2020-10-05

Welcome to Week 29!

Let us start the week with an up-tempo piece. Here is Willie Nelson's 'I Gotta Get Drunk' performed by Joana Casanova and the Joan Chamorro group from Barcelona. Some of the players come from the St. Andreu school that Chamorro founded, and I am always amazed at the vocalists whose English is a second language. There are some wonderful instrumental solos in this arrangement: https://www.youtube.com/watch?v=zkjQQq9K8BI Here is the country version with Willie and George Jones: https://www.youtube.com/watch?v=HKD_o4gbcSE

Most of the COVID-19 news has centered on the treatment of President Trump at Walter Reed Hospital. You know where to find information on this.

The Washington Post has a Q & A with Pfizer CEO Albert Bourla. For those of you who are traveling here are some tips: how to clean your hotel room & how to stay healthy on a plane. Unfortunately, travel may not be much fun with all the pandemic related construction cutbacks. Here is some really good news. No we don't have a COVID-19 vaccine yet, but the US is on track for the fewest lightning fatalities in a single year! Unrelated to COVID-19, three researchers received the Nobel Prize in Medicine for discoveries related to Hepatitis C.

The New York Times has an interesting op-ed on how imperfect measures can do the most good from a doc at the Hutch in Seattle. Good points here. Unfortunately, New York City is going to have to do some selective shutdowns. As a student dies from COVID-19 complications, Appalachian State tries to get serious about the virus. The movie chain Cineworld (owner of Regal Theaters in the US) is closing all 663 movie houses in the US and UK. In addition to COVID-19 concerns, there are no new releases that they can program.

The Lancet has a nice piece describing how advances in HIV can be leveraged for COVID-19.

Kaiser Health News discusses five things to know about a COVID-19 vaccine.

There has been a marked drop off in COVID-19 papers over the past two weeks. I don't want to be clogging up folks in boxes with short emails. I will continue to evaluate news and scientific developments. *This might warrant shifting away from a daily newsletter*. Of course, I do realize that my readers depend on the daily music selections!!!

MODELING

- Nothing

NEWLY REGISTERED CLINICAL TRIALS

- I promise to check this week!

CLINICAL TRIAL RESULTS

- Nothing

DRUG DEVELOPMENT

- Efforts to mitigate COVID-19 include screening of existing antiviral molecules that could be re-purposed to treat SARS-CoV-2 infections. Although SARS-CoV-2 propagates efficiently in African green monkey kidney (Vero) cells, antivirals such as nucleos(t)ide analogs (nucs) often exhibit decreased activity in these cells due to inefficient metabolization. Limited SARS-CoV-2 replication and propagation occurs in human cells, which are the most relevant testing platforms. By performing serial passages of a SARS-CoV-2 isolate in the human hepatoma cell line clone Huh7.5, we selected viral populations with improved viability in human cells. Culture adaptation led to the emergence of a significant number of high frequency changes (>90% of the viral population) in the region coding for the spike glycoprotein, including a deletion of nine amino acids in the N-terminal domain and 3 amino acid changes (E484D, P812R, and Q954H). We demonstrated that the Huh7.5-adapted virus exhibited a >3-Log10 increase in infectivity titers (TCID50) in Huh7.5 cells, with titers of ~8 Log10TCID50/mL, and >2-Log10 increase in the human lung cancer cell line Calu-1, with titers of ~6 Log10TCID50/mL. Culture adaptation in Huh7.5 cells further permitted efficient infection of the otherwise SARS-CoV-2 refractory human lung cancer cell line A549, with titers of ~6 Log10TCID50/mL. *The enhanced ability of the virus to replicate and propagate in human cells permitted screening of a panel of nine nucs, including broad-spectrum compounds. Remdesivir, EIDD-2801 and to a limited extent galidesivir showed antiviral effect across these human cell lines, whereas sofosbuvir, uprifosbuvir, valopicitabine, mericitabine, ribavirin, and favipiravir had no apparent activity. Importance: The cell culture adapted variant of the SARS-CoV-2 virus obtained in the present study, showed significantly enhanced replication and propagation in various human cell lines, including lung derived cells otherwise refractory for infection with the original virus. This SARS-CoV-2 variant will be a valuable tool permitting investigations across human cell types, and studies of identified mutations could contribute to our understanding of viral pathogenesis. In particular, the adapted virus can be a good model for investigations of viral entry and cell tropism for SARS-CoV-2, in which the spike glycoprotein plays a central role. Further, as shown here with the use of remdesivir and EIDD-2801, two nucs with significant inhibitory effect against SARS-CoV-2, large differences in the antiviral activity are observed depending on the cell line.* Thus, it is essential to select the most relevant target cells for pre-clinical screenings of antiviral compounds, facilitated by using a virus with broader tropism. [**note: from Denmark, this is a good finding for drug development. They tested a number of compounds and found that remdesivir and the Merck experimental drug EIDD-2801 had antiviral activity while several others did not.**]
  https://www.biorxiv.org/content/10.1101/2020.10.04.325316v1
- The coronavirus mouse hepatitis virus (MHV)-1 causes pneumonitis in mice which shares many pathological characteristics with human SARS-CoV infection. Previous studies have shown that the amino acid gamma-aminobutyric acid (GABA) has anti-inflammatory effects. We tested whether oral treatment with GABA could modulate the MHV-1 induced pneumonitis in susceptible A/J mice. As expected, MHV-1-inoculated control mice became severely ill (as measured by weight loss, clinical score, and the ratio of lung weight to body weight) and >60% of them succumbed to the infection. In contrast, mice that received GABA immediately after MHV-1 inoculation became only mildly ill and all of them recovered. When GABA treatment was initiated after the appearance of illness (3 days post-MHV-1 infection), we again observed that GABA treatment significantly reduced the severity of illness and greatly increased the frequency of recovery. Therefore, the engagement of GABA receptors (GABA-Rs) prevented the MHV-1

infection-induced severe pneumonitis and death in mice. Given that GABA-R agonists, like GABA and homotaurine, are safe for human consumption, stable, inexpensive, and available worldwide, they are promising candidates to help prevent severe illness stemming from SARS-CoV-2 infection and other coronavirus strains. [**note: this is an animal study on the use of GABA to treat a coronavirus infection. It is not clear to me that this can be extrapolated to SARS-CoV-2 infections. Nonetheless, GABA is cheap and as with any other drug candidates, "This Is Why We Do Clinical Trials."**]
https://www.biorxiv.org/content/10.1101/2020.10.04.325423v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing

DIAGNOSTIC DEVELOPMENT

- Nothing

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2020-10-06

Yesterday there was a Willie Nelson song and I posted a link to the duet that Willie and George Jones did. It occurred to me that I have not featured Jones, an outstanding country singer who lived life to its fullest and maybe then some. There are a lot of good sound clips of Jones but not as many videos. Here is a good sample. George and his one time wife Tammy Wynette singing 'Golden Ring': https://www.youtube.com/watch?v=Q9KniULwvjE Here is 'White Lightning' from 1959 (yes, that is a young Johnny Cash in this band): https://www.youtube.com/watch?v=WE5pM1HXxlI One of my faves is 'He Stopped Loving Her Today': https://www.youtube.com/watch?v=1R2F9f2Cl6Y and finally a music video 'Wrong's What I Do Best' (I sometimes feel this is one of my traits): https://www.youtube.com/watch?v=d6YGmHE-2XA Wait there's more!! Here is a 2004 all-star tribute: https://www.youtube.com/watch?v=o1h0U23JCBk with some great performances by wonderful country singers.

Kara Swisher who covers technology for The New York Times has a new podcast, "Sway," which is quite good. Her current interview is with Moncef Slaoui, who chairs 'Operation Warp Speed.' Slaoui spent his industrial career in the vaccines division of GSK. This is a very good conversation and Slaoui goes into some detailed conversations about whether he has a conflict of interest and what the scientific standards are for deployment of a COVID-19 vaccine. There is a transcript of the discussion for those who don't want to listen. It turns out that Slaoui is a Democrat and he effectively skewers (my term not his), Senator Warren for implying he cannot be impartial. DO READ OR LISTEN TO THIS. The Times reports on a new study showing one third of hospitalized COVID-19 patients experienced some type of altered mental function. This one is a bit disturbing (well, more than a bit). The Trump Administration is blocking FDA's request to modify the standards for issuance of an Emergency Use Authorization for an experimental COVID-19 vaccine. What appears to be the contentious point is the requirement for two months of follow up for those in the clinical trial so that better information on safety and efficacy can be ascertained. For those of us who have worked in drug safety and regulatory affairs, the two month

follow up is reasonable and one manufacturer has indicated that it will observe this requirement. Here is a useful article on how the immune system responds to SARS-CoV-2.

