Post discusses the loss of trust in science because of conflicting narratives on COVID-19. Here is a personal account from one whose mental health has improved because of the pandemic. Iran is tightening its mask mandate as cases rise. While the mainstream news may be driving you mad, this newsletter seeks a more balanced approach. You can always just listen to the music selections and turn off the rest of the noise 😄. This one is not COVID-19 related but is such a 'cool' technology (literally) that I'm posting because of the science involved. It's a nanofilm technology that can help create a cooling system that augments traditional AC and refrigeration. Great innovation!

STAT has a story about two Black university leaders who were doing the right thing urging their universities to join a COVID-19 vaccine trial. The backlash was swift showing that the Tuskegee experiment is still an issue for this community. Here is a video story on how dexamethasone works in the treatment of COVID-19.

Nature has a feature on face masks and what the data says. Here is a story on China's COVID-19 vaccine deployment.

I scour the world for important news!! Here is an interview in Die Zeit with German virologist Chrisian Drosten! (It should come up in English, if not you can read it in German as I did😉) There is some very good practical advice here.

MODELING

- Early school closures were a consistent, nationwide response to the COVID-19 pandemic in mid-March due to the role that children play in spreading influenza. This left us with limited

understanding of COVID-19 transmission in children until several states reopened schools for the 2020-2021 school year. While early school closures were likely beneficial in protecting children in the initial stages of the pandemic in the U.S., long-term closures pose significant cumulative effects in children who rely on schools for instruction and additional social services, and for parents who need to balance work and childcare obligations. Reopening schools safely is a high priority for many interested stakeholders. Proposed in-person school reopening plans include traditional, 100% school capacity, five days per week instruction, hybrid scenarios with reduced in-person instruction and virtual learning, and various reduced school capacity schedules with non-pharmaceutical interventions in place. To assess the potential impacts of different reopening plans, we created a modified SIR-type transmission model that captures multiple known pathways of COVID-19 transmission in a 100,000-person community. *Our results show that plans that utilize consecutive days in school and divide students into separated smaller cohorts who attend school together, as well as plans that emphasize distance learning, are better able to suppress disease spread and reduce risk from an introduced infective into the community. Plans with more consecutive school days are protective for both the schoolchildren and surrounding community by acting to separate the larger intermixing population into smaller intermixing subpopulations. The "Five-Day Switch" plan, which separates students into two cohorts, each of whom attend in-person learning for five consecutive days followed by five days of distance learning, best captures these protective attributes. All modeled plans assumed initially disease-free communities and that children's interactions with the community are greatly reduced during instructional days, both for in-person and distance learning.* [**note: this model for k-12 school reopening is from a group at Los Alamos. A five day switch plan is the best approach.**] https://www.medrxiv.org/content/10.1101/2020.10.07.20208710v1

- Wastewater-based monitoring for SARS-CoV-2 holds promise as tool to inform public health-decision making. Testing at individual building-level could be an efficient, passive means of preventing early detection of new cases in congregate living settings, but this approach has not been validated. Sample collection protocols were developed and refined during preliminary sampling from a hospital and a local municipal wastewater treatment plant. Molecular diagnostic methods were compared side-by-side to assess feasibility, performance and sensitivity. Optimized sample collection and processing protocols were then used to monitor two occupied dormitory complexes (n=105 and 66) over eight weeks. Wastewater results were validated using known case counts from external clinical testing of building occupants. Results confirm that ultracentrifugation from a 24 hour composite collection had a sensitivity of 95% and a specificity of 100%. However, if the detection of convalescent shedding is considered a false positive then the sensitivity would be 95.2% but the specificity would drop to 52%. *We determined a highly sensitive method for detecting SARS-CoV-2 shedding in building wastewater however our methods could not distinguish new infectious cases from persistent convalescent shedding of SARS-CoV-2 RNA. Future work must focus on methods to distinguish new infections from convalescent shedding to widely deploy this promising wastewater surveillance tool*. [**note: this wastewater sampling paper is from Univ of Virginia and points to an important caveat in using this – how to differentiate increases in active infections from convalescent shedding.**] https://www.medrxiv.org/content/10.1101/2020.10.10.20210484v1
- In anticipation of COVID-19 vaccine deployment, we use an age-structured mathematical model to investigate the benefits of optimizing age-specific dose allocation to suppress SARS-CoV-2

transmission. *Across 179 countries, we find that the highest priority individuals are typically those between 30 and 59 years of age because of their high contact rates and higher risk of infection and disease. We reaffirm that vaccination alone may be insufficient to achieve herd immunity in some settings, and that additional intervention measures may be required.* Nevertheless, we show that optimizing the allocation of vaccine doses can more than double their effectiveness. [**note: here is a vaccine administration model from Australia as to which age group should be vaccinated first.**]
https://www.medrxiv.org/content/10.1101/2020.10.08.20208108v1

- Introduction The impact of school opening on the SARS-CoV-2 pandemic is still unknown. This study aims to provide preliminary information about the number of SARS-CoV-2 cases among students attending Italian schools. Methods Data are extracted and analysed from an open access, online dataset that monitor, on a daily basis, media news about SARS-CoV-2 infections of students attending Italian schools. Results As of 5 October 2020, a total of 1350 cases of SARS-CoV-2 infections have been registered in the Italian territory schools (involving 1059 students, 145 teachers and 146 other school members), for a total of 1212 out of 65104 (1.8%) Italian schools involved. National schools reported only 1 case of SARS-CoV-2 infection in more than 90% of cases, and only in one high school a cluster of more than 10 cases have been described (P 0.015). The detection of one or more SARS-CoV-2 infections leaded to the closure of 192 (14.2%) entire schools, more frequently nursery/kindergartens (P <0.0005). Discussion *Our preliminary data support low transmission of SARS-CoV-2 within schools, at least among younger students. However, entire schools are frequently closed in the fear of larger outbreaks. Continuous monitoring of school settings, hopefully through daily updated open access datasets, are needed to better understand the impact of schools on the pandemic, and provide guidelines that better consider different risks within different age groups*. [**note: here is early data on infection in Italian schools one month after reopening. Constant surveillance will be required in all school reopening situations to identify outbreaks and what control practices work.**]
https://www.medrxiv.org/content/10.1101/2020.10.10.20210328v1

NEWLY REGISTERED CLINICAL TRIALS

- The week has just started; be patient.

CLINICAL TRIAL RESULTS

- Although anticoagulants such as unfractionated heparin and low molecular weight heparin (LMWH, e.g. enoxaparin) are both being used for therapeutic mitigation of COVID associated coagulopathy (CAC), differences in their clinical outcomes remain to be investigated. Here, we employ automated neural networks supplemented with expert curation (Augmented Curation) for retrospectively analyzing the complete electronic health records (EHRs) of 671 hospitalized COVID-19 patients administered either enoxaparin or unfractionated heparin, but not both. We find that COVID-19 patients administered unfractionated heparin but not enoxaparin have higher rates of mortality (risk ratio: 2.6; 95% C.I.: [1.2-5.4]; p-value: 0.02; BH adjusted p-value: 0.09), thrombotic events (risk ratio: 5.7, 95% C.I.: [2.1, 33.9], p-value: 0.024), acute kidney injury (risk ratio: 5.5; 95% C.I.: [1.2-17.7]; p-value: 0.02; BH adjusted p-value: 0.10), and bacterial pneumonia (risk ratio undefined; 95% C.I.: [1.0, 292]; p-value:0.02; BH adjusted p-value:0.10), compared to patients administered enoxaparin but not unfractionated heparin. *Notably, even*

after controlling for potential confounding factors such as demographics, comorbidities, admission diagnosis, initial ICU status, and initial level of oxygen support, the above differences between the enoxaparin and unfractionated heparin patient cohorts remain statistically significant. This study emphasizes the need for mechanistically investigating differential modulation of the COVID-associated coagulation cascades by enoxaparin versus unfractionated heparin. [**note: the use of anticoagulants was identified as a key therapeutic intervention early on. However, the choice compounds is appearing to be important as this study shows.**] https://www.medrxiv.org/content/10.1101/2020.10.06.20208025v1

- Background: Biomarkers to predict Coronavirus disease-19 (COVID-19) outcome early at infection are urgently needed to improve prognosis and treatment. Zinc balances immune responses and also has a proven direct antiviral action against some viruses. Importantly, zinc deficiency (ZD) is a common condition in elderly and individuals with chronic diseases, two groups with more severe COVID-19 outcomes. We hypothesize that serum zinc content (SZC) influences COVID-19 disease progression and thus might represent a useful biomarker. Methods: We run a retrospective observational study with 249 COVID-19 patients admitted in Hospital del Mar. We have studied COVID-19 severity and progression attending to SZC at admission. In parallel we have studied SARS-CoV2 replication in the Vero E6 cell line modifying zinc concentrations. Findings*: Our study demonstrates a correlation between serum zinc levels and COVID-19 outcome. Serum zinc levels lower than 50 mcgg/dl at admission correlated with worse clinical presentation, longer time to reach stability and higher mortality*. Our in vitro results indicate that low zinc levels favor viral expansion in SARS-CoV2 infected cells. Interpretation: SZC is a novel biomarker to predict COVID-19 outcome. We encourage performing randomized clinical trials to study zinc supplementation as potential prophylaxis and treatment with people at risk of zinc deficiency. [**note: make sure you have enough zinc in your diet!! This observational study from Barcelona points to zinc deficiency being a factor in COVID-19 outcomes. Much more study needs to be done to validate this as a biomarker.**] https://www.medrxiv.org/content/10.1101/2020.10.07.20208645v1
- Background: Patients with severe COVID-19 have overwhelmed healthcare systems worldwide. We hypothesized that Machine Learning (ML) models could be used to predict risks at different stages of management (at diagnosis, hospital admission and ICU admission) and thereby provide insights into drivers and prognostic markers of disease progression and death. Methods: From a cohort of approx. 2.6 million citizens in the two regions of Denmark, SARS-CoV-2 PCR tests were performed on subjects suspected for COVID-19 disease; 3944 cases had at least one positive test and were subjected to further analysis. A cohort of SARS-CoV-2 positive cases from the United Kingdom Biobank was used for external validation. Findings: The ML models predicted the risk of death (Receiver Operation Characteristics Area Under the Curve, ROC-AUC) of 0.904 at diagnosis, 0.818, at hospital admission and 0.723 at Intensive Care Unit (ICU) admission. Similar metrics were achieved for predicted risks of hospital and ICU admission and use of mechanical ventilation. *We identified some common risk factors, including age, body mass index (BMI) and hypertension as driving factors, although the top risk features shifted towards markers of shock and organ dysfunction in ICU patients. The external validation indicated fair predictive performance for mortality prediction, but suboptimal performance for predicting ICU admission. Interpretation: ML may be used to identify drivers of progression to more severe disease and for prognostication patients in patients with COVID-19. Prognostic features included age, BMI and*

hypertension, although markers of shock and organ dysfunction became more important in more severe cases. We provide access to an online risk calculator based on these findings. [**note: this Danish study comes up with a risk prediction model based on a cohort of 5500 COVID-19 patients.**] https://www.medrxiv.org/content/10.1101/2020.10.06.20207209v1

DRUG DEVELOPMENT

- The coronavirus disease 2019 (COVID-19) pandemic has highlighted the urgent need for effective preventive vaccination to reduce burden and spread of severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV-2) in humans. Intranasal vaccination is an attractive strategy to prevent COVID-19 as the nasal mucosa represents the first-line barrier to SARS-CoV-2 entry before viral spread to the lung. Although SARS-CoV-2 vaccine development is rapidly progressing, the current intramuscular vaccines are designed to elicit systemic immunity without conferring mucosal immunity. Here, we show that AdCOVID, an intranasal adenovirus type 5 (Ad5)-vectored vaccine encoding the receptor binding domain (RBD) of the SARS-CoV-2 spike protein, elicits a strong and focused immune response against RBD through the induction of mucosal IgA, serum neutralizing antibodies and CD4+ and CD8+ T cells with a Th1-like cytokine expression profile. Therefore, AdCOVID, which promotes concomitant systemic and local mucosal immunity, represents a promising COVID-19 vaccine candidate. [**note: here is animal data on the nasal COVID-19 vaccine developed by Altimmune (they are right up the road from me in Gaithersburg!). Good immunity and it may be a more appealing delivery system.**] https://www.biorxiv.org/content/10.1101/2020.10.10.331348v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- A large number of hospitalized COVID-19 patients show neurological symptoms such as ischemic- and hemorrhagic stroke as well as encephalitis, and SARS-CoV-2 can directly infect endothelial cells leading to endotheliitis across multiple vascular beds. These findings suggest an involvement of the brain- and peripheral vasculature in COVID-19, but the underlying molecular mechanisms remain obscure. To understand the potential mechanisms underlying SARS-CoV-2 tropism for brain vasculature, we constructed a molecular atlas of the expression patterns of SARS-CoV-2 viral entry-associated genes (receptors and proteases) and SARS-CoV-2 interaction partners in human (and mouse) adult and fetal brain as well as in multiple non-CNS tissues in single-cell RNA-sequencing data across various datasets. We observed a distinct expression pattern of the cathepsins B (CTSB) and -L (CTSL) - which are able to substitute for the ACE2 co-receptor TMPRSS2 - in the human vasculature with CTSB being mainly expressed in the brain vasculature and CTSL predominantly in the peripheral vasculature, and these observations were confirmed at the protein level in the Human Protein Atlas and using immunofluorescence stainings. This expression pattern of SARS-CoV-2 viralentry associated proteases and SARS-CoV-2 interaction partners was also present in endothelial cells and microglia in the fetal brain, suggesting a developmentally establishedSARS-CoV-2 entry machinery in the human vasculature. At both the adult and fetal stages, we detected a distinct pattern of SARS-CoV-2 entry associated genes' transcripts in brain vascular endothelial cells and microglia, providing a potential explanation for an inflammatory response in the brain endothelium upon SARS-CoV-2 infection. Moreover, CTSB was co-expressed in adult and fetal brain endothelial cells with genes and pathways involved in innate immunity and inflammation, angiogenesis, blood-brain-barrier

permeability, vascular metabolism, and coagulation, providing a potential explanation for the role of brain endothelial cells in clinically observed (neuro)vascular symptoms in COVID-19 patients. *Our study serves as a publicly available single-cell atlas of SARS-CoV-2 related entry factors and interaction partners in human and mouse brain endothelial- and perivascular cells, which can be employed for future studies in clinical samples of COVID-19 patients*. [**note: this research presents possible modes of cellular entry of SARS-CoV-2 into the brain and may explain some of the observed neurological symptoms.**] https://www.biorxiv.org/content/10.1101/2020.10.10.334664v1