The Washington Post notes the CDC has now posted guidance on airborne transmission of SARS-CoV-2. What a stunner! Here is one Michigan man who was finally released from the hospital after a six month battle with COVID-19. CONGRATS to UCLA astrophysicist Andrea Ghez and two of her colleagues for winning the Nobel Prize for Physics for discoveries about black holes! Professor Ghez is the fourth woman to win this prize.

The Lancet carries a study from the UK RECOVERY group that shows the HIV medication lopinavir/ritonavir for treatment of COVID-19 was not associated with reductions in 28-day mortality, duration of hospital stay, or risk of progressing to invasive mechanical ventilation or death. In plain English, it don't work. Here is a commentary on the study. For the pathologists who read the newsletter here is a post-mortem neuropathology of German COVID-19 cases. "SARS-CoV-2 RNA and proteins can be detected in the CNS. The brain shows mild neuropathological changes with pronounced neuroinflammation in the brainstem being the most common finding. However, the presence of SARS-CoV-2 in the CNS was not associated with the severity of neuropathological changes." Here is a commentary on the paper.

Kaiser Health News discusses how university dorm resident assistants have become COVID Cops. Here is a useful analysis about what businesses should do to make people feel safe. Saliva tests for SARS-CoV-2 are beginning to catch on.

MODELING

- The airborne transmission of SARS-CoV-2 through virus-containing aerosol particles has been established as an important pathway for Covid-19 infection. Suitable measures to prevent such infections are imperative, especially in situations when a high number of persons convene in closed rooms. Here we tested the efficiency and practicability of operating air purifiers in a high school classroom while regular classes were taking place. Four air purifiers equipped with HEPA filters were installed in a classroom. We monitored the total aerosol number concentration for particles > 3 nm at two locations in the room (uCPC), the aerosol size distribution in the range from 10 nm to 10 µm (SMPS and OPS), aerosol mass (PM10 derived from OPS) as well as the CO_2 concentration in the room. For comparison, we performed similar measurements in parallel by operating a uCPC and an OPS in a neighboring class room without air purifiers. The measurements show that in times when classes were conducted with windows and door closed, the aerosol concentration was reduced by more than 90 % within less than 30 minutes when running the purifiers with a volume flow of 1027 m^3/h in the classroom with a volume of 186 m^3. The reduction was homogeneous throughout the room, as well as homogeneous for all particle sizes. The measurements are supplemented by a basic calculation estimating the maximum concentration levels of virus-containing aerosol from a highly contagious person speaking in a closed room with and without air purifiers. The measurements and the calculation demonstrate that air purifiers represent a well suited measure to reduce the risks of airborne transmission of SARS-CoV-2 substantially. Staying for two hours in a closed room with a super infective person, we estimate that the inhaled dose is reduced by a factor of six when using air purifiers with a total air exchange rate of 5.7 h^{-1}. Nevertheless, in closed rooms with a high person density

frequent ventilation supplying fresh air is necessary to keep the CO_2 concentration levels below the maximum permissible values. [**note: here is some useful information from Germany on the use of using air purifiers in a school classroom. Certainly, it appears to help. I wonder how many schools have these in place. This is the first of this type of research I've seen.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20205633v1

- With the advent of rapid international travel, disease can now spread between nations faster than ever. As such, when outbreaks occur in foreign states, pressure mounts to reduce the risk of importing cases to the home nation. In a previous paper, we developed a model to investigate the potential effectiveness of deploying screening at airports during outbreaks of influenza, SARS, and Ebola. We also applied the model to the current COVID-19 outbreak. This model simulated the testing of travellers (assumed not to be displaying symptoms prior to boarding their flight) as they arrived at their destination. The model showed that the reduction in risk of case importation that screening alone could deliver was minimal across most scenarios considered, with outputs indicating that screening alone could detect at most 46.4%, 12.9%, and 4.0% of travellers infected with influenza, SARS and Ebola respectively, while the model also reported a detection rate of 12.0% for COVID-19. In this paper, we present a brief modification to this model allowing us to assess the added impact that quarantining incoming travelers for various periods may have on reducing the risk of case importation. Primary results show that requiring all travellers to undergo 5 days of self-isolation on arrival, after which they are tested again, has the potential to increase rates of detection to 100%, 87.6%, 81.7% and 41.3% for travellers infected with influenza, SARS, COVID-19 and Ebola respectively. Extending the period of self-isolation to 14 days increases these potential detection rates to 100%, 100%, 99.5% and 91.8% respectively. [**note: screening travelers is not as effective as quarantine in this model.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20205757v1
- It is desirable to better characterize and understand how ventilation improvements in office spaces could offer significant protection against transmission of COVID-19. It is also desirable to understand how ventilation in office spaces compares to outdoor settings. An attempt to find this information from online searches that included medical journals, private industry, and US government provided materials failed to find specific quantitative estimates and recommendations, which motivated this study. This study uses measured amounts of SARS-CoV-2 in the air of a hospital room with COVID-19 patients from a published and peer-reviewed study and known Influenza A challenge doses from a published and peer-reviewed study and known ASHRAE Office Ventilation standards and an Outdoor Air Exchange model to estimate the time necessary to cause various exposure levels and resulting infection potential in various indoor and outdoor settings of both Influenza A and COVID-19. While these estimations have unknown error margins and cannot be considered authoritative, they may have utility in comparing various environments and relative risk factors. The estimates in this study also present an initial framework and specific quantitative examples for better understanding of the effects of ventilation on aerosolized transmission, and the immunology related to challenge doses, and the potential for low-level viral load exposure to result in some level of immunity without symptoms of illness (asymptomatic infection). Specific quantitative examples of exposure viral load versus symptoms and immune response may Increase public understanding and consciousness of concepts such as viral load, exposure time, challenge dose levels, shedding quantities, immune seroconversion, and re-challenge and could achieve new levels of personal

hygiene that complement centuries-old adages such as wash your hands. [**note: this is a useful modeling paper from a pair of California researchers. They present a variety of scenarios that are helpful in decision making.**]
https://www.medrxiv.org/content/10.1101/2020.10.03.20206110v1

NEWLY REGISTERED CLINICAL TRIALS

- The week isn't even half over!!! I will check it, maybe even tomorrow.

CLINICAL TRIAL RESULTS

- BACKGROUND The characteristics of COVID-19 outbreak and high fatality rate of COVID-19 infection have attracted the attention of scientists due to the strong interactions between components of metabolic syndrome, metabolic abnormalities, and viral pathobiology of COVID-19. Combined metabolic cofactors supplementation (CMCS) consisting of L-serine, N-acetyl-L-cysteine (NAC), nicotinamide riboside (NR), and L-carnitine tartrate is being studied for the treatment of patients with COVID-19. METHODS We conducted a placebo-controlled, phase-2 clinical trial involving ambulatory COVID-19 patients. A total of 100 patients were randomly assigned on a 3:1 basis to hydroxychloroquine plus CMCS or hydroxychloroquine plus placebo. The total treatment period for the hydroxychloroquine was 5 days, and for the CMCS/placebo was 14 days. Clinical status was evaluated daily by phone, using a binomial scale for subject reported presence or absence for multiple COVID-19 related symptoms. Plasma samples for clinical chemistry analyses were collected on day 0 and day 14. RESULTS A total of 93 patients completed the trial. The combination of CMCS and hydroxychloroquine significantly reduced the average complete recovery time compared with hydroxychloroquine and placebo (6.6 days vs 9.3 days, respectively). Moreover, there was a significant reduction in ALT, AST and LDH levels on day 14 compared to day 0 in the hydroxychloroquine plus CMCS group. The adverse effects were uncommon and self-limiting. CONCLUSIONS In patients with mild-to-moderate COVID-19, CMCS resulted in a significant reduction in recovery time and liver enzymes associated with hepatic function compared to placebo. We observed that CMSC is associated with a low incidence of adverse events. [**note: I don't know what to make of this Turkish study. They do note in the discussion that HCQ has not demonstrated any efficacy in RCTs but it is recommended care in their country.**]
https://www.medrxiv.org/content/10.1101/2020.10.02.20202614v1
- Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 remains a global threat with few proven efficacious treatments. Transfusion of convalescent plasma collected from donors who have recovered from COVID-19 disease has emerged as a promising therapy and has been granted emergency use authorization by the U.S. Food and Drug Administration (FDA). We recently reported results from interim analysis of a propensity-score matched study suggesting that early treatment of COVID-19 patients with convalescent plasma containing high titer anti-spike protein receptor binding domain (RBD) IgG significantly decreases mortality. We here present results from 60-day follow up of our cohort of 351 transfused hospitalized patients. Prospective determination of ELISA anti-RBD IgG titer facilitated selection and transfusion of the highest titer units available. Retrospective analysis by the Ortho VITROS IgG assay revealed a median signal/cutoff (S/C) ratio of 24.0 for transfused units, a value far exceeding the recently FDA-required cutoff of 12.0 for designation of high titer

convalescent plasma. *With respect to altering mortality, our analysis identified an optimal window of 44 hours post-hospitalization for transfusing COVID-19 patients with high titer convalescent plasma. In the aggregate, the analysis confirms and extends our previous preliminary finding that transfusion of COVID-19 patients soon after hospitalization with high titer anti-spike protein RBD IgG present in convalescent plasma significantly reduces mortality*. [**note: here if further data from a Texas group on the utility of convalescent plasma. Transfusion soon after hospitalization is the way to go.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20206029v1

- The evidence pertaining to the effects of asthma on Coronavirus disease 2019 outcomes has been unclear. To improve our understanding of the clinically important association of asthma and Coronavirus disease 2019. Methods: A matched cohort study was performed using data from the Mass General Brigham Health Care System (Boston, MA). Adult (age ≥ 18 years) patients with confirmed Coronavirus disease 2019 and without chronic obstructive pulmonary disease, cystic fibrosis, or interstitial lung disease between March 4, 2020 and July 2, 2020 were analyzed. Up to 5 non-asthma comparators were matched to each asthma patient based on age (within 5 years), sex, and date of positive test (within 7 days). The primary outcomes were hospitalization, mechanical ventilation, and death, using multivariable Cox-proportional hazards models accounting for competing risk of death, when appropriate. Patients were followed for these outcomes from diagnosis of Coronavirus disease 2019 until July 2, 2020. Results: Among 562 asthma patients, 199 (21%) were hospitalized, 15 (3%) received mechanical ventilation, and 7 (1%) died. Among the 2686 matched comparators, 487 (18%) were hospitalized, 107 (4%) received mechanical ventilation, and 69 (3%) died. The adjusted Hazard Ratios among asthma patients were 0.99 (95% Confidence Internal 0.80, 1.22) for hospitalization, 0.69 (95% Confidence Internal 0.36, 1.29) for mechanical ventilation, and 0.30 (95% Confidence Internal 0.11, 0.80) for death. Conclusions: In this matched cohort study from a large Boston-based healthcare system, asthma was associated with comparable risk of hospitalization and mechanical ventilation but a lower risk of mortality. [**note: from the Mass General, further evidence that asthma does not pose a greater risk and in fact there may be lower mortality in these patients.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20205724v1

DRUG DEVELOPMENT

- Background and Objectives In the absence of a vaccine or specific antiviral drugs against SARS-CoV-2 COVID-19 convalescent plasma became one of the experimental treatment options in many countries. Aim of this study was to assess the impact of different pathogen reduction technologies on the immunological properties of COVID-19 convalescent plasma. Materials and Methods In our experiment 140 doses of plasma collected by plasmapheresis from COVID-19 convalescent donors were subjected to pathogen reduction with one of three different methods: methylene blue (M), riboflavin (R), and amotosalen (A). To conduct a paired two-sample comparison each plasma dose was divided into 2 that were treated by one of these technologies. The titres of SARS-CoV2 neutralizing antibodies (NtAbs) and levels of specific immunoglobulins to RBD, S- and N- proteins of SARS-CoV-2 were measured before and after pathogen reduction. Results All methods reduced NtAbs titers significantly but not at the same grade: among units with the initial titre 80 or above, 81% of units had unchanged titres while 19% decreased by 1 step after methylene blue; 60% unchanged and 40% - decreased by 1 step

after amotosalen; 43% unchanged, 67% a one-step decrease and 6% - a two-step decrease after riboflavin. Pairwise two-sample comparisons (M vs A, M vs R and A vs R) revealed the most prominent and statistically significant decrease in all studied parameters (except anti-RBD) following pathogen reduction with riboflavin. Conclusion Pathogen reduction with amotosalen and methylene blue provides the greater likelihood of preserving the immunological properties of the COVID-19 convalescent plasma compared to riboflavin. [**note: this is a valuable paper from Russia. One of the issues with convalescent plasma might be the presence of pathogens. Processing steps need to maximize pathogen reduction and not impact the potency of the neutralizing antibody titer. It's one of the reasons why mAb therapy may be preferred as the biological product is easier to standardize.**]
https://www.medrxiv.org/content/10.1101/2020.10.02.20205567v1

- A large number of studies are being conducted to evaluate the efficacy and safety of candidate vaccines against novel coronavirus disease-2019 (COVID-19). Most Phase 3 trials have adopted virologically confirmed symptomatic COVID-19 disease as the primary efficacy endpoint, although laboratory-confirmed SARS-CoV-2 is also of interest. In addition, it is important to evaluate the effect of vaccination on disease severity. To provide a full picture of vaccine efficacy and make efficient use of available data, we propose using SARS- CoV-2 infection, COVID-19, and severe COVID-19 as dual or triple primary endpoints. We demonstrate the advantages of this strategy through realistic simulation studies. Finally, we show how this approach can provide rigorous interim monitoring of the trials and efficient assessment of the durability of vaccine efficacy. [**note: here is a proposal for evaluating vaccine efficacy that is intriguing.]** https://www.medrxiv.org/content/10.1101/2020.10.02.20205906v1
- Effective and affordable treatments for patients suffering from coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), are needed. We report in vitro efficacy of Artemisia annua extracts as well as artemisinin, artesunate, and artemether against SARS-CoV-2. The latter two are approved active pharmaceutical ingredients of anti-malarial drugs. Proof-of-concept for prophylactic efficacy of the extracts was obtained using a plaque-reduction assay in VeroE6 cells. Subsequent concentration-response studies using a high-throughput antiviral assay, based on immunostaining of SARS-CoV-2 spike glycoprotein, revealed that pretreatment and treatment with extracts, artemisinin, and artesunate inhibited SARS-CoV-2 infection of VeroE6 cells. In treatment assays, artesunate (50% effective concentration (EC50): 7 µg/mL) was more potent than the tested plant extracts (128-260 µg/mL) or artemisinin (151 µg/mL) and artemether (>179 µg/mL), while generally EC50 in pretreatment assays were slightly higher. The selectivity index (SI), calculated based on treatment and cell viability assays, was highest for artemisinin (54), and roughly equal for the extracts (5-10), artesunate (6) and artemether (<7). Similar results were obtained in human hepatoma Huh7.5 cells. Peak plasma concentrations of artesunate exceeding EC50 values can be achieved. Clinical studies are required to further evaluate the utility of these compounds as COVID-19 treatment. [**note: from Germany, information on *in vitro* efficacy of Artemisia annua extracts. Some of these are in clinical trials.**] https://www.biorxiv.org/content/10.1101/2020.10.05.326637v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- SARS-CoV-2 pandemic, the fourth pandemic of the decade, has underscored gaps in global pandemic preparedness and the need for generalizable tests to avert overwhelming healthcare systems worldwide, irrespective of a virus. We integrated 4,780 blood transcriptome profiles from patients infected with one of 16 viruses across 34 independent cohorts from 18 countries, and 71 scRNA-seq profiles of 264,224 immune cells across three independent cohorts. *We found a myeloid cell-dominated conserved host response associated with severity. It showed increased hematopoiesis, myelopoiesis, and myeloid-derived suppressor cells with increased severity. We identified four gene modules that delineate distinct trajectories associated with mild and severe outcomes, and show the interferon response was decoupled from protective host response during severe viral infection. These modules distinguished non-severe from severe viral infection with clinically useful accuracy. Together, our findings provide insights into immune response dynamics during viral infection, and identify factors that may influence patient outcomes*. [**note: this paper looks across patients infected with a number of different viruses and leads to identification of factors that may differentiate severity of infection. More good information on the immune response.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20205880v1
- SARS-CoV-2 infection of human airway epithelium activates genetic programs that lead to progressive hyperinflammation in COVID-19 patients. Here we report on the transcriptomic response of airway epithelium to interferons and its suppression by the JAK inhibitors Baricitinib and Ruxolitinib. There is a debate on the regulation of the conventional versus the novel intronic promoter inducing the short ACE2 isoform. Through RNA-seq and ChIP-seq analyses for activating chromatin marks and Polymerase II, we define the interferon-activated intronic regulatory region. Our results also support that the conventional ACE2 promoter is controlled by interferon. [**note: information the relationship of interferon and progressive inflammation.**] https://www.biorxiv.org/content/10.1101/2020.10.04.325415v1