- The search for potential antibody-based diagnostics, vaccines, and therapeutics for pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has focused almost exclusively on the spike (S) and nucleocapsid (N) proteins. Coronavirus membrane (M), orf3a, and orf8 proteins are also humoral immunogens in other coronaviruses (CoVs) but remain largely uninvestigated for SARS-CoV-2. Here we show that SARS-CoV-2 infection induces robust antibody responses to epitopes throughout the SARS-CoV-2 proteome, particularly in M, in which one epitope achieved near-perfect diagnostic accuracy. We map 79 B cell epitopes throughout the SARS-CoV-2 proteome and demonstrate that anti-SARS-CoV-2 antibodies appear to bind homologous peptide sequences in the 6 known human CoVs. *Our results demonstrate previously unknown, highly reactive B cell epitopes throughout the full proteome of SARS-CoV-2 and other CoV proteins, especially M, which should be considered in diagnostic, vaccine, and therapeutic development*. [**note: from Univ of Wisconsin, the landscape of antibody binding to SARS-CoV-2.]** https://www.biorxiv.org/content/10.1101/2020.10.10.334292v1

DIAGNOSTIC DEVELOPMENT

- In a large cohort of ambulatory confirmed COVID-19 patients with multiple self-collected sample time points, we compared 202 matched nasal-oropharyngeal swabs and oral salivary fluid sample pairs by RT-PCR. Nasal-oropharyngeal swabs were more sensitive than this salivary sample type (oral crevicular fluid) suggesting that not all saliva sample types have equivalent sensitivity. *However, all samples that were Vero E6-TMPRSS2 cell culture positive (e.g., infectious virus) were also oral fluid RT-PCR positive suggesting that oral fluid may find the patients most likely to transmit disease to others*. [**note: this study on salivary and nasal specimens is from Johns Hopkins. The linkage of oral samples to cultured virus is important and may point to a good test to show potential viral transmission.**] https://www.medrxiv.org/content/10.1101/2020.10.07.20208520v1

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2020-10-13

Of course, you are all asking yourself, why the newsletter writer has omitted one of the best known female vocalists of all time. He has featured lots of singers, some of whom are pretty obscure. Well this has certainly got to end right here and now. With the recent spate of country music vocalists, you have already guessed the genre. It is none other than Porter Wagoner's favorite (even though he sued her for breech of contract when she decided to go solo), Dolly Parton. I don't think I've ever seen her without a great smile on her face. Here is the single that brought her the early fame, 'Jolene' from the Porter Wagoner show in 1973: https://www.youtube.com/watch?v=OTuQ9cKGSdM Parton did a great

comedic turn in the movie, '9 to 5' with Jane Fonda and Lily Tomlin as well as singing the title tune: https://www.youtube.com/watch?v=UbxUSsFXYo4 Here is Dolly and Porter singing 'Just Someone I used to Know': https://www.youtube.com/watch?v=UDlcxHU4Zi8 Finally, one of my faves 'Coat of Many Colors': https://www.youtube.com/watch?v=w_-YbWHs6DE Enjoy!!!

The New York Times Science Tuesday section is full of good COVID-19 articles today. Carl Zimmer writes on the clinical trial set up for COVID-19 vaccines and why we might not know which one of them is best. Here is a story on Heidi Larson who founded the Vaccine Confidence Project designed to build trus in vaccines. Science writer, Donald McNeill has a dose of optimism as the pandemic rages on. This network of scientists are chasing a super antibody against COVID-19. Here is a good interactive article unveiling some of the mysteries of COVID-19. What happens when COVID-19 symptoms don't go away? Some COVID-19 patients require extensive rehabilitation.

The Washington Post reports on the second wave of COVID-19 cases in Europe. An 89 year old Dutch woman died of an apparent reinfection form COVID-19. The Netherlands is rolling out new restrictions in light of a surge of viral infections.

JAMA have two research letters, one on excess deaths from COVID-19 and other causes over the March – July period and a second article comparing all-cause mortality in the US and 18 comparison countries. Here is an editorial on this topic along with a general editorial on the toll from COVID-19. The US has not done a good job of responding to the pandemic.

STAT writes about the pause of the J & J vaccine trial because of an unexplained illness in a trial subject. There is also an opinion piece on the placebo response which may be a hidden risk in COVID-19 trials.

The Lancet has a commentary of potential treatments for post-intensive care syndrome arising from COVID-19. Here is a commentary on what reinfections mean for COVID-19. This is clearly something to continually monitor.

Medscape have the cautionary story of rushed vaccine development.

MODELING

- A better understanding of SARS-CoV-2 transmission from children and adolescents is crucial for informing public health mitigation strategies. *We conducted a retrospective cohort study among household contacts of primary cases defined as children and adolescents aged 7-19 years with laboratory evidence of SARS-CoV-2 infection acquired during an overnight camp outbreak. Among household contacts, we defined secondary cases using the Council of State and Territorial Epidemiologists definition. Among 526 household contacts of 224 primary cases, 48 secondary cases were identified, corresponding to a secondary attack rate of 9% (95% confidence interval [CI], 7%-12%). Our findings show that children and adolescents can transmit SARS-CoV-2 to adult contacts and other children in a household setting*. [**note: here is a CDC study that tracks a camp outbreak of SARS-CoV-2 that occurred in Georgia. It confirms that the virus can be transmitted to adult and child contacts in a household setting (unsurprising but good to have this documented).**] https://www.medrxiv.org/content/10.1101/2020.10.10.20210492v1

NEWLY REGISTERED CLINICAL TRIALS

- It's only Tuesday, you will get an update this week.

CLINICAL TRIAL RESULTS

- Stroke and central nervous system dysfunction are cardinal symptoms in critically ill corona virus disease 19 (COVID-19) patients. In an autopsy series of 32 COVID-19 patients, we investigated whether carotid arteries were infected with SARS-CoV-2 by employing genomic, virologic, histochemical and transcriptomic analyses. We show that SARS-CoV-2 productively infects and modulates vascular responses in carotid arteries. This finding has far reaching implications for the understanding and clinical treatment of COVID-19. [**note: here is an autopsy series from Germany showing SARS-CoV-2 can infect and modulate vascular response in carotid arteries.**] https://www.biorxiv.org/content/10.1101/2020.10.10.334458v1

DRUG DEVELOPMENT

- The human membrane protein Angiotensin-converting enzyme 2 (hACE2) acts as the main receptor for host cells invasion of the new coronavirus SARS-CoV-2. The viral surface glycoprotein Spike binds to hACE2, which triggers virus entry into cells. As of today, the role of hACE2 for virus fusion is not well understood. Blocking the transition of Spike from its prefusion to post-fusion state might be a strategy to prevent or treat COVID-19. Here we report a single particle cryo-electron microscopy analysis of SARS-CoV-2 trimeric Spike in presence of the human ACE2 ectodomain. The binding of purified hACE2 ectodomain to Spike induces the disassembly of the trimeric form of Spike and a structural rearrangement of its S1 domain to form a stable, monomeric complex with hACE2. This observed hACE2 dependent dissociation of the Spike trimer suggests a mechanism for the therapeutic role of recombinant soluble hACE2 for treatment of COVID-19. [**note: from Switzerland, structural investigation the ACE2 – Spike protein interaction. It suggests that recombinant soluble ACE2 might play a therapeutic role. There are trials ongoing with this approach.**] https://www.biorxiv.org/content/10.1101/2020.10.12.336016v1
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VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The simultaneous measurement of multiple modalities, known as multimodal analysis, represents an exciting frontier for single-cell genomics and necessitates new computational methods that can define cellular states based on multiple data types. Here, we introduce "weighted-nearest neighbor analysis", an unsupervised framework to learn the relative utility of each data type in each cell, enabling an integrative analysis of multiple modalities. We apply our procedure to a CITE-seq dataset of hundreds of thousands of human white blood cells alongside a panel of 228 antibodies to construct a multimodal reference atlas of the circulating immune system. We demonstrate that integrative analysis substantially improves our ability to resolve cell states and validate the presence of previously unreported lymphoid subpopulations. Moreover, we demonstrate how to leverage this reference to rapidly map new datasets, and to interpret immune responses to vaccination and COVID-19. Our approach represents a broadly applicable strategy to analyze single-cell multimodal datasets, including paired measurements of RNA and chromatin state, and to look beyond the transcriptome towards a unified and multimodal definition of cellular identity. Availability: Installation instructions, documentation,

tutorials, and CITE-seq datasets are available at http://www.satijalab.org/seurat [**note: I'm not sure I fully grasp this paper but suspect that it offers considerable utility in looking what goes on inside cells. It is good that the effort is publicly available.**] https://www.biorxiv.org/content/10.1101/2020.10.12.335331v1

- The spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds the cell surface protein ACE2 to mediate fusion of the viral membrane with target cells. S comprises a large external domain, a transmembrane domain (TMD) and a short cytoplasmic tail. To elucidate the intracellular trafficking of S protein in host cells we applied proteomics to identify cellular factors that interact with its cytoplasmic tail. We confirm interactions with components of the COPI, COPII and SNX27/retromer vesicle coats, and with FERM domain actin regulators and the WIPI3 autophagy component. The interaction with COPII promotes efficient exit from the endoplasmic reticulum (ER), and although COPI-binding should retain S in the early Golgi system where viral budding occurs, the binding is weakened by a suboptimal histidine residue in the recognition motif. As a result, S leaks to the surface where it accumulates as it lacks an endocytosis motif of the type found in many other coronaviruses. It is known that when at the surface S can direct cell:cell fusion leading to the formation of multinucleate syncytia. Thus, the trafficking signals in the cytoplasmic tail of S protein indicate that syncytia formation is not an inadvertent by-product of infection but rather a key aspect of the replicative cycle of SARS-CoV-2 and potential cause of pathological symptoms. [**note: here is further information on the Spike protein syncytia formation which may be mportant to replication.**] https://www.biorxiv.org/content/10.1101/2020.10.12.335562v1
- The SARS-CoV-2 spike (S) protein is the target of vaccine design efforts to end the COVID-19 pandemic. Despite a low mutation rate, isolates with the D614G substitution in the S protein appeared early during the pandemic, and are now the dominant form worldwide. Here, we analyze the D614G mutation in the context of a soluble S ectodomain construct. Cryo-EM structures, antigenicity and proteolysis experiments suggest altered conformational dynamics resulting in enhanced furin cleavage efficiency of the G614 variant. Furthermore, furin cleavage alters the conformational dynamics of the Receptor Binding Domains (RBD) in the G614 S ectodomain, demonstrating an allosteric effect on the RBD dynamics triggered by changes in the SD2 region, that harbors residue 614 and the furin cleavage site. Our results elucidate SARS-CoV-2 spike conformational dynamics and allostery, and have implications for vaccine design. [**note: from Duke more information on the D614G mutation which is now the dominant version of SARS-CoV-2**] https://www.biorxiv.org/content/10.1101/2020.10.11.335299v1
- Severe acquired respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of coronavirus disease (COVID-19). In severe COVID-19 cases, higher antibody titers against seasonal coronaviruses have been observed than in mild cases. To investigate antibody cross-reactivity as potential explanation for severe disease, we determined the kinetics, breadth, magnitude and level of cross-reactivity of IgG against SARS-CoV-2 and seasonal CoV nucleocapsid and spike from 17 severe COVID-19 cases at the clonal level. *Although patients mounted a mostly type-specific SARS-CoV-2 response, B-cell clones directed against seasonal CoV dominated and strongly increased over time. Seasonal CoV IgG responses that did not neutralize SARS-CoV-2 were boosted well beyond detectable cross-reactivity, particularly for HCoV-OC43 spike. These findings support a back-boost of poorly protective coronavirus-specific antibodies in severe COVID-19 patients that may negatively impact de novo SARS-CoV-2 immunity, reminiscent of*

original antigenic sin. [**note: this is from Erasmus Univ in Rotterdam and shows how some severe COVID-19 patients may mount a increased immune response against seasonal coronavirus strains that may impact immunity against SARS-CoV-2.** https://www.medrxiv.org/content/10.1101/2020.10.10.20210070v1

- T-cell immunity is likely to play a role in protection against SARS-CoV-2 by helping generate neutralizing antibodies. We longitudinally studied CD4 T-cell responses to the M, N, and S structural proteins of SARS-CoV-2 in 21 convalescent individuals. Within the first two months following symptom onset, a majority of individuals (81%) mount at least one CD4 T-cell response, and 48% of individuals mount detectable SARS-CoV-2-specific peripheral T follicular helper cells (pTfh, defined as CXCR5+PD1+ CD4 T cells). SARS-CoV-2-specific pTfh responses across all three protein specificities correlate with antibody neutralization with the strongest correlation observed for S protein-specific responses. When examined over time, pTfh responses increase in frequency and magnitude in convalescence, and robust responses with magnitudes greater than 5% were detected only at the second convalescent visit, an average of 38 days post-symptom onset. These data deepen our understanding of antigen-specific pTfh responses in SARS-CoV-2 infection, suggesting that M and N protein-specific pTfh may also assist in the development of neutralizing antibodies and that pTfh response formation may be delayed in SARS-CoV-2 infection. [**note: looks like today is antibody Tuesday! Here is more information on other cell factors that are important in the response to viral infection.**] https://www.medrxiv.org/content/10.1101/2020.10.07.20208488v1

DIAGNOSTIC DEVELOPMENT

- Nothing of note today.

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2020-10-14

I've now featured Linda, Dolly, and Emmylou and now it is time to take a look at the famous trio that was formed by them. They had gotten together during the 1970s but an album was not to be released until 1987, simply titled 'Trio.' It was a critical and sales hit. The video is not great, but here they are on Johnny Carson after the record was released: https://www.youtube.com/watch?v=ENJHxo1w_10 Here they are a little more than a decade later on Letterman right after Trio 2 was issued: https://www.youtube.com/watch?v=o2XcBL6kWn8 Here is 'High Sierra' from Trio 2: https://www.youtube.com/watch?v=Cnieh0Y1V-o One of the earliest collaborations was on Dolly's variety show in the mid-1970s and here is 'The Sweetest Gift': https://www.youtube.com/watch?v=wWEQDyrbphE Finally, a full length documentary on the formation of the trio: https://www.youtube.com/watch?v=uQ7StOs2xY0

The Washington Post has a good interactive article on how genetic sequencing of SARS-CoV-2 variants has provided important information and how the US has failed to tap this. The J&J vaccine and Lilly monoclonal antibody trials have been halted because of safety concerns. This stuff happens all the time in drug/vaccine trials when a safety issue comes up. The AstraZeneca/Oxford trial was briefly halted last month. The Director General of the World Health Organization argues that it is unethical to try to reach herd immunity. But wait, here is an effort driven by three scientists right here in the good old US of A. The approach called 'Focused Protection' is set forth online I the Great Barrington Declaration. I am not

signing this for the simple reason that it's stupid and overlooks the fundamental problem that even 'healthy' people may require hospitalization, over taxing the system. Their argument that everything should open up is also stupid as many of us are not going to settings that are crowded and poorly ventilated. They should take a hard look at the airline industry that has been open for business and losing billions of dollars because people are not going to fly unless absolutely necessary. Enough of this stupidity. COVID-19 cases are on the rise in the US sparking concerns about a second wave (the US really never got over the first wave). Make sure you have adequate humidity in your abode this winter; a Japanese study suggests the virus does not linger in air in higher humidity situations. An outbreak of COVID-19 from this Canadian fitness studio suggests that masks are important for preventing spread (the did not have a mask requirement). Russia hospital occupancy is at 90% because of COVID-19 infections.