DIAGNOSTIC DEVELOPMENT

- Individuals can test positive for SARS-CoV-2 by real-time polymerase chain reaction (RT-PCR) after no longer being infectious.1-8 Positive SARS-CoV-2 antigen-based testing exhibits a temporal pattern that corresponds with active, replicating virus and could therefore be a more accurate predictor of an individuals potential to transmit SARS-CoV-2.2,3,9 Using the BD Veritor System for Rapid Detection of SARS-CoV-2 later flow antigen detection test, we demonstrate a higher concordance of antigen-positive test results with the presence of cultured, infectious virus when compared to RT-PCR. When compared to infectious virus isolation, the sensitivity of antigen-based testing is similar to RT-PCR. *The correlation between SARS-CoV-2 antigen and SARS-CoV-2 culture represents a significant advancement in determining the risk for potential transmissibility beyond that which can be achieved by detection of SARS-CoV-2 genomic RNA. Coupled with a rapid time-to-result, low cost, and scalability, antigen-based testing should facilitate effective implementation of testing and public health interventions that will better contain COVID-19*. [**note: this may address a critical gap in diagnostic testing. RT-PCR tests can show up as positive even if the person is not capable of viral transmission. Of course scale up mass testing really makes this work.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20205708v1
- We evaluated saliva (SAL) specimens for SARS-CoV-2 RT-PCR testing by comparison of 459 prospectively paired nasopharyngeal (NP) or mid-turbinate (MT) swabs from 449 individuals

with the aim of using saliva for asymptomatic screening. Samples were collected in a drive-through car line for symptomatic individuals (N=380) and in the emergency department (ED) (N=69). The percent positive and negative agreement of saliva compared to nasopharyngeal swab were 81.1% (95% CI: 65.8% – 90.5%) and 99.8% (95% CI: 98.7% – 100%), respectively. The sensitivity increased to 90.0% (95% CI: 74.4% – 96.5%) when considering only samples with moderate to high viral load (Cycle threshold (Ct) for the NP <=34). Pools of five saliva specimens were also evaluated on three platforms: bioMérieux NucliSENS easyMAG with ABI 7500Fast (CDC assay), Hologic Panther Fusion, and Roche COBAS 6800. The median loss of signal upon pooling was 2-4 Ct values across the platforms. *The sensitivity of detecting a positive specimen in a pool compared with testing individually was 100%, 93%, and 95% for CDC 2019-nCoV Real-Time RT-PCR, Panther Fusion® SARS-CoV-2 assay, and cobas® SARS-CoV-2 test respectively, with decreased sample detection trending with lower viral load. We conclude that although pooled saliva testing, as collected in this study, is not quite as sensitive as NP/MT testing, saliva testing is adequate to detect individuals with higher viral loads in an asymptomatic screening program, does not require swabs or viral transport media for collection, and may help to improve voluntary screening compliance for those individuals averse to various forms of nasal collections*. [**note: this is from NIH and shows that while pooled saliva testing is not quite as good as nasopharyngeal swab collection, it can be useful in detecting those with higher viral loads.**] https://www.medrxiv.org/content/10.1101/2020.10.02.20204859v1

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2020-10-07

It will be no surprise that today's featured musical performer is Tammy Wynette who was for a short period of her tumultuous life (and what a life it was) marred to George Jones. Jones was the third of her five husbands and even after their divorce they recorded some tracks. Here are some of the iconic performance. 'Stand by Your Man' : https://www.youtube.com/watch?v=f2KP9fYZUWA 'D-I-V-O-R-C-E' https://www.youtube.com/watch?v=912DKxD0H1U 'Til I Can Make It On My Own': https://www.youtube.com/watch?v=eaYu2d9EM00 and finally 'I Don't Wanna Play House': https://www.youtube.com/watch?v=uCc9tJlRUZ4 Wynette never gave less than 100% either in the studio or on the stage.

US COVID-19 STATISTICS - **Infection Rate: 2.3%; CFR: 2.8%** (IR is slowly increasing; to achieve herd immunity it needs to get to between 25-70% depending on which model one follows. My guess is that the CFR will eventually end up at 0.3-0.6%; slightly worse than the 1957-58 Asian Flu epidemic)

One of the blogs I regularly read is Tyler Cowen's Marginal REVOLUTION. Yesterday, there was an interesting post on a paper done by a group regarding a cost/benefit analysis of clinical trial designs for COVID-19 candidates. The paper is quite interesting but has one major drawback, they do not factor in the need for doing safety assessments. As most of us who have been involved in drug safety know, much of the time and effort in vaccine trials is spent ascertaining the safety of the vaccine. This is a principal reason for the large number of subjects in vaccine trials.

The New York Times has a first person account of a COVID-19 hallucination. A company that develops and sells clinical trial software was hit by a ransomware attack. Remember, don't click on suspect links (I double check all the ones in the newsletter before sending it out). Here is a story of a small

businessman who owns eight budget hotels and how he is trying to keep things above water during the pandemic. University reopenings in the UK are not going smoothly. North Dakota is running out of hospital beds as COVID-19 cases spike in the state. For those health economists, here is an estimate of the cost of President Trump's COVID-19 treatment. THIS and THIS explain in blunt terms why America will continue to fail in curbing the COVID-19 pandemic this year.

Both the Pfizer and Moderna vaccines have reported 'serious' side effects. I use the term 'serious' as that is what the headline says. This points out the necessity of completing the trials and evaluating all AEs (adverse events for my readers who are not from the pharma industry) before an experimental use authorization is considered. The FDA guidance document requiring further follow up on vaccine clinical trial subjects has officially posted.

The Washington Post has a story on the FDA vaccine guidance noted above. The UK health secretary has warned of a serious COVID-19 problem as new infections and hospitalization rise. The Nobel Prize in chemistry goes to Berkeley biochemist Jennifer Doudna and French microbiologist Emmanuelle Charpentier for their work developing the CRISPR gene editing tool.

STAT reports on a new Lilly study with a duo of monoclonal antibodies that is effective in reducing levels of SARS-CoV0-2. Here is the story of a recruiter for a COVID-19 vaccine trial.

Lots of interesting stuff today including a new update to a herd immunity model!

MODELING

- Background: The World Health Organization has identified contact tracing and isolation (CTI) as a key strategy to slow transmission of SARS-CoV-2. Structured agent-based models (ABMs) provide a means to investigate the efficacy of such strategies in heterogeneous populations and to explore the impact of factors such as changes in test turnaround times (TaT). Methods: We developed a structured ABM to simulate key SARS-CoV-2 transmission and Covid-19 disease progression dynamics in populations of 10,000 agents. We ran 10,000 simulations of each of three scenarios: (1) No CTI with a TaT of two days, (2) CTI with a TaT of two days, and (3) CTI with a TaT of eight days. We conducted a secondary analysis in which TaT values were varied from two to 11. The primary outcome for all analyses was mean total infections. Results: CTI reduced the mean number of infections from 5,577 to 4,157 (a relative reduction of 25.5%) when TaT was held steady at two days. CTI with a TaT of eight days resulted in a mean of 5,163 infections (a relative reduction of 7.4% compared to no CTI and a TaT of two days). In the secondary analysis, every additional day added to the TaT increased the total number of infections - with the greatest increase in infections between four and five days, and the smallest increase between ten and eleven days. Conclusions: *In a structured ABM that simulates key dynamics of Covid-19 transmission and disease progression, CTI results in a substantial reduction in the mean number of total infections. The benefit is greater with shorter TaT times, but remained substantial even with TaTs of eight days. The results suggest that CTI may play a critical role in reducing the size of outbreaks and that TaTs should be kept as short as possible in order to maximise this benefit*. [**note: here is contact tracing and isolation model from South Africa. As I've noted, this approach can be a useful tool to manage the pandemic as long as it**

is coupled with other public health measures.]
https://www.medrxiv.org/content/10.1101/2020.10.06.20207761v1

- Face masks are recommended to reduce community transmission of SARS CoV 2. One of the primary benefits of face masks and other coverings is as source control devices to reduce the expulsion of respiratory aerosols during coughing, breathing, and speaking. Face shields have been proposed as an alternative to face masks, but information about face shields as source control devices is limited. We used a cough aerosol simulator with a headform to propel small aerosol particles (0 to 7 μm) into different face coverings. *An N95 respirator blocked 99% of the cough aerosol, a procedure mask blocked 59%, a 3-ply cloth face mask blocked 51%, and a polyester neck gaiter blocked 47% as a single layer and 60% when folded into a double layer. In contrast, the face shield blocked 2% of the cough aerosol. Our results suggest that face masks and neck gaiters are preferable to face shields as source control devices for cough aerosols*. [**note: this is from NIOSH. Remember to Mask-Up when you go out in public.**]
https://www.medrxiv.org/content/10.1101/2020.10.05.20207241v1
- Heat treatment denatures viral proteins that comprise the virion, making virus incapable of infecting a host. Coronavirus (CoV) virions contain single-stranded RNA genomes with a lipid envelope and 4 proteins, 3 of which are associated with the lipid envelope and thus are thought to be easily denatured by heat or surfactant-type chemicals. Prior studies have shown that a temperature of as low as 75 oC and treatment duration of 15 min can effectively inactivate CoV. The applicability of a CoV heat inactivation method greatly depends on the length of time of a heat treatment and the temperature needed to inactivate the virus. With the goal of finding conditions where sub-second heat exposure of CoV can sufficiently inactivate CoV, we designed and developed a simple system that can measure sub-second heat inactivation of CoV. The system is composed of capillary stainless-steel tubing immersed in a temperature-controlled oil bath followed by an ice bath, through which virus solution can be flowed at various speeds. *Flowing virus solution at different speeds, along with a real-time temperature monitoring system, allows the virus to be accurately exposed to a desired temperature for various durations of time. Using mouse hepatitis virus (MHV), a beta-coronavirus, as a model system, we identified that 85.2 oC for 0.48 s exposure is sufficient to obtain > 5 Log_{10} reduction in viral titer (starting titer: 5 x 10^7 PFU/mL), and that when exposed to 83.4 oC for 0.95 s, the virus was completely inactivated (zero titer, > 6 Log_{10} reduction*). [**note: here is a study on sub-second heat inactivation of coronavirus.**] https://www.biorxiv.org/content/10.1101/2020.10.05.327528v1
- As the world recovers from the lockdown imposed by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, returning to shared indoor spaces is considered a formidable risk. It is now clear that transmission of SARS-CoV-2 is driven by respiratory microdroplets expelled by infected persons, which can become suspended in the air. Several layering technologies are being explored to mitigate indoor transmission in the hopes of re-opening business, schools and transportation systems. Here we coupled the water adsorptive and photocatalytic capacity of novel Metal Organic Frameworks (MOFs) to demonstrate the capture and inactivation of SARS-CoV-2. *Discussion is given on the methods of analysis and the differences between the photocatalytic activity of several MOFs, and the difference between MOF induced photocatalysis and ultra violet photolysis of SARS-CoV-2. Our results are intended to provide support to industry looking for alternative methods secure indoor spaces*. [**note: here**

is some good work on the use of a novel surface material that can perhaps be used for viral inactivation.] https://www.medrxiv.org/content/10.1101/2020.10.01.20204214v1

- The current Covid-19 Pandemic caused by the highly contagious SARS-CoV-2 virus has proven extremely difficult to prevent or control. Currently there are few treatment options and very few long-lasting disinfectants available to prevent the spread. While masks and protective clothing and social distancing may offer some protection, their use has not always halted or slowed the spread. Several vaccines are currently undergoing testing; however there is still a critical need to provide new methods for inactivating the virus before it can spread and infect humans. In the present study we examined the inactivation of SARS-CoV-2 by synthetic conjugated polymers and oligomers developed in our laboratories as antimicrobials for bacteria, fungi and non-enveloped viruses. Our results show that we can obtain highly effective light induced inactivation with several of these oligomers and polymers including irradiation with near-UV and visible light. With both the oligomers and polymers, we can reach several logs of inactivation with relatively short irradiation times. Our results suggest several applications involving the incorporation of these materials in wipes, sprays, masks and clothing and other Personal Protection Equipment (PPE) that can be useful in preventing infections and the spreading of this deadly virus and future outbreaks from similar viruses. [**note: looks like today is good for researchers looking at new materials for viral inactivation. Here is more work from Texas looking at some conjugated polymers and oligomers that can be used as antimicrobials.**] https://www.medrxiv.org/content/10.1101/2020.09.29.20204164v1
- Objectives. To understand what levels of herd immunity are required in the COVID-19 pandemic, given spatial population heterogeneity, to best inform policy and action. Methods. Using a network of counties in the United States connected by transit data we considered a set of coupled differential equations for susceptible-infectious-removed populations. We calculated the classical herd immunity level plus a version reflecting the heterogeneity of connections in the network by running the model forward in time until the epidemic completed. Results. Necessary levels of herd immunity vary greatly from county to county. A population weighted average for the United States is 47.5% compared to a classically estimated level of 77.1%. Conclusions. Common thinking argues that the nation needs to achieve at least 60% herd immunity to emerge from the COVID-19 pandemic. *Heterogeneity in contact structure and individual variation in infectivity, susceptibility, and resistance are key factors that reduce the disease-induced herd immunity levels to 34.2-47.5% in our models.* Looking forward toward vaccination strategies, these results suggest we should consider not just who is vaccinated but where those vaccinations will do the most good. [**note: here is an updated herd immunity model from a trio of UnitedHealthcare researchers. They are looking at a possible value in the lower third of the range based on a population weighted model.**] https://www.medrxiv.org/content/10.1101/2020.10.05.20207100v1

NEWLY REGISTERED CLINICAL TRIALS

- The week isn't over yet.

CLINICAL TRIAL RESULTS

- Background: Early observational studies suggested that the use of the renin angiotensin system (RAS) inhibitors, specifically angiotensin converting enzyme inhibitors or angiotensin receptor

blockers, may increase the risk of infection with SARS-CoV-2 and adversely affect the prognosis or survival of infected patients. To explore the impact of RAS inhibitor use on the risk of SARS-CoV-2 infection and the prognosis of SARS-CoV-2 infected patients, from all published studies. Methods and Findings: A systematic review and meta-analysis of the use of RAS inhibitors in relation to infection with SARS-CoV-2 and/or the severity and mortality associated with COVID-19 was conducted. English language bibliographic databases PubMed, Web of Science, OVID Embase, Scopus, MedRxiv, BioRxiv, searched from Jan 1st, 2020 to July 20th, 2020. 58 observational studies (69,200 COVID-19 patients and 3,103,335 controls) were included. There was no difference in the susceptibility to SARS-CoV-2 infection between RAS inhibitor users and non-users (unadjusted OR 1.05, 95% CI 0.90 to 1.21), (adjusted OR 0.93, 95% CI 0.85 to 1.02), (adjusted HR 1.07, 95% CI 0.87 to 1.31). There was no significant difference in the severe Covid-19 case rate between RAS inhibitor users and non-users (unadjusted OR 1.05, 95% CI 0.81 to 1.36), (adjusted OR 0.76, 95% CI 0.52 to 1.12), or in mortality due to COVID-19 between RAS inhibitor users and non-users (unadjusted OR 1.12, 95% CI 0.88 to 1.44), (adjusted OR 0.97, 95% CI 0.77 to 1.23), (adjusted HR 0.62, 95% CI 0.34 to 1.14). Conclusions: In the most comprehensive analysis of all available data to date, treatment with RAS inhibitors was not associated with increased risk of infection, severity of disease, or mortality due to COVID-19. *The best available evidence suggests that these treatments should not be discontinued on the basis of concern about risk associated with COVID-19*. [**note: here is a large cohort study showing the use of renin angiotensin system drugs do not pose any increased risk for COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.10.03.20206375v1