The New York Times has a short clip on the Great Barrington Declaration. I guess we really don't need the CDC after all since letting the virus run wild negates the need for track and trace and other public health measures. As I said above, this is stupid (maybe I will be proven wrong, who knows?). Outbreaks in the Midwest and Mountain West have pushed the case load to the highest level since late August, straining hospitals. Maybe New York City office space will make a comeback as some big tech companies are increasing their footprint in the Big Apple. Reinfections with SARS-CoV-2 are likely to be quite rare.

The Atlantic has an article about socializing indoors during the colder pandemic months. Maybe teens have handled the pandemic quarantine OK. Here are some tips to keep the fall surge from becoming a winter catastrophe.

The Lancet have an article on the New Zealand national response to COVID-19. Here is an editorial commentary on this response.

MODELING

- Airborne spread of COVID-19 by infectious aerosol is all but certain. However, easily implemented approaches to assess the actual environmental threat are currently unavailable. We present a simple approach with the potential to rapidly provide information about the prevalence of SARS-CoV-2 in the atmosphere at any location. We used a portable dehumidifier as a readily available and affordable tool to collect airborne virus in the condensate. The dehumidifiers were deployed in selected locations of a hospital ward with patients reporting flu like symptoms which could possibly be due to COVID-19 over three separate periods of one week. Samples were analyzed frequently for both virus envelope protein and SARS-CoV-2 RNA. In several samples across separate deployments, condensate from dehumidifiers tested positive for the presence of SARS-CoV-2 antigens and confirmed using two independent assays. RNA was detected, but not attributable to SARS-CoV-2. *Our results point to a facile pool testing method to sample air in any location in the world and assess the presence and concentration of the infectious agent in order to obtain quantitative risk assessment of exposure, designate zones as hot spots and minimize the need for individual testing which may often be time consuming, expensive and laborious*. [**note: here is a cool low cost way to sample air for SARS-CoV-2 using**

low cost dehumidiers! This receives the DIY award for this week.]
https://www.medrxiv.org/content/10.1101/2020.10.08.20208785v1

- Background: The early stages of the COVID-19 pandemic illustrated that SARS-CoV-2, the virus that causes the disease, has the potential to spread exponentially. Therefore, as long as a substantial proportion of the population remains susceptible to infection, the potential for new epidemic waves persists even in settings with low numbers of active COVID-19 infections, unless sufficient countermeasures are in place. In this study, we examine the Australian state of New South Wales, a setting with prolonged low transmission, high mobility, non-universal mask usage, and a well-functioning test-and-trace system. We investigate how vulnerable the state would be to resurgences in COVID-19 transmission under variations in the levels of testing, tracing, and mask usage. Methods: We use a stochastic agent-based model, calibrated to the New South Wales epidemic and policy environment, to simulate possible epidemic outcomes over October 1 to December 31, 2020, under a range of assumptions about contact tracing efficacy, testing rates, and mask uptake. Results: *We find that the relative impact of masks is greatest when testing and tracing rates are lower (and vice versa). With very high testing rates (90% of people with symptoms, plus 90% of people with a known history of contact with a confirmed case), we estimate that the epidemic would remain under control until at least the end of 2020, with as little as 70-110 new infections estimated over October 1 to December 31 under high mask uptake scenarios, or 340-1400 without masks, depending on the efficacy of community contact tracing. However, across comparable levels of mask uptake and contact tracing, the number of infections over this period would be up to 6 times higher if the testing rate was 80% instead of 90%, 17 times higher if the testing rate was 65%, or more than 100 times higher with a 50% testing rate. Conclusions: Our work suggests that testing, tracing and masks can all be effective means of controlling transmission in dynamic community settings. A multifaceted strategy that combines all three, alongside continued hygiene and distancing protocols, is likely to be the most robust means of controlling community-based transmission of SARS-CoV-2*. [**note: here is a model from Australia on how robust track and trace can prevent COVID-19 resurgences. Too bad large areas of the US have not learned this.**]
https://www.medrxiv.org/content/10.1101/2020.10.09.20209429v1
- College reopening decisions during the SARS-CoV-2 pandemic represent a trade-off between competing risks to students, faculty and staff, and college finances. Additionally, risks taken in reopening colleges can impose significant burdens on individuals living in surrounding communities. Many colleges that reopened for in-person instruction have reported frequent SARS-CoV-2 outbreaks. *La Crosse County, Wisconsin experienced a substantial SARS-CoV-2 outbreak (2,002 cases in September 2020) that coincided with the return to in-person instruction at three local academic institutions. Genomic sequencing of SARS-CoV-2 cases in La Crosse during that period found rapid expansion of two viral substrains. Although the majority of cases were among college-age individuals, from a total of 111 genomes sequenced we identified rapid transmission of the virus into more vulnerable populations. Eight sampled genomes represented two independent transmission events into two skilled nursing facilities, resulting in two fatalities. Our study highlights the very significant risks imposed by college administrator reopening decisions, not just on college-associated populations, but on vulnerable individuals in surrounding communities*. [**note: this is the first surveillance study I've seen based on data from a college reopening. Community spread of the virus was observed and it got into two**

nursing facilities! So much for the theory that the young pose no risk.]
https://www.medrxiv.org/content/10.1101/2020.10.12.20210294v1

- The rapid development of vaccines against the SARS-CoV-2 virus is an unprecedented achievement. Once vaccines become mass produced, they will have to be distributed to almost the entire population to prevent deaths and permit prompt economic recovery. The necessity to vaccinate a large number of people in a short period of time, and possibly with insufficient vaccine doses to cover most, creates in itself a new challenge for governments and health authorities: which population groups (by age or other criteria) should be targeted first and what sequence must be followed, if any at all, to achieve the minimum number of fatalities? In this work, we demonstrate the importance and impact of optimally planning the priorities for vaccine deployment by population groups using a modified SEIR-type model for the COVID-19 outbreak considering age-related groups. Finding the absolute guaranteed best solution of the mathematical optimisation problem may be hard, if even possible, and would likely require intense computational resources for every possible case study scenario. In this work, several strategies are evaluated and compared, in an attempt to approach the most effective possible vaccination priority sequence in an example case study using demographic and epidemiological data from Spain. The minimum total fatalities at the end of the vaccination campaign is the objective pursued. The population groups classifications are established based on relevant differences in mortality (due to their age) and risk-related behaviour such as their number of daily person-to-person interactions. Assuming a capacity limited constant vaccination rate, vaccination distribution strategies were evaluated for different vaccine effectiveness levels and different percentages of final vaccine population coverage. Our results unambiguously show how planning vaccination by priority groups can achieve dramatic reductions in total fatalities (more than 70% in some cases) compared to no prioritisation. The results also indicate in all cases, for all vaccine effectiveness and coverage values evaluated, that the criteria for groups vaccination priority should not be those with the highest mortality but rather those the highest number of daily person-to-person interactions. Strikingly, our results show in all cases, that prioritisation of groups with the highest mortality but less social interactions, may lead to significantly larger numbers of final total fatalities, even higher as if no group priorities were established at all. The explanation, clearly displayed by the mechanistic model, is that vaccination avoids infections that reduce mortality not only from the vaccinated group itself but also from the projected secondary and subsequent infections inflicted on the rest of the population by those vaccinated in that group. Precisely this amplification effect (exponential nature of the curve) appears to cause the larger reduction in total fatalities if the groups with the most interactions are vaccinated first. The possible contradiction of these results with some published recommendations highlight the importance of conducting an open comprehensive and rigorous analysis of this problem leaving behind any subjective preconceptions. [**note: I think this is the first paper from Abu Dubai, a model for vaccine deployment**]
https://www.medrxiv.org/content/10.1101/2020.10.12.20211094v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a platform trial to conduct a series of randomized, double-blind, placebo-controlled trials using common assessments and endpoints in hospitalized adults diagnosed with COVID-19. BET is a proof-of-concept study with the intent of identifying promising treatments to enter a more

definitive study. The study will be conducted in up to 40 sites throughout the US. The study will compare different investigational therapeutic agents to a common control arm and determine which have relatively large effects. In order to maintain the double blind, each intervention will have a matched placebo. However, the control arm will be shared between interventions and may include participants receiving the matched placebo for a different intervention. The goal is not to determine clear statistical significance for an intervention, but rather to determine which products have clinical data suggestive of efficacy and should be moved quickly into larger studies. Estimates produced from BET will provide an improved basis for designing the larger trial, in terms of sample size and endpoint selection. Products with little indication of efficacy will be dropped on the basis of interim evaluations. In addition, some interventions may be discontinued on the basis of interim futility or efficacy analyses. One or more interventions may be started at any time. The number of interventions enrolling are programmatic decisions and will be based on the number of sites and the pace of enrollment. At the time of enrollment, subjects will be randomized to receive any one of the active arms they are eligible for or placebo. Approximately 100 subjects will be assigned to each arm entering the platform and a given site will generally have no more than 3 interventions at once. The BET-B stage will evaluate the combination of remdesivir with lenzilumab vs remdesivir with a lenzilumab placebo. The primary objective is to evaluate the clinical efficacy of different investigational therapeutics relative to the control arm in adults hospitalized with COVID-19 according to clinical status (8-point ordinal scale) at Day 8. [**note: this is a very big NIAID sponsored trial. I imagine that other drugs will be entering this system. It would have been nice had this been put in place several months ago.**] NCT04583969 and here is another one studying remdesivir and Risankizumab. NCT04583956

- Phase II: Early viral responses to triazavirin In hospitalised patients with mild-moderate COVID-19, in addition to standard of care therapy, treatment with triazavirin 250mg three times daily for five days, the slope of increase of the Ct values of serial nasopharyngeal swabs to 12 days after initiation of treatment will be ≥24% higher than in hospitalised patients receiving standard of care treatment only. Phase III: Efficacy of triazavirin to improve clinical outcomes In hospitalised patients with mild-moderate laboratory proven COVID-19, in addition to standard of care therapy, treatment with triazavirin 250mg three times daily for five days will reduce a composite outcome - death; ICU admission or mechanical ventilation; or prolonged duration of admission- by ≥29% when compared to the composite outcome in hospitalised patients receiving standard of care therapy only. [**note: this is a South African trial of a Russian broad spectrum antiviral.**] NCT04581915
- COVID-19 is a new disease caused by SARS-CoV-2 that was identified in 2019. Some people who get sick with COVID-19 become ill requiring hospitalization. There are some medicines that may help with recovery. Researchers want to see if a drug called fostamatinib may help people who are hospitalized with COVID-19. [**note: this is another NIH-sponsored trial.**] NCT04579393

CLINICAL TRIAL RESULTS

- Today, COVID-19 pandemic has brought countries' health services into sharp focus. Despite the low incidence of cases(1.2%) and high mortality rate(2.4%) among Turkish population, the low mortality rate(0.3%) despite the high incidence(11.5%) declared in healthcare workers drew our group's attention. Therefore, we aimed to report the characteristics of infected health-care workers and investigate the relationship between BCG vaccine and tuberculosis history with COVID-19 mortality in infected health-care worker population. Method: This study was

conducted in three hospitals to assess the clinical presentations, disease severity and correlation with BCG vaccine and tuberculous history in COVID-19 positive health-care workers by an online questionnaire platform. The relationship between characteristics and tuberculosis history were investigated according to hospitalization status of the patients. Result: Total of 465 infected healthcare workers included in the study. The rate of history of direct care and contact to tuberculosis patient, presence of previous tuberculosis treatment and BCG scar, presence of radiological infiltrations was significantly higher in hospitalized healthcare workers. The ratio of direct care and direct contact to the patient with tuberculosis, and presence of family history of tuberculosis were statistically significantly higher in patients with radiological infiltrations. Conclusion: *Although COVID-19 risk and incidence are higher among healthcare workers compared to the normal population due to higher virus load, we think that the lower mortality rate seen in infected healthcare workers results from healthcare workers' frequent exposure to tuberculosis bacillus and the mortality-reducing effects of BCG vaccine, despite the higher hospitalization rate and radiological infiltrations due to over-triggered immune system*. [**note: here is an interesting observational study from Turkey on healthcare workers and BCG vaccine and or exposure to tuberculosis bacillus. Infection in the cohort is higher than the general population but mortality is much lower. There could be confounders in this study and there are several trials going on looking at BCG vaccine as a prophylaxis agent.**] https://www.medrxiv.org/content/10.1101/2020.10.08.20209403v1

- COVID-19 patients may exhibit neuropsychiatric and/or neurological symptoms. We found that anxiety and cognitive impairment are manifested by 28-56% of SARS-CoV-2-infected individuals with mild or no respiratory symptoms and are associated with altered cerebral cortical thickness. Using an independent cohort, we found histopathological signs of brain damage in 19% of individuals who died of COVID-19. All of the affected brain tissues exhibited foci of SARS-CoV-2 infection, particularly in astrocytes. Infection of neural stem cell-derived astrocytes changed energy metabolism, altered key proteins and metabolites used to fuel neurons and for biogenesis of neurotransmitters, and elicited a secretory phenotype that reduces neuronal viability. *Our data support the model where SARS-CoV-2 reaches the brain, infects astrocytes and triggers neuropathological changes that contribute to the structural and functional alterations in the brain of COVID-19 patients*. [**note: here is more information on neurological symptoms, this time from Brazil. I am struck by the high percent of patients who had anxiety or cognitive impairment. It is worth noting that seasonal influenza can also lead to neurological symptoms.**] https://www.medrxiv.org/content/10.1101/2020.10.09.20207464v1
- Develop and validate models that predict mortality of SARS-CoV-2 infected patients admitted to the hospital. Design: Retrospective cohort study Setting: A multicenter cohort across ten Dutch hospitals including patients from February 27 to June 8 2020. Participants: SARS-CoV-2 positive patients (age ≥ 18) admitted to the hospital. Main Outcome Measures: 21-day mortality evaluated by the area under the receiver operatory curve (AUC), sensitivity, specificity, positive predictive value and negative predictive value. The predictive value of age was explored by comparison with age-based rules used in practice and by excluding age from analysis. Results: 2273 patients were included, of whom 516 had died or discharged to palliative care within 21 days after admission. *Five feature sets, including premorbid, clinical presentation and laboratory & radiology values, were derived from 80 features. Additionally, an ANOVA-based data-driven feature selection selected the ten features with the highest F-values: age, number of home*

medications, urea nitrogen, lactate dehydrogenase, albumin, oxygen saturation (%), oxygen saturation is measured on room air, oxygen saturation is measured on oxygen therapy, blood gas pH and history of chronic cardiac disease. A linear logistic regression (LR) and non-linear tree-based gradient boosting (XGB) algorithm fitted the data with an AUC of 0.81 (95% confidence interval 0.77 to 0.85) and 0.82 (0.79 to 0.85), respectively, using the ten selected features. Both models outperformed age-based decision rules used in practice (AUC of 0.69, 0.65 to 0.74 for age > 70). Furthermore, performance remained stable when excluding age as predictor (AUC of 0.78, 0.75 to 0.81) Conclusion: Both models showed excellent performance and had better test characteristics than age-based decision rules, using ten admission features readily available in Dutch hospitals. The models hold promise to aid decision making during a hospital bed shortage. [**note: here is a retrospective Dutch cohort study that looks at factors that can predict mortality in individual COVID-19 patients.**]
https://www.medrxiv.org/content/10.1101/2020.10.10.20210591v1