- Background: While COVID-19 remains largely unclear and mortality continues to raise, early effective approaches prior to complications lack, as well as researches for characterization and therapeutical potential options in actual early COVID-19. Although females seem to be less affected than females, hyperandrogenic (HA) phenotype, like polycystic ovary syndrome (PCOS), idiopathic hirsutism, congenital adrenal hyperplasia (CAH) female androgenetic alopecia (AGA), or idiopathic HA may be at higher risk due to its inherent enhanced androgenic activity. The present study aimed to evaluate the effects of any early pharmacological approach to females diagnosed with COVID-19 before seven days of symptoms, as well as investigate whether HA is an additional risk factor in this population. Materials and methods: Females with symptoms for less than seven days confirmed for COVID-19 through positive real-time polymerase chain reaction (rtPCR-SARS-CoV-2) were classified and divided as non-HA, HA, and HA using spironolactone (HA-spiro) groups. Patients were questioned for baseline characteristics, 23 different diseases, 44 drug classes and vaccines, 28 different symptoms, and eight different parameters to measure COVID-19 related clinical outcomes. Treatment was then provided, including azithromycin 500mg/day for five days in all cases, associated with hydroxychloroquine 400mg/day for five days, nitazoxanide 500mg twice a day for six days, or ivermectin 0.2mg/kg/day por three days, and optionally spironolactone 100mg twice a day until cure. Patients were assessed for COVID-19 clinical course, clinical and viral duration, and disease progression. Results: In total, 270 females were enrolled, including 195, 67, and eight in non-HA, HA, and HA-spiro groups, respectively. Prevailing symptoms were anosmia (71.1%), ageusia (67.0%), headache (48.1%), myalgia (37.4%), dry cough (36.3%), nasal congestion or rhinorrhea (34.1%), fatigue (33.3%), weakness (29.5%), hyporexia (27.8%), thoracic pain (24.8%), diarrhea (24.1%) and dizziness (21.5%). Earliest symptoms (days) were dizziness (1.0 +- 0.2 day),

abdominal pain (1.1 +- 0.3); conjunctival hyperemia (1.1 +- 0.5), nasal congestion or rhinorrhea (1.2 +- 0.5), headache (1.2 +- 0.5), dry cough (1.2 +- 61617; 0.5), myalgia (1.2 +- 0.4), nauseas (1.3 +- 0.5) and weakness (1.3 +- 0.5). Time-to-treat, positive rtPCR, and duration of symptoms with and without anosmia and ageusia were significantly lower in HA-spiro than non-HA, HA, and overall non-users. Time-to-treat was similar while all duration of symptoms and positive rtPCR-SARS-CoV-2 were significantly shorter in non-HA than HA. Spironolactone users were more likely to be asymptomatic than non-users during COVID-19. Fewer non-HA than HA females were affected by anosmia, ageusia, dry cough, fatigue, weakness and hyporexia. Ageusia, weakness and myalgia lasted shorter in non-HA than HA. None of the patients needed hospitalization or any other COVID-19 complication. Conclusions: *A sensitive, early detection of COVID-19 followed by a pharmaceutical approach with different drug combinations yielded irrefutable differences compared to sex-, age-, body mass index (BMI)-, and disease-matched non-treated controls in terms of clinical outcomes, ethically disallowing placebo-control randomized clinical trials in the early stage of COVID-19 due to the marked improvements. HA females presented more severe and prolonged clinical manifestations, although none progressed to worse outcomes. Spironolactone mitigated the additional risks due to HA*. [**note: this is an open label study of antiandrogen and non-antiandrogen as early interventions in females with mild to moderate COVID-19.]**
https://www.medrxiv.org/content/10.1101/2020.10.05.20206870v1

DRUG DEVELOPMENT

- The COVID-19 pandemic by non-stop infections of SARS-CoV-2 has continued to ravage many countries worldwide. Here we report the discovery of suramin, a 100-year-old drug, as a potent inhibitor of the SARS-CoV-2 RNA dependent RNA polymerase (RdRp) through blocking the binding of RNA to the enzyme. In biochemical assays, suramin and its derivatives are at least 20-fold more potent than remdesivir, the currently approved nucleotide drug for COVID-19. The 2.6 Å cryo-EM structure of the viral RdRp bound to suramin reveals two binding sites of suramin, with one site directly blocking the binding of the RNA template strand and the other site clash with the RNA primer strand near the RdRp catalytic active site, therefore inhibiting the viral RNA replication. Furthermore, suramin potently inhibits SARS-CoV-2 duplication in Vero E6 cells. These results provide a structural mechanism for the first non-nucleotide inhibitor of the SARS-CoV-2 RdRp and a rationale for repurposing suramin for treating COVID-19. [**note: here is another drug repurposing study from China. It's interesting that this drug with a complex chemical structure was first made by Bayer AG scientists 100 years ago.**]
https://www.biorxiv.org/content/10.1101/2020.10.06.328336v1
- Since December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2/2019-nCoV) has spread quickly worldwide, with more than 29 million cases and 920,000 deaths. Interestingly, coronaviruses were found to subvert and hijack the autophagic process to allow their viral replication. One of the spotlights had been focused on the autophagy inhibitors as a target mechanism effective in the inhibition of SARS-CoV-2 infection. Consequently, chloroquine (CQ) and hydroxychloroquine (HCQ), a derivative of CQ, was suggested as the first potentially be therapeutic strategies as they are known to be autophagy inhibitors. Then, they were used as therapeutics in SARS-CoV-2 infection along with remdesivir, for which the FDA approved emergency use authorization. Here, we investigated the antiviral activity and associated

mechanism of GNS561, a small basic lipophilic molecule inhibitor of late-stage autophagy, against SARS-CoV-2. Our data indicated that GNS561 showed the highest antiviral effect for two SARS-CoV-2 strains compared to CQ and remdesivir. Focusing on the autophagy mechanism, we showed that GNS561, located in LAMP2-positive lysosomes, together with SARS-CoV-2, blocked autophagy by increasing the size of LC3-II spots and the accumulation of autophagic vacuoles in the cytoplasm with the presence of multilamellar bodies characteristic of a complexed autophagy. Finally, our study revealed that the combination of GNS561 and remdesivir was associated with a strong synergistic antiviral effect against SARS-CoV-2. Overall, our study highlights GNS561 as a powerful drug in SARS-CoV-2 infection and supports that the hypothesis that autophagy inhibitors could be an alternative strategy for SARS-CoV-2 infection. [**note: here is a report on an experimental drug that shows good *in vitro* activity through autophagy inhibition. The company is Genoscience Pharma, a French company.**]
https://www.biorxiv.org/content/10.1101/2020.10.06.327635v1

- BACKGROUND In-depth investigations of the safety and immunogenicity of inactivated SARS-CoV-2 vaccines are needed. METHOD In a phase I randomized, double-blinded, and placebo-controlled trial involving 192 healthy adults 18-59 years of age, two injections of three different doses (50 EU, 100 EU and 150 EU) of an inactivated SARS-CoV-2 vaccine or the placebo were administered intramuscularly with a 2- or 4-week interval between the injections. The safety and immunogenicity of the vaccine were evaluated within 28 days. FINDING In this study, 191 subjects assigned to three doses groups or the placebo group completed the 28-day trial. There were 44 adverse reactions within the 28 days, most commonly mild pain and redness at the injection site or slight fatigue, and no abnormal variations were observed in 48 cytokines in the serum samples of immunized subjects. The serum samples diluted from 1:32 to 1:4096 and incubated with the virus did not show antibody-dependent enhancement effects (ADEs) with regard to human natural killer cells, macrophages or dendritic cells. At day 14, the seroconversion rates had reached 92%, 100% and 96% with geometric mean titers (GMTs) of 18.0, 54.5 and 37.1, and at day 28, the seroconversion rates had reached 80%, 96% and 92% with GMTs of 10.6, 15.4 and 19.6in 0, 14 and 0, 28 procedures, respectively. Seroconversion was associated with the synchronous upregulation of ELISA antibodies against the S protein, N protein and virion and a cytotoxic T lymphocyte (CTL) response. Transcriptome analysis shaped the genetic diversity of immune response induced by the vaccine. INTERPRETATION *In a population aged 18-59 years, this inactivated SARS-CoV-2 vaccine was safe and immunogenic*. [**note: here is clinical trial data on the Chinese inactivated SARS-CoV-2 vaccine.**]
https://www.medrxiv.org/content/10.1101/2020.09.27.20189548v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Understanding how SARS-CoV-2 spreads within the respiratory tract is important to define the parameters controlling the severity of COVID-19. We examined the functional and structural consequences of SARS-CoV-2 infection in a reconstituted human bronchial epithelium model. SARS-CoV-2 replication caused a transient decrease in epithelial barrier function and disruption of tight junctions, though viral particle crossing remained limited. Rather, SARS-CoV-2 replication led to a rapid loss of the ciliary layer, characterized at the ultrastructural level by axoneme loss and misorientation of remaining basal bodies. The motile cilia function was compromised, as measured in a mucociliary clearance assay. Epithelial defense mechanisms,

including basal cell mobilization and interferon-lambda induction, ramped up only after the initiation of cilia damage. Analysis of SARS-CoV-2 infection in Syrian hamsters further demonstrated the loss of motile cilia in vivo. This study identifies cilia damage as a pathogenic mechanism that could facilitate SARS-CoV-2 spread to the deeper lung parenchyma. [**note: here is a study of how SARS-CoV-2 can damage cilia within the respiratory track of Syrian hamsters. This might explain why it spreads to deeper lung locations.**]
https://www.biorxiv.org/content/10.1101/2020.10.06.328369v1