DRUG DEVELOPMENT

- The current CoVid-19 crisis is revealing the strengths and the weaknesses of the world's capacity to respond to a global health crisis. A critical weakness has resulted from the excessive centralization of the current biomanufacturing capacities, a matter of great concern, if not a source of nationalistic tensions. On the positive side, scientific data and information have been shared at an unprecedented speed fuelled by the preprint phenomena, and this has considerably strengthened our ability to develop new technology-based solutions. In this work we explore how, in a context of rapid exchange of scientific information, plant biofactories can serve as a rapid and easily adaptable solution for local manufacturing of bioreagents, more specifically recombinant antibodies. For this purpose, we tested our ability to produce, in the framework of an academic lab and in a matter of weeks, milligram amounts of six different recombinant monoclonal antibodies against SARS-CoV-2 in Nicotiana benthamiana. For the design of the antibodies we took advantage, among other data sources, of the DNA sequence information made rapidly available by other groups in preprint publications. mAbs were all engineered as single-chain fragments fused to a human gamma Fc and transiently expressed using a viral vector. In parallel, we also produced the recombinant SARS-CoV-2 N protein and its Receptor Binding Domain (RBD) in planta and used them to test the binding specificity of the recombinant mAbs. Finally, for two of the antibodies we assayed a simple scale-up production protocol based on the extraction of apoplastic fluid. Our results indicate that gram amounts of anti-SARS-CoV-2 antibodies could be easily produced in little more than 6 weeks in repurposed greenhouses with little infrastructure requirements using N. benthamiana as production platform. Similar procedures could be easily deployed to produce diagnostic reagents and, eventually, could be adapted for rapid therapeutic responses. [**note: this is from Spain and demonstrates pilot production of SARS-CoV-2 related proteins in plants. This is not a new technology but it's good to see it being used here.**]
https://www.biorxiv.org/content/10.1101/2020.10.13.331306v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Antibody-dependent enhancement (ADE) has been reported in several virus infections including dengue fever virus, severe acute respiratory syndrome (SARS) and Middle East respiratory

syndrome (MERS) coronavirus infection. To study whether ADE is involved in COVID-19 infections, in vitro pseudotyped SARS-CoV-2 entry into Raji cells, K562 cells, and primary B cells mediated by plasma from recovered COVID-19 patients were employed as models. The enhancement of SARS-CoV-2 entry into cells was more commonly detected in plasma from severely-affected elderly patients with high titers of SARS-CoV-2 spike protein-specific antibodies. Cellular entry was mediated via the engagement of FcγRII receptor through virus-cell membrane fusion, but not by endocytosis. Peptide array scanning analyses showed that antibodies which promote SARS-CoV-2 infection targeted the variable regions of the RBD domain. To further characterize the association between the spike-specific antibody and ADE, an RBD-specific monoclonal antibody (7F3) was isolated from a recovered patient, which potently inhibited SARS-Cov-2 infection of ACE-2 expressing cells and also mediated ADE in Raji cells. Site-directed mutagenesis the spike RBD domain reduced the neutralization activity of 7F3, but did not abolish its binding to the RBD domain. Structural analysis using cryo-electron microscopy (Cryo-EM) revealed that 7F3 binds to spike proteins at a shift-angled pattern with one up and two down RBDs, resulting in partial overlapping with the receptor binding motif (RBM), while a neutralizing monoclonal antibody that lacked ADE activity binds to spike proteins with three up RBDs, resulting in complete overlapping with RBM. *Our results revealed that ADE mediated by SARS-CoV-2 spike-specific antibodies could result from binding to the receptor in slightly different pattern from antibodies mediating neutralizations. Studies on ADE using antibodies from recovered patients via cell biology and structural biology technology could be of use for developing novel therapeutic and preventive measures for control of COVID-19 infection*. [**note: this is a useful paper from China that looks at potential antibody-dependent enhancement where antibodies to the virus may end up facilitating infection. This can be both a clinical concern as well as an issue in vaccine development. Although there is some *in vitro* evidence in this paper, it appears not to be a general issue. It will be something to look out for as vaccine trials proceed.**] https://www.medrxiv.org/content/10.1101/2020.10.08.20209114v1

- Substitution for aspartic acid by glycine at position 614 in the spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the ongoing pandemic, appears to facilitate rapid viral spread. The G614 variant has now replaced the D614-carrying virus as the dominant circulating strain. *We report here cryo-EM structures of a full-length S trimer carrying G614, which adopts three distinct prefusion conformations differing primarily by the position of one receptor-binding domain (RBD). A loop disordered in the D614 S trimer wedges between domains within a protomer in the G614 spike. This added interaction appears to prevent premature dissociation of the G614 trimer, effectively increasing the number of functional spikes and enhancing infectivity. The loop transition may also modulate structural rearrangements of S protein required for membrane fusion*. These findings extend our understanding of viral entry and suggest an improved immunogen for vaccine development. [**note: more information on the D614G Spike mutation.**] https://www.biorxiv.org/content/10.1101/2020.10.13.337980v1
- An unexpected observation among the COVID-19 pandemic is that smokers constituted only 1.4-18.5% of hospitalized adults, calling for an urgent investigation to determine the role of smoking in SARS-CoV-2 infection. Here, we show that cigarette smoke extract (CSE) and carcinogen benzo(a)pyrene (BaP) increase ACE2 mRNA but trigger ACE2 protein catabolism. BaP induces an aryl hydrocarbon receptor (AhR)-dependent upregulation of the ubiquitin E3 ligase Skp2 for

ACE2 ubiquitination. ACE2 in lung tissues of non-smokers is higher than in smokers, consistent with the findings that tobacco carcinogens downregulate ACE2 in mice. *Tobacco carcinogens inhibit SARS-CoV-2 Spike protein pseudovirions infection of the cells. Given that tobacco smoke accounts for 8 million deaths including 2.1 million cancer deaths annually and Skp2 is an oncoprotein, tobacco use should not be recommended and cessation plan should be prepared for smokers in COVID-19 pandemic*. {**note: from China, a mechanisitic understanding of why there are fewer smokers hospitalized for COVID-19. Note the conclusion!!!**]
https://www.biorxiv.org/content/10.1101/2020.10.13.337774v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

>>

2020-10-15 (Another milestone today with **Newsletter #200**; I really want to get out of the pandemic newsletter business!😆)

It's been a couple of weeks since I posted a video of my favorite Catalan jazz group. Here is Joan Chamorro playing as part of the Andrea Motis quintet at a date in New York City last year. Some great jazz standards along with a couple of original cmpositions by Andrea: https://www.youtube.com/watch?v=Yikb5jVAYWY She is also a new mother (baby boy Cel was born October 2) and here is one of her last performances before the baby arrived: https://www.youtube.com/watch?v=TijmJAEgRtE

The Washington Posts tells you how to form a pod that will help get through the winter and they are setting up a newsletter service to help you remember what day it is. Of course, my loyal readers don't need anything such as this as you are reminded of the date each day when you get a fresh helping of COVID-19 news at no cost!!!! We forget that there are many rural areas that have no or very poor Internet access; this may be a good infrastructure project to undertake.

The New York Times has an op-ed on how to save Halloween. Is President Trump immune from SARS-CoV-2? This is not meant as a political commentary but something I've wondered about given the treatment regimen that was employed. He received the large dose of the Regeneron mAb duo. Would this, coupled with the other drugs that were administered inhibited his immune system from producing its own antibodies against the virus or created the 'immune memory' needed to ward off subsequent infections. I'm not sure what the half life of the mAb treatment and there was a preprint in the last several days on the development of a longer lasting antibody. This is an interesting scientific question.

CNN point to Danish and Canadian studies that seem to confirm differential response to COVID-19 in terms of blood types. Very early on in the pandemic it was noted that those with Type O seemed not be getting as ill as those with Types A or AB.

The New England Journal of Medicine has the results of the safety and immunogenicity of the two Pfizer mRNA vaccine candidates. The results of the UK RECOVERY trial of HCQ has been published. It is not an effective treatment. Also published is the final report of the remdesivir study.

STAT have a story on the use of Artificial Intelligence (AI) tools and whether they will be able to differentiate COVID-19 from seasonal influenza.

The Lancet have an article on the use of a track and trace program on the Isle of Wight (admittedly a small area geographically and population wise). It included the use of a contact tracing app. Here is a commentary on this report. Here is a position statement from a group of public health officials and epidemiologists *against* the herd immunity approach that has recently been in the news. Here is the money quote, "This is a dangerous fallacy unsupported by scientific evidence." More is at the John Snow Memorandum website (Snow developed the water theory of transmission of cholera. In 1854, he famously removed the handle of a water pump to try to curtail transmission of cholera during an outbreak in London).

Kaiser Health News have an article on pandemic stress.

There has been a significant uptick in articles over the past few days with some pretty good findings.

MODELING

- We investigate the role of aerosols in the transmission of SARS-CoV-2 in public spaces. Direct measurement of aerosol concentrations however has proven technically difficult; we propose the use of handheld particle counters as a novel and easily applicable method to measure aerosol concentrations. This allow us to perform measurements in typical public spaces, each differing in volume, number of people and ventilation rate. These data are used to estimate the relation between aerosol persistence time and the risk of infection with SARS-CoV-2. [**note: this is cool. These Amsterdam researchers measure small droplet aerosol concentration in public spaces suing handheld particle counters.**
https://www.medrxiv.org/content/10.1101/2020.10.13.20211839v1
- To reduce the transmission of SARS-CoV-2 most countries closed schools, despite uncertainty if school closures are an effective containment measure. At the onset of the pandemic, Swedish upper secondary schools moved to online instruction while lower secondary school remained open. This allows for a comparison of parents and teachers differently exposed to open and closed schools, but otherwise facing similar conditions. Leveraging rich Swedish register data, we connect all students and teachers in Sweden to their families and study the impact of moving to online instruction on the incidence of SARS-CoV-2 and COVID-19. *We find that among parents, exposure to open rather than closed schools resulted in a small increase in PCR-confirmed infections [OR 1.15; CI95 1.03-1.27]. Among lower secondary teachers the infection rate doubled relative to upper secondary teachers [OR 2.01; CI95 1.52-2.67]. This spilled over to the partners of lower secondary teachers who had a higher infection rate than their upper secondary counterparts [OR 1.30; CI95 1.00-1.68]. When analyzing COVID-19 diagnoses from healthcare visits and the incidence of severe health outcomes, results are similar for teachers but somewhat weaker for parents and teachers' partners. The results for parents indicate that keeping lower secondary schools open had minor consequences for the transmission of SARS-CoV-2 in society. The results for teachers suggest that measures to protect teachers could be considered*. [**note: this study is from Sweden and looks the effect of school closures in the country which was only partial.**]
https://www.medrxiv.org/content/10.1101/2020.10.13.20211359v1

- The upcoming flu season in the northern hemisphere merging with the current COVID-19 pandemic may raise a potentially severe threat to public health. However, little is known about the consequences of the co-infection of influenza A virus (IAV) and SARS-CoV-2. Through experimental co-infection of IAV with either pseudotyped or SARS-CoV-2 live virus, we found that IAV pre-infection significantly promoted the infectivity of SARS-CoV-2 in a broad range of cell types. Intriguingly, such enhancement of SARS-CoV-2 infectivity was only seen under co-infection with IAV but not with several other viruses including Sendai virus, human rhinovirus, human parainfluenza virus, human respiratory syncytial virus, or human enterovirus 71. IAV infection rather than interferon signaling induced elevated expression of ACE2 essential for such enhancement of SARS-CoV-2 infectivity. *Remarkably, we further confirmed that the pre-infection of IAV indeed resulted in an increased SARS-CoV-2 viral load and more severe lung damage in hACE2-transgenic mice. This study illustrates that the co-infection of IAV aggravates SARS-CoV-2 infection and disease severity, which in turn suggests that preventing the convergence of flu season and COVID-19 pandemic would be of great significance*. [**note: GET YOUR FLU SHOT ASAP!!!!**] https://www.biorxiv.org/content/10.1101/2020.10.14.335893v1
- A growing number of studies provide insight into how SARS-CoV-2 spreads1-7. Yet, many factors that characterize its transmissibility remain unclear, including mechanistic correlates of overdispersion, viral kinetics, the extent to which respiratory droplets and aerosols carry viable virus and the infectiousness of asymptomatic, presymptomatic and pediatric cases7. Here, we developed a comprehensive dataset of respiratory viral loads (rVLs) via systematic review and investigated these factors using meta-analyses and modeling. By comparing cases of COVID-19, SARS and influenza A(H1N1)pdm09, we found that heterogeneity in rVL was associated with overdispersion and facilitated the distinctions in individual variation in infectiousness among these emergent diseases. For COVID-19, case heterogeneity was broad throughout the infectious period, although rVL tended to peak at 1 day from symptom onset (DFSO) and be elevated for 1-5 DFSO. While most cases presented minimal risk, highly infectious ones could spread SARS-CoV-2 by talking, singing or breathing, which shed virions at comparable rates via droplets and aerosols. Coughing shed considerable quantities of virions, predominantly via droplets, and greatly increased the contagiousness of many symptomatic cases relative to asymptomatic ones. Asymptomatic and symptomatic infections showed similar likelihoods of expelling aerosols with SARS-CoV-2, as did adult and pediatric cases. Children tended to be less contagious by droplet spread than adults based on tendencies of symptomatology rather than rVL. Our findings address longstanding questions on SARS-CoV-2 transmissibility and present pertinent considerations for disease control. [**note: here is a good overview of transmissibility and shedding of SARS-CoV-2 via droplets and aerosols.**]
  https://www.medrxiv.org/content/10.1101/2020.10.13.20212233v1

NEWLY REGISTERED CLINICAL TRIALS

- You got the news yesterday!

CLINICAL TRIAL RESULTS

- **Objectives**: The role of innate lymphoid cells (ILCs) in coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is unknown. Understanding the immune response in COVID-19 could contribute to unravel the pathogenesis

and identification of treatment targets. To describe the phenotypic landscape of circulating ILCs in COVID-19 patients and to identify ILC phenotypes correlated to serum biomarkers, clinical markers, and laboratory parameters relevant in COVID-19. **Methods**: Blood samples collected from moderately (n=11) and severely ill (n=12) COVID-19 patients as well as healthy control donors (n=16), were analyzed with 18-parameter flow cytometry. Using supervised and unsupervised approaches, we examined the ILC activation status and homing profile. Clinical and laboratory parameters were obtained from all COVID-19 patients and serum biomarkers were analyzed with multiplex immunoassays. **Results**: ILCs were largely depleted from the circulation of COVID-19 patients compared with healthy controls. Remaining circulating ILCs from patients revealed increased frequencies of ILC2 in moderate COVID-19, with a concomitant decrease of ILC precursors (ILCp), as compared with controls. ILC2 and ILCp showed an activated phenotype with increased CD69 expression, whereas expression levels of the chemokine receptors CXCR3 and CCR4 were significantly altered in ILC2 and ILCp, and ILC1, respectively. The activated ILC profile of COVID-19 patients was associated with soluble inflammatory markers, while frequencies of ILC subsets were correlated with laboratory parameters that reflect the disease severity. **Conclusion**: This study provides insights into the potential role of ILCs in immune responses against SARS-CoV-2, particularly linked to the severity of COVID-19. [**note: this Swedish study shows correlates of innate lymphoid cell composition with disease severity.**] https://www.medrxiv.org/content/10.1101/2020.10.13.20211367v1

- Studies of adaptive immunity to SARS-CoV-2 include characterisation of lethal, severe and mild cases. Understanding how long immunity lasts in people who have had mild or asymptomatic infection is crucial. Healthcare worker (HCW) cohorts exposed to and infected by SARS-CoV-2 during the early stages of the pandemic are an invaluable resource to study this question. The UK COVIDsortium is a longitudinal, London hospital HCW cohort, followed from the time of UK lockdown; weekly PCR, serology and symptom diaries allowed capture of asymptomatic infection around the time of onset, so duration of immunity could be tracked. Here, we conduct a cross-sectional, case-control, sub-study of 136 HCW at 16-18 weeks after UK lockdown, with 76 having had laboratory-confirmed SARS-CoV-2 mild or asymptomatic infection. *Neutralising antibodies (nAb) were present in 90% of infected HCW sampled after the first wave; titres, likely to correlate with functional protection, were present in 66% at 16-18 weeks. T cell responses tended to be lower in asymptomatic infected HCW than those reporting case-definition symptoms of COVID-19, while nAb titres were maintained irrespective of symptoms. T cell and antibody responses were discordant. HCW lacking nAb also showed undetectable T cells to Spike protein but had T cells of other specificities. Our findings suggest that the majority of HCW with mild or asymptomatic SARS-CoV-2 infection carry nAb complemented by multi-specific T cell responses for at least 4 months after mild or asymptomatic SARS-CoV-2 infection*. [**note: this UK study of healthcare workers with mild or asymptomatic infections show T cell responses and neutralizing antibodies. This is a good sign.**]
  https://www.medrxiv.org/content/10.1101/2020.10.13.20211763v1
- The clinical course of COVID-19 is very heterogeneous: Most infected individuals can be managed in an outpatient setting, but a substantial proportion of patients requires intensive care, resulting in a high rate of fatalities. Recently, an association between contact to small children and mild course of COVID-19 was reported. We performed an observational study to assess the impact of previous infections with seasonal coronaviruses on COVID-19 severity. 60

patients with confirmed COVID-19 infections were included (age 30 - 82 years; 52 males, 8 females): 19 inpatients with critical disease, 16 inpatients with severe or moderate disease and 25 outpatients (age and gender matched to inpatients). Patients with critical disease had significantly lower levels of HCoV OC43- (p=0.016) and HCoV HKU1-specific (p=0.023) antibodies at the first encounter compared to other COVID-19 patients. Our results indicate that previous infections with seasonal coronaviruses might protect against a severe course of disease. This finding should be validated in other settings and could contribute to identify persons at risk before an infection. [**note: this is from Germany and hints that previous infection with seasonal coronaviruses may be protective against severe COVID-19. Obviously this finding requires more validation.**]
https://www.medrxiv.org/content/10.1101/2020.10.12.20211599v1

- Background SARS-Cov-2 is a new virus causing a pandemic of primarily respiratory illness designated as Coronavirus Disease 2019 (COVID-19). This disease is associated with excess mortality, particularly among the elderly, raising concerns for public health. It is crucial to identify whether existing medications could protect against adverse outcomes of COVID-19 infection. Methods We performed a population-based study among members of Clalit Health Services (CHS), the largest healthcare provider in Israel. CHS centrally manages electronic health records (EHR) including medication purchases for over 4.5 million insurees. Since the disease outbreak through October 10, 2020, 8,681 adult patients aged between 18 and 95 have been hospitalized for COVID-19, among them 3,777 in severe condition. Two case-control matched cohorts were assembled to assess which drugs taken by patients in the month preceding a SARS-CoV-2 positive test affected risks of COVID-19 hospitalization and disease severity. Significance of the associations was assessed using Fisher's exact test and Benjamini-Hochberg correction for multiple testing. Findings We identified several drugs and products sold in pharmacies that are significantly associated with reduced odds ratios of SARS-CoV-2 hospitalization and disease severity: notably ubiquinone (OR:0.25, p<0.001), ezetimibe (OR=0.51, P<0.001), rosuvastatin (OR=0.75, p<0.001) and flecainide (OR=0.30, p<0.01). Additionally, acquisition of surgical masks, latex gloves and several ophthalmological products, including eye wipes were associated with decreased risk for hospitalization. *Interpretation Ubiquinone, ezetimibe and rosuvastatin, all related to the cholesterol synthesis pathway, are associated with a protective effect against COVID-19 complications. These medications are associated with reduced hospitalization rate and decreased severity in hospitalized patients*. These findings set the basis for specific prospective randomized control trials that should be carried out to carefully assess their protective effects. [**note: this is an observational study from Israel and suggests there are several drugs that might be protective against severe COVID-19. Only clinical trials can really confirm this.**]
https://www.medrxiv.org/content/10.1101/2020.10.13.20211953v1
- SARS-CoV-2 infection induces severe disease in a subpopulation of patients, but the underlying mechanisms remain unclear. We demonstrate robust IgM autoantibodies that recognize angiotensin converting enzyme-2 (ACE2) in 18/66 (27%) patients with severe COVID-19, which are rare (2/52; 3.8%) in hospitalized patients who are not ventilated. The antibodies do not undergo class-switching to IgG, suggesting a T-independent antibody response. Purified IgM from anti-ACE2 patients activates complement. Pathological analysis of lung obtained at autopsy shows endothelial cell staining for IgM in blood vessels in some patients. We propose that vascular endothelial ACE2 expression focuses the pathogenic effects of these autoantibodies on

blood vessels, and contributes to the angiocentric pathology observed in some severe COVID-19 patients. These findings may have predictive and therapeutic implications. [**note: this is from Johns Hopkins and suggests that IgM autoantibodies may be another predictor of severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.10.13.20211664v1

DRUG DEVELOPMENT

- The COVID19 pandemic has resulted in 1,092,342 deaths as of 14th October 2020, indicating the urgent need for a vaccine. This study highlights novel protein sequences generated by shot gun sequencing protocols that could serve as potential antigens in the development of novel subunit vaccines and through a stringent inclusion criterion, we characterized these protein sequences and predicted their 3D structures. *We found distinctly antigenic sequences from the SARS-CoV-2 that have led to identification of 4 proteins that demonstrate an advantageous binding with Human leukocyte antigen-1 molecules. Results show how previously unexplored proteins may serve as better candidates for subunit vaccine development due to their high stability and immunogenicity, reinforce by their HLA-1 binding propensities and low global binding energies. This study thus takes a unique approach towards furthering the development of vaccines by employing multiple consensus strategies involved in immuno-informatics technique*. [**note: this is from a Nigerian and Indian group of researchers and offers a different approach to vaccine development.**] https://www.biorxiv.org/content/10.1101/2020.10.14.339689v1
- Coronavirus disease 2019 (COVID-19) is a severe acute respiratory syndrome (SARS) caused by a virus known as SARS-Coronavirus 2 (SARS-CoV2). Without a targeted-medicine, this disease has been causing a massive humanitarian crisis not only in terms of mortality, but also imposing a lasting damage to social life and economic progress of humankind. Therefore, an immediate therapeutic strategy needs to be intervened to mitigate this global crisis. Here, we report a novel KepTide(TM) (Knock-End Peptide) therapy that nullifies SARS-CoV2 infection. SARS-CoV2 employs its surface glycoprotein spike (S-glycoprotein) to interact with angiotensin converting enzyme-2 (ACE-2) receptor for its infection in host cells. Based on our in-silico-based homology modeling study validated with a recent X-ray crystallographic structure (PDB ID:6M0J), we have identified that a conserved motif of S-glycoprotein that intimately engages multiple hydrogen-bond (H-bond) interactions with ACE-2 enzyme. *Accordingly, we designed a peptide, termed as ACIS (ACE-2 Inhibitory motif of Spike), that displayed significant affinity towards ACE-2 enzyme as confirmed by biochemical assays such as BLItz and fluorescence polarization assays. Interestingly, more than one biochemical modifications were adopted in ACIS in order to enhance the inhibitory action of ACIS and hence called as KEpTide(TM). Consequently, a monolayer invasion assay, plaque assay and dual immunofluorescence analysis further revealed that KEpTide(TM) efficiently mitigated the infection of SARS-CoV2 in vitro in VERO E6 cells. Finally, evaluating the relative abundance of ACIS in lungs and the potential side-effects in vivo in mice, our current study discovers a novel KepTideTM therapy that is safe, stable, and robust to attenuate the infection of SARS-CoV2 virus if administered intranasally*. [**note: here is a new approach to a viral inhibitor from Sotira Scientific. It is a nasal spray that blocks binding.**] https://www.biorxiv.org/content/10.1101/2020.10.13.337584v1
- SARS-CoV-2 infection results in viral burden in the upper and lower respiratory tract, enabling transmission and often leading to substantial lung pathology. Delivering the antiviral treatment directly to the lungs has the potential to improve lung bioavailability and dosing efficiency. As

the SARS-CoV-2 Receptor Binding Domain (RBD) of the Spike (S) is increasingly deemed to be a clinically validated target, RBD-specific B cells were isolated from patients following SARS-CoV-2 infection to derive a panel of fully human monoclonal antibodies (hmAbs) that potently neutralize SARS-CoV-2. The most potent hmAb, 1212C2 was derived from an IgM memory B cell, has high affinity for SARS-CoV-2 RBD which enables its direct inhibition of RBD binding to ACE2. The 1212C2 hmAb exhibits in vivo prophylactic and therapeutic activity against SARS-CoV-2 in hamsters when delivered intraperitoneally, achieving a meaningful reduction in upper and lower respiratory viral burden and lung pathology. Furthermore, liquid nebulized inhale treatment of SARS-CoV-2 infected hamsters with as low as 0.6 mg/kg of inhaled dose, corresponding to approximately 0.03 mg/kg of lung deposited dose, mediated a reduction in respiratory viral burden that is below the detection limit, and mitigated lung pathology. The therapeutic efficacy achieved at an exceedingly low-dose of inhaled 1212C2 supports the rationale for local lung delivery and achieving dose-sparing benefits as compared to the conventional parenteral route of administration. Taken together, these results warrant an accelerated clinical development of 1212C2 hmAb formulated and delivered via inhalation for the prevention and treatment of SARS-CoV-2 infection. [**note: if this research transfers to humans it would be important as the amount to therapeutic mAb would be lower than that delivered by injection (President Trump received an 8 gram dose of the Regeneron product).**
https://www.biorxiv.org/content/10.1101/2020.10.14.339150v1

- Passive transfer of convalescent plasma or serum is a time-honored strategy for treating infectious diseases. Human convalescent plasma containing antibodies against SARS-CoV-2 is currently being used to treat COVID-19 patients. However, most patients have been treated outside of randomized clinical trials making it difficult to determine the efficacy of this approach. Here, we assessed the efficacy of convalescent sera in a newly developed African green monkey model of COVID-19. Groups of SARS-CoV-2-infected animals were treated with pooled convalescent sera containing either high or low to moderate anti-SARS-CoV-2 neutralizing antibody titers. Differences in viral load and disease pathology were minimal between monkeys that received the lower titer convalescent sera and untreated controls. However, and importantly, lower levels of SARS-CoV-2 in respiratory compartments, reduced gross and histopathological lesion severity in the lungs, and reductions in several parameters associated with coagulation and inflammatory processes were observed in monkeys that received convalescent sera versus untreated controls. *Our data support human studies suggesting that convalescent plasma therapy is an effective strategy if donors with high level of antibodies against SARS-CoV-2 are employed and if recipients are at an early stage of disease*. [**note: here is non-human primate data showing the efficacy of convalescent plasma against COVID-19. There are a number of registered clinical trials that I hope will be completed despite the FDA EUA for this product.**]
  https://www.biorxiv.org/content/10.1101/2020.10.14.340091v1
- An effective vaccine to address the global pandemic of coronavirus disease 2019 (COVID-19) is an urgent public health priority. Novel synthetic mRNA and vector-based vaccine technologies offer an expeditious development path alternative to traditional vaccine approaches. Here we describe the efforts to utilize an mRNA platform for rational design and evaluations of mRNA vaccine candidates based on Spike (S) glycoprotein of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the virus causing COVID-19. Several mRNA constructs expressing

various structural conformations of S-protein, including wild type (WT), a pre-fusion stabilized mutant (2P), a furin cleavage-site mutant (GSAS) and a double mutant form (2P/GSAS), were tested in a preclinical animal model for their capacity to elicit neutralizing antibodies (nAbs). The lead 2P/GSAS candidate was further assessed in dose-ranging studies in mice and Cynomolgus macaques. The selected 2P/GSAS vaccine formulation, now designated MRT5500, elicited potent nAbs as measured in two types of neutralization assays. In addition, MRT5500 elicited TH1-biased responses in both mouse and non-human primate species, a result that helps to address a hypothetical concern regarding potential vaccine-associated enhanced respiratory diseases associated with TH2-biased responses. These data position MRT5500 as a viable vaccine candidate for clinical development against COVID-19. [**note: here is animal data for the Sanofi mRNA vaccine. They also have a protein subunit vaccine under development.**]
https://www.biorxiv.org/content/10.1101/2020.10.14.337535v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 is a novel coronavirus which has caused the COVID-19 pandemic. Other known coronaviruses show a strong pattern of seasonality, with the infection cases in humans being more prominent in winter. Although several plausible origins of such seasonal variability have been proposed, its mechanism is unclear. SARS-CoV-2 is transmitted via airborne droplets ejected from the upper respiratory tract of the infected individuals. It has been reported that SARS-CoV-2 can remain infectious for hours on surfaces. As such, the stability of viral particles both in liquid droplets as well as dried on surfaces is essential for infectivity. Here we have used atomic force microscopy to examine the structural stability of individual SARS-CoV-2 virus like particles at different temperatures. *We demonstrate that even a mild temperature increase, commensurate with what is common for summer warming, leads to dramatic disruption of viral structural stability, especially when the heat is applied in the dry state. This is consistent with other existing non-mechanistic studies of viral infectivity, provides a single particle perspective on viral seasonality, and strengthens the case for a resurgence of COVID-19 in winter*. [**note: from Univ of Utah, temperature stability of SARS-CoV-2. It can be disrupted at warmer summer-like temperatures but beware this winter!**]
https://www.biorxiv.org/content/10.1101/2020.10.12.336818v1
- The coronavirus disease 2019 pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is an ongoing global public crisis. Although viral RNA modification has been reported based on the transcriptome architecture, the types and functions of RNA modification are still unknown. In this study, we evaluated the roles of RNA N6-methyladenosine (m6A) modification in SARS-CoV-2. Our methylated RNA immunoprecipitation sequencing (MeRIP-Seq) analysis showed that SARS-CoV-2 RNA contained m6A modification. Moreover, SARS-CoV-2 infection not only increased the expression of methyltransferase-like 3 (METTL3) but also altered its distribution. Modification of METTL3 expression by short hairpin RNA or plasmid transfection for knockdown or overexpression, respectively, affected viral replication. Furthermore, the viral key protein RdRp interacted with METTL3, and METTL3 was distributed in both the nucleus and cytoplasm in the presence of RdRp. RdRp appeared to modulate the sumoylation and ubiquitination of METTL3 via an unknown mechanism. Taken together, our findings demonstrated that the host m6A modification complex interacted with viral proteins to modulate SARS-CoV-2 replication. [**note: from China, here is information on**

methyltransferase modification of the virus. It's still unclear what the full impact of this is.] https://www.biorxiv.org/content/10.1101/2020.10.14.338558v1