- The interactions between antibodies, SARS-CoV-2 and immune cells contribute to the pathogenesis of COVID-19 and protective immunity. To understand the differences between antibody responses in mild versus severe cases of COVID-19, we analyzed the B cell responses in patients 1.5 months post SARS-CoV-2 infection. Severe and not mild infection correlated with high titers of IgG against Spike receptor binding domain (RBD) that were capable of viral inhibition. B cell receptor (BCR) sequencing revealed two VH genes, VH3-38 and VH3-53, that were enriched during severe infection. Of the 22 antibodies cloned from two severe donors, six exhibited potent neutralization against live SARS-CoV-2, and inhibited syncytia formation. Using peptide libraries, competition ELISA and RBD mutagenesis, we mapped the epitopes of the neutralizing antibodies (nAbs) to three different sites on the Spike. Finally, we used combinations of nAbs targeting different immune-sites to efficiently block SARS-CoV-2 infection. Analysis of 49 healthy BCR repertoires revealed that the nAbs germline VHJH precursors comprise up to 2.7% of all VHJHs. *We demonstrate that severe COVID-19 is associated with unique BCR signatures and multi-clonal neutralizing responses that are relatively frequent in the population. Moreover, our data support the use of combination antibody therapy to prevent and treat COVID-19*. [**note: here is a study on antibody generation from two severe COVID-19 patients in Israel.]** https://www.biorxiv.org/content/10.1101/2020.10.06.323634v1
- The SARS-CoV-2 nucleocapsid (N) protein is the most immunogenic of the structural proteins and plays essential roles in several stages of the virus lifecycle. It is comprised of two major structural domains: the RNA binding domain, which interacts with viral and host RNA, and the oligomerization domain which assembles to form the viral core. Here, we investigate the assembly state and RNA binding properties of the full-length nucleocapsid protein using native mass spectrometry. We find that dimers, and not monomers, of full-length N protein bind RNA, implying that dimers are the functional unit of ribonucleoprotein assembly. In addition, we find that N protein binds RNA with a preference for GGG motifs which are known to form short stem loop structures. Unexpectedly, we found that N undergoes autoproteolytic processing within the linker region, separating the two major domains. This process results in the formation of at least five proteoforms that we sequenced using electron transfer dissociation, higher-energy collision induced dissociation and corroborated by peptide mapping. The cleavage sites identified are in highly conserved regions leading us to consider the potential roles of the resulting proteoforms. We found that monomers of N-terminal proteoforms bind RNA with the same preference for GGG motifs and that the oligomeric state of a C-terminal proteoform ($N_{156-419}$) is sensitive to pH. We used mass spectrometry to show that N binds to a monoclonal antibody raised against full-length N. No antibody interactions were detected for N proteoforms without C-terminal residues, therefore locating antigenic regions towards the C-terminus. We then tested interactions of the proteoforms with the immunophilin cyclophilin A, a key component in coronavirus replication. We found that N_{1-209} and N_{1-273} bind directly to cyclophilin

A, an interaction that is abolished by the approved immunosuppressant drug cyclosporin A. *We propose that the proteoforms generated via autoproteolysis evade antibody detection through removal of the antigenic C-terminus and facilitate interactions with structured RNA or cyclophilin thereby enabling the virus to proliferate*. [**note: here is more on the Nucleocapsid protein and how it might help the virus evade antibody detection.**]
https://www.biorxiv.org/content/10.1101/2020.10.06.328112v1

DIAGNOSTIC DEVELOPMENT

- Serological detection of antibodies to SARS-CoV-2 is essential for establishing rates of seroconversion in populations, detection of seroconversion after vaccination, and for seeking evidence for a level of antibody that may be protective against COVID-19 disease. Several high-performance commercial tests have been described, but these require centralised laboratory facilities that are comparatively expensive, and therefore not available universally. Red cell agglutination tests have a long history in blood typing, and general serology through linkage of reporter molecules to the red cell surface. They do not require special equipment, are read by eye, have short development times, low cost and can be applied as a Point of Care Test (POCT). We describe a red cell agglutination test for the detection of antibodies to the SARS-CoV-2 receptor binding domain (RBD). *We show that the Haemagglutination Test (HAT) has a sensitivity of 90% and specificity of 99% for detection of antibodies after a PCR diagnosed infection. The HAT can be titrated, detects rising titres in the first five days of hospital admission, correlates well with a commercial test that detects antibodies to the RBD, and can be applied as a point of care test. The developing reagent is composed of a previously described nanobody to a conserved glycophorin A epitope on red cells, linked to the RBD from SARS-CoV-2. It can be lyophilised for ease of shipping. We have scaled up production of this reagent to one gram, which is sufficient for ten million tests, at a cost of ~0.27 UK pence per test well. Aliquots of this reagent are ready to be supplied to qualified groups anywhere in the world that need to detect antibodies to SARS-CoV-2, but do not have the facilities for high throughput commercial tests*. [**note: kudos to this Oxford group for coming up with an agglutination test for SARS-CoV—2 antibodies. This will have utility in areas that lack sophisticated diagnostic equipment.**]
https://www.medrxiv.org/content/10.1101/2020.10.02.20205831v1

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2020-10-08

Mother Maybelle Carter had a profound impact on both country and folk music. Her performing career began in 1927 when she was 18 years old and ran to 1973 with the issuance of her final recording. She was an innovative guitarist and brought back the use of the autoharp as a performance instrument. 'Wildwood Flower' provides a good example of her guitar playing: https://www.youtube.com/watch?v=XE80Ed59uCY Here she is playing the autoharp on the Johnny Cash (her son-in-law) show: https://www.youtube.com/watch?v=RAeT1JmJNTg Also from the Johnny Cash show is Maybelle with her cousin Sara who with her husband made up the original Carter family singing 'You Are My Flower': https://www.youtube.com/watch?v=ks8lNZ2ZRtw Finally, here is a tribute to Mother Maybelle featuring Emmy Lou Harris, Ricky Skaggs, and Linda Ronstadt: https://www.youtube.com/watch?v=WNZs2vR6Eg8

The Washington Post points to a major problem with the US system in that states set criteria for what constitutes a COVID-19 outbreak. It is another reason why it is so difficult to control the virus in America. This is just depressing; the pandemic is amplifying the anti-vaccine movement in the US and abroad. Caution on interpreting antibody tests has to be exercised following administration of humanized monoclonals. This is not meant to be a political post as it concerns President Trump but only to point out the difficulty in assessing the state of the patient based on serology. It also begs the question about whether the President is still infectious as PCR tests can give false positive results based on viral debris. Vermont is seeing an outbreak of COVID-19 among migrant workers. This Florida hospital is bracing for a fall surge in COVID-19 cases. Let us hope it doesn't happen. Italy announces a nationwide mask mandate; good move. Six travelers share their flying experience. Regeneron seek an FDA Emergency Use Authorization for their dual monoclonal antibody treatment for COVID-19, joining Lilly in expanding the use of these treatments.

The New York Times reports on New Zealand's second effort to stamp out the virus. It worked. Notre Dame's president is in trouble for flouting university safety rules regarding the wearing of masks. Nevada has halted the use of some rapid COVID-19 tests because of false positive rates. Boston is delaying school openings because of increases in COVID-19 cases.