- SARS-CoV-2 coronavirus is responsible for Covid-19 pandemic. In the early phase of infection, the single-strand positive RNA genome is translated into non-structural proteins (NSP). One of the first proteins produced during viral infection, NSP1, binds to the host ribosome and blocks the mRNA entry channel. This triggers translation inhibition of cellular translation. In spite of the presence of NSP1 on the ribosome, viral translation proceeds however. The molecular mechanism of the so-called viral evasion to NSP1 inhibition remains elusive. Here, we confirm that viral translation is maintained in the presence of NSP1. The evasion to NSP1-inhibition is mediated by the cis-acting RNA hairpin SL1 in the 5'UTR of SARS-CoV-2. NSP1-evasion can be transferred on a reporter transcript by SL1 transplantation. The apical part of SL1 is only required for viral translation. We show that NSP1 remains bound on the ribosome during viral translation. We suggest that the interaction between NSP1 and SL1 frees the mRNA accommodation channel while maintaining NSP1 bound to the ribosome. *Thus, NSP1 acts as a ribosome gatekeeper, shutting down host translation or fostering SARS-CoV-2 translation depending on the presence of the SL1 5'UTR hairpin. SL1 is also present and necessary for translation of sub-genomic RNAs in the late phase of the infectious program. Consequently, therapeutic strategies targeting SL1 should affect viral translation at early and late stages of infection. Therefore, SL1 might be seen as a genuine 'Achille heel' of the virus*. [**note: here is more on the NSP1 protein and how it can shut down host protein synthesis. We are finding out more about how the virus works *in vivo* and this may offer multiple therapeutic approaches.**] https://www.biorxiv.org/content/10.1101/2020.10.14.339515v1

DIAGNOSTIC DEVELOPMENT

- Nothing new.

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2020-10-16

It's time for some good old-time rock and roll!!! Enough country music for now. One of the great bands of all time is The Rolling Stones. I was still a teenager when they made their first appearance, and they are still around. Lots of iconic songs but maybe this is the best one, 'Sympathy for the Devil', and here are Mick and company in a live performance: https://www.youtube.com/watch?v=ZRXGsPBUV5g I would be hard pressed to decide between that one and 'You Can't Always Get What You Want': https://www.youtube.com/watch?v=Ef9QnZVpVd8 but I prefer this earlier live version where Mick is less affected: https://www.youtube.com/watch?v=c9mGtDGzoSA . I always like the version with the choral introduction the same way it was on the record; here is that version live from Anaheim: https://www.youtube.com/watch?v=t0b74EisBC4 (this must have been taken with a cell phone) and Hyde Park in Chicago: https://www.youtube.com/watch?v=2O8DXLfYquY (there are other videos from this concert tour with local choral groups) Finally the original LP recording: https://www.youtube.com/watch?v=ZUqSNbJuGOw . I better stop before I watch too many more videos of Mick prancing on the stage!

The Washington Post reports on the recent COVID-19 outbreaks in the US and notes that Tony Fauci won't be having his children fly home for Thanksgiving. Our daughter in Oakland will not either and

perhaps I ought to invite the good Doctor over to our house. Here is a short story about how CDC traced an outbreak of COVID-19 to an indoor Florida hockey game. Cases of COVID-19 in Europe now exceed those in the US. This is an important study from the Defense Department on the risk of contracting COVID-19 on an airplane. Wearing masks coupled with a well-designed ventilation system appears to make flying reasonably safe. I look forward to seeing the published paper. This outbreak does not bode well for the upcoming election in the Atlanta Georgia area. What were these Swiss yodelers thinking?

The New York Times notes the President of SUNY Oneonta has resigned after worse COVID-19 outbreak of any public university in the state. Former NJ Governor Chris Christie spent time in the ICU for COVID-19 and says he was wrong not to wear masks at the White House. Pfizer stated that an Experimental Use Authorization for their mRNA COVID-19 would not be ready until late November. There is a brief comment on the WHO SOLIDARITY trial and remdesivir not having an impact on mortality (link to the article is below). New York City is trying to stomp out COVID-19 one block at a time. College journalists report on COVID-19 quarantines on their campus. As you know, I have been tracking testing results at Purdue University, a land-grant college with a campus population of 49,700. For the past week, 5827 COVID-19 tests were performed with 177 positive results for a infection rate of 3.04%. This is slightly higher than the overall positive rate of 2.87% from the August 1 start date of the program.

Science have an important perspective on whether SARS-CoV-2 will become endemic.

STAT have an opinion piece from two Johns Hopkins researchers on the magical thinking of herd immunity. Here is how you might plan for Thanksgiving. Three Boston MDs comment on how systems issues at hospitals need to be the focus of safety as a second surge looms.

The Lancet have early stage results from the Chinese inactivated SARS-CoV-2 vaccine. This is the second inactivated whole virus vaccine developed in China to enter human trials. "The inactivated SARS-CoV-2 vaccine, BBIBP-CorV, is safe and well tolerated at all tested doses in two age groups. Humoral responses against SARS-CoV-2 were induced in all vaccine recipients on day 42. Two-dose immunisation with 4 µg vaccine on days 0 and 21 or days 0 and 28 achieved higher neutralising antibody titres than the single 8 µg dose or 4 µg dose on days 0 and 14." There is also an associated commentary on this study.

ProPublica have a long story on the fall of the CDC.

MedScape point to a Reuters story on the increased use of rapid antigen tests to contain the second wave of COVID-19 cases.

Derek Lowe comments on the J&J vaccine adverse reaction and also on immunity and re-infection.

MODELING

- Nothing new today.

NEWLY REGISTERED CLINICAL TRIALS

- Don't get your hopes up that I will report on this today; I won't.

CLINICAL TRIAL RESULTS

- BACKGROUND WHO expert groups recommended mortality trials in hospitalized COVID-19 of four re-purposed antiviral drugs. METHODS Study drugs were Remdesivir, Hydroxychloroquine,

Lopinavir (fixed-dose combination with Ritonavir) and Interferon-β1a (mainly subcutaneous; initially with Lopinavir, later not). COVID-19 inpatients were randomized equally between whichever study drugs were locally available and open control (up to 5 options: 4 active and local standard-of-care). The intent-to-treat primary analyses are of in-hospital mortality in the 4 pairwise comparisons of each study drug vs its controls (concurrently allocated the same management without that drug, despite availability). Kaplan-Meier 28-day risks are unstratified; log-rank death rate ratios (RRs) are stratified for age and ventilation at entry. RESULTS In 405 hospitals in 30 countries 11,266 adults were randomized, with 2750 allocated Remdesivir, 954 Hydroxychloroquine, 1411 Lopinavir, 651 Interferon plus Lopinavir, 1412 only Interferon, and 4088 no study drug. Compliance was 94-96% midway through treatment, with 2-6% crossover. 1253 deaths were reported (at median day 8, IQR 4-14). Kaplan-Meier 28-day mortality was 12% (39% if already ventilated at randomization, 10% otherwise). Death rate ratios (with 95% CIs and numbers dead/randomized, each drug vs its control) were: Remdesivir RR=0.95 (0.81-1.11, p=0.50; 301/2743 active vs 303/2708 control), Hydroxychloroquine RR=1.19 (0.89-1.59, p=0.23; 104/947 vs 84/906), Lopinavir RR=1.00 (0.79-1.25, p=0.97; 148/1399 vs 146/1372) and Interferon RR=1.16 (0.96-1.39, p=0.11; 243/2050 vs 216/2050). No study drug definitely reduced mortality (in unventilated patients or any other subgroup of entry characteristics), initiation of ventilation or hospitalisation duration. *CONCLUSIONS These Remdesivir, Hydroxychloroquine, Lopinavir and Interferon regimens appeared to have little or no effect on hospitalized COVID-19, as indicated by overall mortality, initiation of ventilation and duration of hospital stay. The mortality findings contain most of the randomized evidence on Remdesivir and Interferon, and are consistent with meta-analyses of mortality in all major trials*. (Funding: WHO. Registration: ISRCTN83971151, NCT04315948) [**note: these are interim results from the WHO SOLIDARITY trial. Interesting that remdesivir seems not to be effective here and no surprise about HCQ.**] https://www.medrxiv.org/content/10.1101/2020.10.15.20209817v1

DRUG DEVELOPMENT

- Antigenic imprinting, which describes the bias of antibody response due to previous immune history, can influence vaccine effectiveness and has been reported in different viruses. Give that COVID-19 vaccine development is currently a major focus of the world, there is a lack of understanding of how background immunity influence antibody response to SARS-CoV-2. This study provides evidence for antigenic imprinting in Sarbecovirus, which is the subgenus that SARS-CoV-2 belongs to. Specifically, we sequentially immunized mice with two antigenically distinct Sarbecovirus strains, namely SARS-CoV and SARS-CoV-2. We found that the neutralizing antibodies triggered by the sequentially immunization are dominantly against the one that is used for priming. Given that the impact of the background immunity on COVID-19 is still unclear, our results will provide important insights into the pathogenesis of this disease as well as COVID-19 vaccination strategy. [**note: this Chinese paper at sequential immunization in an animal model with to different coronaviruses. The immune response appears to be greater in the 'priming' virus vaccine. I'm unsure what this means in the real world.**] https://www.biorxiv.org/content/10.1101/2020.10.14.339465v1
- SARS-CoV-2, a positive single-stranded RNA virus, caused the COVID-19 pandemic. Although its sense-mRNA architecture was reported, its anti-sense strand was unexplored. Here, we deeply sequenced both strands of RNA and found SARS-CoV-2 transcription is strongly biased to form

the sense strand. During negative strand synthesis, apart from canonical sub-genomic ORFs, numerous non-canonical fusion transcripts are formed, driven by 3-15 nt sequence homology scattered along the genome but more prone to be inhibited by SARS-CoV-2 RNA polymerase inhibitor Remdesivir. The drug also represses more of the negative than the positive strand synthesis as supported by a mathematic simulation model and experimental quantifications. Overall, this study opens new sights into SARS-CoV-2 biogenesis and may facilitate the anti-viral drug design. [**note: another paper from China, this one looking at the inhibitory effect of remdesivir.**] https://www.biorxiv.org/content/10.1101/2020.10.15.325050v1

- The Spike protein of SARS-CoV-2, its receptor binding domain (RBD), and its primary receptor ACE2 are extensively glycosylated. The impact of this post-translational modification on viral entry is yet unestablished. We expressed different glycoforms of the Spike-protein and ACE2 in CRISPR-Cas9 glycoengineered cells, and developed corresponding SARS-CoV-2 pseudovirus. We observed that N- and O-glycans had only minor contribution to Spike-ACE2 binding. However, these carbohydrates played a major role in regulating viral entry. Blocking N-glycan biosynthesis at the oligomannose stage using both genetic approaches and the small molecule kifunensine dramatically reduced viral entry into ACE2 expressing HEK293T cells. Blocking O-glycan elaboration also partially blocked viral entry. Mechanistic studies suggest multiple roles for glycans during viral entry. Among them, inhibition of N-glycan biosynthesis enhanced Spike-protein proteolysis. This could reduce RBD presentation on virus, lowering binding to host ACE2 and decreasing viral entry. Overall, chemical inhibitors of glycosylation may be evaluated for COVID-19. [**note: this one is from SUNY Buffalo and shows the importance of glycosylation for viral entry. Perhaps this is a good therapeutic target.**] https://www.biorxiv.org/content/10.1101/2020.10.15.339838v1
- SARS-CoV-2, the coronavirus that causes COVID-19, evades the human immune system by capping its RNA. This process protects the viral RNA and is essential for its replication. Multiple viral proteins are involved in this RNA capping process including the nonstructural protein 16 (nsp16) which is an S-adenosyl-L-methionine (SAM)-dependent 2'-O-methyltransferase. Nsp16 is significantly active when in complex with another nonstructural protein, nsp10, which plays a key role in its stability and activity. Here we report the development of a fluorescence polarization (FP)-based RNA displacement assay for nsp10-nsp16 complex in 384-well format with a Z'-Factor of 0.6, suitable for high throughput screening. In this process, we purified the nsp10-nsp16 complex to higher than 95% purity and confirmed its binding to the methyl donor SAM, product of the reaction, SAH, and a common methyltransferase inhibitor, sinefungin using Isothermal Titration Calorimetry (ITC). The assay was further validated by screening a library of 1124 drug-like compounds. This assay provides a cost-effective high throughput method for screening nsp10-nsp16 complex for RNA-competitive inhibitors towards developing COVID-19 therapeutics. [**note: these Toronto scientists report on the development of a high throughput screening system for the nsp1-nsp16 complex that can be used for drug development.**] https://www.biorxiv.org/content/10.1101/2020.10.14.340034v1
- The novel coronavirus SARS-CoV-2 has been identified as the causal agent of COVID-19 and stands at the center of the current global human pandemic, with death toll exceeding one million. The urgent need for a vaccine has led to the development of various immunization approaches. mRNA vaccines represent a cell-free, simple and rapid platform for immunization, and therefore have been employed in recent studies towards the development of a SARS-CoV-2

vaccine. In this study, we present the design of a lipid nanoparticles (LNP)-encapsulated receptor binding domain (RBD) mRNA vaccine. Several ionizable lipids have been evaluated in vivo in a luciferase mRNA reporter assay, and two leading LNPs formulation have been chosen for the subsequent RBD mRNA vaccine experiment. Intramuscular administration of LNP RBD mRNA elicited robust humoral response, high level of neutralizing antibodies and a Th1-biased cellular response in BALB/c mice. These novel lipids open new avenues for mRNA vaccines in general and for a COVID19 vaccine in particular. [**note; it looks like Israel is getting into the nRNA vaccine race with a different type of lipid formulation.**]
https://www.biorxiv.org/content/10.1101/2020.10.15.341537v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The COVID-19 (Coronavirus disease-2019) pandemic, caused by the SARS-CoV-2 coronavirus, is a significant threat to public health and the global economy. SARS-CoV-2 is closely related to the more lethal but less transmissible coronaviruses SARS-CoV-1 and MERS-CoV. Here, we have carried out comparative viral-human protein-protein interaction and viral protein localization analysis for all three viruses. Subsequent functional genetic screening identified host factors that functionally impinge on coronavirus proliferation, including Tom70, a mitochondrial chaperone protein that interacts with both SARS-CoV-1 and SARS-CoV-2 Orf9b, an interaction we structurally characterized using cryo-EM. Combining genetically-validated host factors with both COVID-19 patient genetic data and medical billing records identified important molecular mechanisms and potential drug treatments that merit further molecular and clinical study. [**note: this is from the big group headed up by UCSF group. Several of their earlier papers have been linked in the newsletter.**]
https://science.sciencemag.org/content/early/2020/10/14/science.abe9403
- The innate immune system efficiently defends the human host against viral pathogens. Thus, viruses evolved strategies to counteract immune activation. Here, we systematically analysed the impact of 29 SARS-CoV-2 encoded proteins on three major arms of our cell-intrinsic innate immune defences: interferon (IFN) induction, cytokine signalling and autophagy. Subsequent mechanistic analyses revealed that SARS-CoV-2 proteins target the respective signalling cascades at multiple steps. For example, we show that Nsp14 reduces endogenous IFN receptor levels and ORF3a and ORF7a perturb the late endosomal/trans-Golgi network. Our data demonstrates that most antagonistic activities are conserved between proteins encoded by SARS-CoV-2, the closely related bat RaTG13-CoV and the highly pathogenic SARS-CoV-1. *However, SARS-CoV-1 Nsp15 is strikingly more potent in suppressing IFN induction and signalling than its SARS-CoV-2 counterpart. This may help explain the lower pathogenicity of SARS-CoV-2, which facilitated its rapid spread. Overall our analyses revealed that IFN-γ and IFN-λ1 signalling are antagonised the least, leaving SARS-CoV-2 highly susceptible to these two cytokines. Their combination synergistically potentiated the anti-viral effects against SARS-CoV-2 at low concentrations. Taken together, our results allow an explanation for differences in susceptibility towards IFNs and provide evidence that rational immune activation may be an effective future therapeutic strategy against SARS-CoV-2*. [**note: from Germany, this paper may explain the role of interferons in suppressing viral infection. It is interesting that there seems to be a difference between SARS-CoV-1 and SARS-CoV-2 in this regard.**]
https://www.biorxiv.org/content/10.1101/2020.10.15.340612v1

- Cell entry of the pandemic virus SARS-CoV-2 is mediated by its spike protein S. As main antigenic determinant, S protein is in focus of antibody-based prophylactic and therapeutic strategies. Besides particle-cell fusion, S mediates fusion between infected and uninfected cells resulting in syncytia formation. Here we present quantitative assay systems covering not only particle-cell and cell-cell fusion, but also demonstrating fusion-from-without (FFWO), the formation of syncytia induced by S-containing viral particles in absence of newly synthesized S protein. Based on complementation of split β-galactosidase and virus-like-particles (VLPs) displaying S protein, this assay can be performed at BSL-1. All three assays provided readouts with a high dynamic range and signal-to-noise ratios covering several orders of magnitude. The data obtained confirm the enhancing effect of trypsin and overexpression of angiotensin-converting enzyme 2 (ACE2) on membrane fusion. *Neutralizing antibodies as well as sera from convalescent patients inhibited particle-cell fusion with high efficiency. Cell-cell fusion, in contrast, was only moderately inhibited despite requiring much lower levels of S protein, which were below the detection limit of flow cytometry and Western blot. The data indicate that syncytia formation as a pathological consequence in tissues of Covid-19 patients can proceed at low levels of S protein and may not be effectively prevented by antibodies*. [**note: this is a German study that looks at an alternative cell entry for the virus that appears not to be well controlled by antibodies. I'm not sure how prevalent this is in real world situations but it's something worth further research.**] https://www.biorxiv.org/content/10.1101/2020.10.15.340604v1

DIAGNOSTIC DEVELOPMENT

- The ongoing coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, poses a severe threat to humanity. Rapid and comprehensive analysis of both pathogen and host sequencing data is critical to track infection and inform therapies*. In this study, we performed unbiased metatranscriptomic analysis of clinical samples from COVID-19 patients using a newly-developed RNA-seq library construction method (TRACE-seq), which utilizes tagmentation activity of Tn5 on RNA/DNA hybrids. This approach avoids the laborious and time-consuming steps in traditional RNA-seq procedure, and hence is fast, sensitive and convenient. We demonstrated that TRACE-seq allowed integrated characterization of full genome information of SARS-CoV-2, putative pathogens causing coinfection, antibiotic resistance and host response from single throat swabs*. We believe that the integrated information will deepen our understanding of pathogenesis and improve diagnostic accuracy for infectious diseases. [**note: this paper is from China and is a new approach to diagnostic testing. It's an interesting technology and I look forward to seeing what the sensitivity and specificity are.**] https://www.biorxiv.org/content/10.1101/2020.10.15.340794v1

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2020-10-17

I'm going to stay with rock for another day (maybe more, we'll see). One of the great super groups of the 1960s was Cream with Eric Clapton, Jack Bruce and Ginger Baker. I wrote music reviews for the school paper at UC Santa Barbara which had the benefit of scoring a free pair of tickets to the on-campus concerts and saw the group in person in April 1968. I think this was the last tour before the

broke up and the performance was electrifying. It was great when Bruce and Clapton left the stage during 'Toad' so that Baker would have the drum solo alone. Here is 'Crossroads' from a San Francisco performance just before they came down the coast: https://www.youtube.com/watch?v=7HfkSzsyh1E I always liked 'White Room': https://www.youtube.com/watch?v=0bcrkiCPNso The group got back together for a final set of concerts at the Royal Albert Hall in London. Here is another one of my faves 'Sunshine of Your Love': https://www.youtube.com/watch?v=vyftaay-pFA Good memories for me looking at these clips!!! I hope all my readers enjoy this classic rock.

The Washington Post has a story on the lack of treatment options for the 'long haulers.' I would like to see some quantitative data on how many patients fall into this category along with a breakdown by sex and age. Should you get a COVID-19 test if you just have cold symptoms? If we had a more robust testing system in the US the answer would be easy. Some folks are choosing to move to the country. Hawaii has a new travel program to encourage tourism. I hope it works!!!

The New York Times has the cautionary story of a Montana city that was once virus free but now is in the middle of an outbreak. This Israeli charity group is trying to help out the ultra-Orthodox get in home health care. A plan for a COVID-19 vaccine distribution has been released that will use CVS and Walgreens to sever long term care facilities. The Navy carrier USS Theodore Roosevelt is confronting it's second COVID-19 outbreak. I really don't understand why there is resistance to increasing virus testing on the part of the Trump Administration. It makes no sense. On interesting finding is there appears to be a drop in preterm births during the first phase of the pandemic.

The Lancet have a rapid review of cytokine elevation in severe COVID-19 and compare it to other inflammatory syndromes. "The systemic inflammatory profile of COVID-19 is distinct from that of non-COVID-19 ARDS, sepsis, and CAR T cell-induced cytokine release syndrome; applying the descriptor cytokine storm to COVID-19 might be particularly problematic. Alternative models of organ dysfunction in COVID-19, such as endovasculitis, direct viral injury and lymphodepletion, or viral-induced immunosuppression, might be worth considering."

Politico have an interesting article on the FDA Vaccine Advisory Committee.

ProPublica have a story on how the government funded vaccine trials will be handled. Pfizer is not part of Operation Warp Speed.

MODELING

- Public health measures and private behaviour are based on reported numbers of SARS-CoV-2 infections. Some argue that testing influences the confirmed number of infections. OBJECTIVES/METHODS. Do time series on reported infections and the number of tests allow one to draw conclusions about actual infection numbers? A SIR model is presented where the true numbers of susceptible, infectious and removed individuals are unobserved. Testing is also modelled. RESULTS. Official confirmed infection numbers are likely to be biased and cannot be compared over time. The bias occurs because of different reasons for testing (e.g. by symptoms, representative or testing travellers). The paper illustrates the bias and works out the effect of

the number of tests on the number of reported cases. The paper also shows that the positive rate (the ratio of positive tests to the total number of tests) is uninformative in the presence of non-representative testing. CONCLUSIONS. A severity index for epidemics is proposed that is comparable over time. This index is based on Covid-19 cases and can be obtained if the reason for testing is known. [**note: this paper from Germany purports to remove the testing bias in SARS-CoV-2 statistics. I 'm not sure I accept the premise.**]
https://www.medrxiv.org/content/10.1101/2020.10.14.20212431v1

- Unprecedented sequencing efforts have, as of October 2020, produced over 100,000 genomes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that is responsible for the ongoing COVID-19 crisis. Understanding the trends in SARS-CoV-2 evolution is paramount to control the pandemic. Although this extensive data availability quickly facilitated the development of vaccine candidates1, major challenges in the analysis of this enormous dataset persist, limiting the ability of public health officials to translate science into policy. Having evolved over a short period of time, the SARS-CoV-2 isolates show low diversity, necessitating analysis of trees built from genome-scale data. Here we provide a complete ancestral genome reconstruction for SARS-CoV-2 leveraging Fitch Traceback2. *We show that the ongoing evolution of SARS-CoV-2 over the course of the pandemic is characterized primarily by purifying selection. However, a small set of sites, including the extensively studied spike 6143, harbor mutations which recurred on multiple, independent occasions, indicative of positive selection. These mutations form a strongly connected network of apparent epistatic interactions. The phylogenetic tree of SARS-CoV-2 consists of 7 major clades which show distinct global and temporal dynamics. Periods of regional diversification of SARS-CoV-2 are short and, despite dramatically reduced travel4, globalization of the virus is apparent.* [**note: here is a good model of the adaptive evolution and globalization of SARS-CoV-2**]
https://www.biorxiv.org/content/10.1101/2020.10.12.336644v1
- Since emerging in late 2019, SARS-CoV-2 has caused a global pandemic, and it may become an endemic human pathogen. Understanding the impact of environmental conditions on SARS-CoV-2 viability and its transmission potential is crucial to anticipating epidemic dynamics and designing mitigation strategies. Ambient temperature and humidity are known to have strong effects on the environmental stability of viruses, but there is little data for SARS-CoV-2, and a general quantitative understanding of how temperature and humidity affect virus stability has remained elusive. Here, we characterise the stability of SARS-CoV-2 on an inert surface at a variety of temperature and humidity conditions, and introduce a mechanistic model that enables accurate prediction of virus stability in unobserved conditions. *We find that SARS-CoV-2 survives better at low temperatures and extreme relative humidities; median estimated virus half-life was more than 24 hours at 10 °C and 40 % RH, but less than an hour and a half at 27 °C and 65 % RH. Our results highlight scenarios of particular transmission risk, and provide a mechanistic explanation for observed superspreading events in cool indoor environments such as food processing plants. Moreover, our model predicts observations from other human coronaviruses and other studies of SARS-CoV-2, suggesting the existence of shared mechanisms that determine environmental stability across a number of enveloped viruses*. [**note: here is more data on viral stability as a function of temperature and humidity.**]
https://www.biorxiv.org/content/10.1101/2020.10.16.341883v1

NEWLY REGISTERED CLINICAL TRIALS

- I already checked this week!

CLINICAL TRIAL RESULTS

- The clinical manifestations of COVID-19 vary broadly, ranging from asymptomatic infection to acute respiratory failure and death. But the predictive biomarkers for characterizing the variability are still lacking. Since emerging evidence indicates that extracellular vesicles (EVs) and extracellular RNAs (exRNAs) are functionally involved in a number of pathological processes, we hypothesize that these extracellular components may be key determinants and/or predictors of COVID-19 severity. To test our hypothesis, we collected serum samples from 31 patients with mild COVID-19 symptoms at the time of their admission. After standard therapy without corticosteroids, 9 of the 31 patients developed severe COVID-19 symptoms. We analyzed EV protein and exRNA profiles to look for correlations between these profiles and COVID-19 severity. Strikingly, we identified three distinct groups of markers (antiviral response-related EV proteins, coagulation-related markers, and liver damage-related exRNAs) with the potential to serve as early predictive biomarkers for COVID-19 severity. Among these markers, EV COPB2 has the best predictive value for severe deterioration of COVID-19 patients in this cohort. This type of information concerning functional extracellular component profiles could have great value for patient stratification and for making early clinical decisions about strategies for COVID-19 therapy. [**note: this one is from Japan and offers another predictive model for COVID-19 severity.**] https://www.medrxiv.org/content/10.1101/2020.10.14.20212340v1

DRUG DEVELOPMENT

- Every year, influenza causes 290.000 to 650.000 deaths worldwide and vaccination is encouraged to prevent infection in high-risk individuals. Interestingly, cross-protective effects of vaccination against heterologous infections have been reported, and long-term boosting of innate immunity (also termed trained immunity) has been proposed as the underlying mechanism. Several epidemiological studies also suggested cross-protection between influenza vaccination and COVID-19 during the current pandemic. However, the mechanism behind such an effect is unknown. Using an established in-vitro model of trained immunity, we demonstrate that the quadrivalent inactivated influenza vaccine used in the Netherlands in the 2019-2020 influenza season can induce a trained immunity response, including an improvement of cytokine responses after stimulation of human immune cells with SARS-CoV-2. In addition, we found that SARS-CoV-2 infection was less common among Dutch hospital employees who had received influenza vaccination during the 2019/2020 winter season (RR = 0,61 (95% CI, 0.4585 - 0.8195, P = 0.001). In conclusion, *a quadrivalent inactivated influenza vaccine can induce trained immunity responses against SARS-CoV-2, which may result in relative protection against COVID-19. These data, coupled with similar recent independent reports, argue for a beneficial effect of influenza vaccination against influenza as well as COVID-19, and suggests its effective deployment in the 2020-2021 influenza season to protect against both infections*. [**note: I'm not sure which topic area this one fits. It shows that getting the flu vaccine may offer some protection from SARS-CoV-2. It would be good to have more observational data on this, particularly from the US. Get your flu shot ASAP.]** https://www.medrxiv.org/content/10.1101/2020.10.14.20212498v1
- The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes Coronavirus Disease 2019 (COVID-19), has caused a global pandemic. Antibodies are powerful

biotherapeutics to fight viral infections; however, discovery of the most potent and broadly acting clones can be lengthy. Here, we used the human apoferritin protomer as a modular subunit to drive oligomerization of antibody fragments and transform antibodies targeting SARS-CoV-2 into exceptionally potent neutralizers. Using this platform, half-maximal inhibitory concentration (IC50) values as low as 9E-14 M were achieved as a result of up to 10,000-fold potency enhancements. *Combination of three different antibody specificities and the fragment crystallizable (Fc) domain on a single multivalent molecule conferred the ability to overcome viral sequence variability together with outstanding potency and Ig-like in vivo bioavailability. This MULTi-specific, multi-Affinity antiBODY (Multabody; or MB) platform contributes a new class of medical countermeasures against COVID-19 and an efficient approach to rapidly deploy potent and broadly-acting therapeutics against infectious diseases of global health importance.* **[note: here is an interesting approach from a Toronto group who create multivalent antibodies against SARS-CoV-2 that are up to 10K more potent. One question I have is whether this might be cleared more quickly than a traditional antibody.**]
https://www.biorxiv.org/content/10.1101/2020.10.15.341636v1

- The virus replication and lung inflammation are basic targets for COVID-19 treatment. To effectively treat COVID-19, the best chemical drug should combine inhibition of SARS-CoV-2 replication and direct suppression of inflammatory cytokine expression together. Our SARS-CoV-2 main protease (Mpro) crystal structure studies revealed Au(I), derived from auranofin (AF) or gold cluster (GA), could specifically bind thiolate of Cys145 of SARS-CoV-2 Mpro. GA or AF could well inhibit Mpro activity and significantly decrease SARS-CoV-2 replication in cell. Cell studies showed that either AF or GA could down-regulate NFκB pathway, therefore significantly inhibit inflammatory cytokine level of IL-6, IL-1β, TNF-α in macrophage and bronchial epithelial cell, respectively. The lung viral load in GA treated COVID-19 mice (15mg/kg.bw) is significantly lower than that in normal saline (NS, 0.9% NaCl) treated COVID-19 mice, and pathological studies revealed GA treatment (score ~1.8) significantly reduced lung inflammatory injury compared with NS treated COVID-19 mice (score ~3). After normal mice were treated by GA (15mg/kg), the Au ingredient well distributed into lungs and there are no pathological changes in main organs when compared with control mice. The toxicity results revealed GA is more safety than auranofin for cell/mice/rat. The rat pharmacokinetics studies show GA is with high bioavailability (> 90%) in vivo. [**note: 'there's gold in them thar hills' as this study from China shows. I had no idea that gold would have such a therapeutic effect.**]
https://www.biorxiv.org/content/10.1101/2020.10.16.342097v1
- We developed a global peptide vaccine against SARS-CoV-2 that addresses the dual challenges of heterogeneity in the immune responses of different individuals and potential heterogeneity of the infecting virus. PolyPEPI-SCoV-2 is a polypeptide vaccine containing nine 30-mer peptides derived from all four major structural proteins of the SARS-CoV-2. Vaccine peptides were selected based on their frequency as HLA class I and class II personal epitopes (PEPIs) restricted to multiple autologous HLA alleles of individuals in an in silico cohort of 433 subjects of different ethnicities. PolyPEPI-SCoV-2 vaccine administered with Montanide ISA 51VG adjuvant generated robust, Th1-biased CD8+ and CD4+ T cell responses against all four structural proteins of the virus, as well as binding antibodies upon subcutaneous injection into BALB/c and CD34+ transgenic mice. In addition, PolyPEPI-SCoV-2-specific, polyfunctional CD8+ and CD4+ T cells were detected ex vivo in each of the 17 asymptomatic/mild COVID-19 convalescents' blood

investigated, 1-5 months after symptom onset. The PolyPEPI-SCoV-2-specific T cell repertoire used for recovery from COVID-19 was extremely diverse: donors had an average of seven different peptide-specific T cells, against the SARS-CoV-2 proteins, 87% of donors had multiple targets against at least three SARS-CoV-2 proteins and 53% against all four. In addition, PEPIs determined based on the complete HLA class I genotype of the convalescent donors were validated, with 84% accuracy, to predict PEPI-specific CD8+ T cell responses measured for the individuals. *Extrapolation of the above findings to a US bone marrow donor cohort of 16,000 HLA-genotyped individuals with 16 different ethnicities (n=1,000 each ethnic group) suggest that PolyPEPI-SCoV-2 vaccination in a general population will likely elicit broad, multi-antigenic CD8+ and CD4+ T cell responses in 98% of individuals, independent of ethnicity, including Black, Asian, and Minority Ethnic (BAME) cohorts*. [**note: looks like Hungary are entering the vaccine race. This approach is interesting in that they create a vaccine with epitopes from all the major SARS-CoV-2 structural proteins. It would be good to see what some human data looks like with this vaccine candidate.**] https://www.biorxiv.org/content/10.1101/2020.10.16.339937v1

- The SARS-COV-2 pandemic and the global spread of coronavirus disease 2019 (COVID-19) urgently calls for efficient and safe antiviral treatment strategies. A straightforward approach to speed up drug development at lower costs is drug repurposing. Here we investigated the therapeutic potential of targeting the host- SARS-CoV-2 interface via repurposing of clinically licensed drugs and evaluated their use in combinatory treatments with virus- and host-directed drugs. We tested the antiviral potential of repurposing the antifungal itraconazole and the antidepressant fluoxetine on the production of infectious SARS-CoV-2 particles in the polarized Calu-3 cell culture model and evaluated the added benefit of a combinatory use of these host-directed drugs with remdesivir, an inhibitor of viral RNA polymerase. Drug treatments were well-tolerated and potent impaired viral replication was observed with all drug treatments. Importantly, both itraconazole-remdesivir and fluoxetine-remdesivir combinations inhibited the production of infectious SARS-CoV-2 particles > 90% and displayed synergistic effects in commonly used reference models for drug interaction. Itraconazole-Remdesivir and Fluoxetine-Remdesivir combinations are promising therapeutic options to control SARS-CoV-2 infection and severe progression of COVID-19. [**note: here is a paper that shows remdesivir inhibiton of SARS-CoV-2 can be augmented by fluoxetine or itraconazole. I've seen other references to fluoxetine utility but I think this is the first for the anti-fungal drug.**] https://www.biorxiv.org/content/10.1101/2020.10.16.342410v1
- The COVID-19 pandemic is expected to have an adverse effect on the progression of multiple cancers, including prostate cancer, due to the ensuing cytokine storm and associated oncogenic signaling. Epidemiological data showing increased severity and mortality of COVID-19 in men suggests a potential role for androgen in SARS-CoV-2 infection. Here, we present evidence for the transcriptional regulation of SARS-CoV-2 host cell receptor ACE2 and co-receptor TMPRSS2 by androgen in mouse tissues and human prostate and lung cell lines. Additionally, we demonstrate the endogenous interaction between TMPRSS2 and ACE2 in human cells and validate ACE2 as a TMPRSS2 substrate. In an overexpression model, and the prostate and lung cells, Camostat – a TMPRSS2 inhibitor, blocked the cleavage of pseudotype SARS-CoV-2 surface Spike without disrupting TMPRSS2-ACE2 interaction. Thus providing evidence for the first time a direct role of TMPRSS2 in priming the SARS-CoV-2 Spike protein, required for viral fusion to the host cell. Importantly, androgen-deprivation, anti-androgens such as enzalutamide/AR-PROTAC,

or Camostat treatment attenuated the SARS-CoV-2 S-mediated entry in lung and prostate cells. *Together, our preclinical data provide a strong rationale for clinical evaluations of the TMPRSS2 inhibitors, androgen-deprivation therapy and androgen receptor antagonists alone or in combination with anti-viral drugs as early as clinically possible to prevent inflammation driven COVID-19 progression*. {**note: this is from Univ of Pennsylvania and suggests that androgen deprivation therapy may be useful as a combination therapy for COVID-19.**]
https://www.biorxiv.org/content/10.1101/2020.10.16.342782v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Virus-specific humoral and cellular immunity act synergistically to protect the host from viral infection. We interrogated the dynamic changes of virological and immunological parameters in 12 patients with symptomatic acute SARS-CoV-2 infection from disease onset to convalescence or death. *We quantified SARS-CoV-2 viral RNA in the respiratory tract in parallel with antibodies and circulating T cells specific for various structural (NP, M, ORF3a and spike) and non-structural proteins (ORF7/8, NSP7 and NSP13). We observed that while rapid induction and quantity of humoral responses were associated with increased disease severity, an early induction of SARS-CoV-2 specific T cells was present in patients with mild disease and accelerated viral clearance*. These findings provide further support for a protective role of SARS-CoV-2 specific T cells over antibodies during SARS-CoV-2 infection with important implications in vaccine design and immune-monitoring. [**note: this is an interesting study that shows rapid induction of specific T cells is present in patients with mild disease and accelerated clearance. How to achieve this in clinical practice is another issue and perhaps a multifactor vaccine would be better than a single protein one.**] https://www.biorxiv.org/content/10.1101/2020.10.15.341958v1
- Characterization of antibody response to SARS-CoV-2 is urgently needed to predict COVID-19 disease trajectories. Ineffective antibodies or antibody-dependent enhancement (ADE) could derail patient immune responses, for example. ELISA and coronavirus antigen microarray (COVAM) analysis epitope-mapped plasma from 86 COVID-19 patients. The experiments identified antibodies to a 21-residue epitope from nucleocapsid (termed Ep9) associated with severe disease, including ICU stay, requirement for ventilators, and death. Furthermore, anti-Ep9 antibodies correlate both with various comorbidities and ADE hallmarks, including increased IL-6 levels and early IgG response. Importantly, anti-Ep9 antibodies can be detected within five days post-symptom onset and sometimes within one day. The results lay the groundwork for a new type of COVID-19 diagnostic for the early prediction of disease severity to guide more effective therapeutic interventions. [**note: this one is from UC Irvine and shows that antibody response to a segment of the nucleocapsid protein is associated with more adverse outcomes. It may result from antibody dependent enhancement.**]
https://www.biorxiv.org/content/10.1101/2020.10.15.341743v1
- Our immune system plays a critical role in preventing, clearing, and treating the virus, but aberrant immune responses can contribute to deleterious symptoms and mortality. Many aspects of immune responses to SARS-CoV-2 are being investigated, but little is known about immune responses to carbohydrates. Since the surface of the virus is heavily glycosylated, pre-existing antibodies to glycans could potentially recognize the virus and influence disease progression. Furthermore, antibody responses to carbohydrates could be induced, affecting disease severity and clinical outcome. In this study, we used a carbohydrate antigen microarray

with over 800 individual components to profile serum anti-glycan antibodies in COVID-19 patients and healthy control subjects. In COVID-19 patients, we observed abnormally high IgG and IgM antibodies to numerous self-glycans, including gangliosides, N-linked glycans, LacNAc-containing glycans, blood group H, and sialyl Lewis X. Some of these anti-glycan antibodies are known to play roles in autoimmune diseases and neurological disorders, which may help explain some of the unusual and prolonged symptoms observed in COVID-19 patients. *The detection of antibodies to self-glycans has important implications for using convalescent serum to treat patients, developing safe and effective SARS-CoV-2 vaccines, and understanding the risks of infection. In addition, this study provides new insight into the immune responses to SARS-CoV-2 and illustrates the importance of including host and viral carbohydrate antigens when studying immune responses to viruses.* [**note: lots of antibody papers today. Here is one that looks at abnormal antibodies to self carbohydrates. These type of reactions are found in autoimmune disorders and this may play role in some COVID-19 patients.**]
https://www.biorxiv.org/content/10.1101/2020.10.15.341479v1

DIAGNOSTIC DEVELOPMENT

- With growing concern of persistent or multiple waves of SARS-CoV-2 in the United States, sensitive and specific SARS-CoV-2 antibody assays remain critical for community and hospital-based SARS-CoV-2 surveillance. Here, we describe the development and application of a multiplex microsphere-based immunoassay (MMIA) for COVD-19 antibody studies, utilizing serum samples from non-human primate SARS-CoV-2 infection models, an archived human sera bank and subjects enrolled at five U.S. military hospitals. The MMIA incorporates prefusion stabilized spike glycoprotein trimers of SARS-CoV-2, SARS-CoV-1, MERS-CoV, and the seasonal human coronaviruses HCoV-HKU1 and HCoV-OC43, into a multiplexing system that enables simultaneous measurement of off-target pre-existing cross-reactive antibodies. We report the sensitivity and specificity performances for this assay strategy at 98% sensitivity and 100% specificity for subject samples collected as early as 10 days after the onset of symptoms. In archival sera collected prior to 2019 and serum samples from subjects PCR negative for SARS-CoV-2, we detected seroprevalence of 72% and 98% for HCoV-HKU1 and HCoV-0C43, respectively. *Requiring only 1.25 uL of sera, this approach permitted the simultaneous identification of SARS-CoV-2 seroconversion and polyclonal SARS-CoV-2 IgG antibody responses to SARS-CoV-1 and MERS-CoV, further demonstrating the presence of conserved epitopes in the spike glycoprotein of zoonotic betacoronaviruses. Application of this serology assay in observational studies with serum samples collected from subjects before and after SARS-CoV-2 infection will permit an investigation of the influences of HCoV-induced antibodies on COVID-19 clinical outcomes*. [**note: here is an interesting multiplex assay system that will detect early SARS-CoV-2 seroconversion.**]
https://www.medrxiv.org/content/10.1101/2020.10.14.20207050v1

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2020-10-18

For reflection Sunday here is another in the Wigmore Hall fall series. This time it is the young German pianist Elisabeth Brauss in a program of Beethoven, Mendelssohn and Prokofiev. This will come as a

respite from the recent rock and country selections. Enjoy:
https://www.youtube.com/watch?v=Vy41ekgUpN4

The Washington Post has a story about the differential mortality between men and women. Pandemic learning is causing problems across the world. The southern part of Italy, left untouched during the first wave, is now being hit hard. It looks like the famed Sturgis motorcycle rally did cause a spike in Midwestern COVID-19 cases.

The New York Times writes about pandemic fatigue. Yes, it is real and it has to be resisted if you want to protect the public health.

The Atlantic has an article by Brown Univ economist Emily Oster on why schools are not super-spreaders. There is a link to the project dashboard in the article that is worth looking at as it covers some of the mitigation measures that schools have taken. She also had a recent piece in The New York Times. It's important to note that this is just a small snapshot of 200,000 students and many large metro school districts have not opened yet this fall (our local county public school system is still all remote learning). I don't know if she has data linked to zip code or area where one can look at community spread of SARS-CoV-2 and whether there is a correlation. Since many children are asymptomatic but still capable of viral transmission, it is unclear how to count those numbers or if they matter. I look forward to following this research as it goes forward. It is truly a shame that this was not a major project of the CDC and is perhaps another example of how far the famed agency has fallen. This is important reading for those with school age children or teachers in their family.

Derek Lowe reports on the WHO SOLIDARITY trial results.

Kaiser Health News shows how musicians who play wind instruments are improvising to keep the band together.

The New England Journal of Medicine has an FDA perspective on considerations for an Emergency Use Authorization for COVID-19 vaccines. Here is a paper on the genome wide association of severe COVID-19 with respiratory failure along with an editorial on this topic.

THERE ARE NO PAPERS TO READ TODAY AS THE SERVER SHOWS NOTHING NEW! 😂 Sundays are always slow but I usually see one or two. I'm sure there will be a large number at the start of the week.

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