I picked up this Twitter thread from the Marginal Revolution blog and it's informative as to how the system in Israel went wrong regarding COVID-19 infections.

The OHDSI group just had a study published on hospitalized patients in the US, South Korea and Spain. "Patients hospitalized with COVID-19 were more likely male, younger, and, in both the US and Spain, had fewer comorbidities and lower medication use than hospitalized influenza patients according to a recent study published by the Observational Health Data Sciences and Informatics (OHDSI) community. This global network study, which included more than 34,000 COVID-19 patients from across three continents, is intended to provide greater detail about the characteristics of patients suffering from the disease, and also to help inform decision-making around the care of hospitalized patients." Well done!!!

Here is an interesting perspective from The New England Journal of Medicine on the stress of Bayesian medicine. I may have linked to the preprint of this paper, but here is the published correspondence on the detection of SARS-CoV-2 with one pot testing that utilizes CRISPR for which a Nobel Prize in Chemistry was just awarded.

STAT have a nice story on the discovery and development of CRISPR. Here is an opinion piece from two Harvard scientists on how a recent mumps outbreak can inform colleges about reopening safely in the time of COVID-19.

The Lancet have an editorial on why we will not be returning to the old normal.

JAMA have a genetic analysis of the COVID-19 outbreak in Southern California. The predominate isolates originated from Europe, similar to the ones found in New York City. Maybe we should call this the 'Milan Flu.' (Sorry, I've been waiting to use this one for some time now. 😉) Here is a proposal on a "return-to-learning natural experiment outlining considerations for researchers seeking to partner with K-12 schools to ensure that ongoing decisions about children, schools, and COVID-19 are based on science, not speculation. There is also a viewpoint on the long-term health consequences of COVID-19. It seems like the USS Theodore Roosevelt SARS-CoV-2 outbreak took place years ago when it was just

last March. Here is an outcomes study of sailors in isolation following that outbreak. "In a confined space congregate setting with young essential workers, COVID-19 is unlikely to be clinically distinguishable from other acute respiratory illness without specific laboratory testing. Asymptomatic (and presymptomatic) spread will limit the effectiveness of symptomatic screening in the absence of other nonpharmaceutical interventions, such as testing, masking, and, as feasible, social distancing. Finally, the rapid increase in case number as incubating cases disembarked, followed by the precipitous decrease in cases, suggests that the shore-based nonpharmaceutical interventions interrupted a probable acceleration in case incidence that would have likely resulted in a substantial disease burden. Lessons learned from the USS TR COVID-19 outbreak may have applicability in other congregate settings staffed by essential workers and in understanding clinical features of the illness in younger adult populations."

MODELING

- Background: Quantifying antibody reactivity to SARS-CoV-2 antigens may help understand its effect on COVID-19 severity at the population level. This antibody reactivity may be particularly prevalent among childcare providers, including pediatric health care workers (HCW) who may be more exposed to circulating coronaviruses. Methods: Cross-sectional study that included adults in the Vancouver area in British Columbia (BC), Canada, between May 17 and June 19, 2020. A novel 10-plex antibody assay (IgG) was used to measure antibody reactivity against the spike protein from circulating coronaviruses (229E, NL63, OC43, and HKU1), SARS-CoV, and four SARS-CoV-2 antigens. Seroreactivity from previous viral exposure was ascertained using this assay, and by measuring total SARS-CoV-2 IgG/M/A antibodies against a recombinant spike (S1) protein using a commercial CLIA assay. Findings: Among 276 participants (71% HCW), three showed evidence of direct viral exposure, yielding an adjusted seroprevalence of 0.6% [95%CI 0.2 to 3.1%], with no difference between HCW and non-HCW, or between paediatric and adult HCW. Among the remaining 273 unexposed individuals, 7.3% [95%CI 4.5% to 11.1%], 48.7 [95%CI 42.7% to 54.8%] and 82.4% [95%CI 77.4% to 86.7%] showed antibody reactivity against SARS-CoV-2 RBD, N or Spike proteins, respectively. This reactivity was evenly distributed as a function of age, sex or between paediatric and adult HCW, and partly correlated with reactivity to circulating coronaviruses (Spearman; range: 0.147 to 0.513 for significant correlation after false-discovery rate adjustment at 5%). Interpretation: *A substantial proportion of individuals in this population showed antibody reactivity against SARS-CoV-2 antigens despite low serological evidence of SARS-CoV-2 exposure.* [**note: here is an antibody reactivity study from metropolitan Vancouver.**] https://www.medrxiv.org/content/10.1101/2020.10.05.20206664v1

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this study is to assess the efficacy of PF-06650833 in addition to standard-of-care compared to standard-of-care treatment alone in improving outcomes in patients with COVID-19. [**note: this is a Pfizer experimental IRAK4 inhibitor. Study is at Yale.**] NCT04575610
- This study aims to evaluate the safety, tolerability and efficacy of molnupiravir (MK-4482) compared to placebo. The primary hypothesis is that molnupiravir is superior to placebo as assessed by the rate of sustained recovery through Day 29 [**note: this is the drug Merck brought in house several months ago for development. It is a 1300 patient combination phase 2/3 study**.] NCT04575584

- The current research is a pilot study to determine the feasibility of recruiting and retaining 40 participants diagnosed with COVID-19. The purpose is to observe the early use of fluoxetine (commonly known as Prozac) to reduce the severity of the COVID-19 illness. Fluoxetine is a drug that has been approved by the U.S. Food and Drug Administration (FDA) since 1987 for various mental health disorders. [**note: it will be good to know the results of this trial. We can level out people's modds and treat COVID-19 at the same time!**] NCT04570449
- The aim of this study will test the safety, tolerability, and efficacy of RLS-0071 for approximately 28 days in comparison to a placebo control in patients with acute lung injury due to COVID-19 pneumonia in early respiratory failure. [**note: don't know much about this drug; the sponsor is ReAlta Life Scinences.]** NCT04574869
- This study will assess the safety and efficacy of TSC as a treatment for participants who are infected with SARS-CoV-2 (COVID-19). [**note: this is a study of trans sodium crocetinate sponsored by Diffusion Pharmaceutical. The compound is from the crocus flower and is responsible for the color in saffron. Trial is in Romania.**] NCT04573322

CLINICAL TRIAL RESULTS

- Nothing today.

DRUG DEVELOPMENT

- Human monoclonal antibodies are safe, preventive and therapeutic tools, that can be rapidly developed to help restore the massive health and economic disruption caused by the Covid−19 pandemic. By single cell sorting 4277 SARS−CoV−2 spike protein specific memory B cells from 14 Covid−19 survivors, 453 neutralizing antibodies were identified and 220 of them were expressed as IgG. Up to 65,9% of monoclonals neutralized the wild type virus at a concentration of >500 ng/mL, 23,6% neutralized the virus in the range of 100 − 500 ng/mL and 9,1% had a neutralization potency in the range of 10 − 100 ng/mL. Only 1,4% neutralized the authentic virus with a potency of 1−10 ng/mL. We found that the most potent neutralizing antibodies are extremely rare and recognize the RBD, followed in potency by antibodies that recognize the S1 domain, the S-protein trimeric structure and the S2 subunit. The three most potent monoclonal antibodies identified were able to neutralize the wild type and D614G mutant viruses with less than 10 ng/mL and are good candidates for the development of prophylactic and therapeutic tools against SARS−CoV−2. [**note: here is an Italian effort to identify and develop high potency monoclonal antibodies.**] https://www.biorxiv.org/content/10.1101/2020.10.07.328302v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Coronaviruses, like SARS-CoV-2, encode a nucleotidyl transferase in the N-terminal NiRAN domain of the non-structural protein (nsp) 12 protein within the RNA dependent RNA polymerase (RdRP). Though the substrate targets of the viral nucleotidyl transferase are unknown, NiRAN active sites are highly conserved and essential for viral replication. We show, for the first time, the detection and sequence location of GMP-modified amino acids in nidovirus RdRP-associated proteins using heavy isotope-assisted MS and MS/MS peptide sequencing. We identified lys-143 in the equine arteritis virus (EAV) protein, nsp7, as a primary site of nucleotidylation in vitro that uses a phosphoramide bond to covalently attach with GMP. In SARS-CoV-2 replicase proteins, we demonstrate a unique O-linked GMP attachment on nsp7 ser-

1, whose formation required the presence of nsp12. It is clear that additional nucleotidylation sites remain undiscovered, which includes the possibility that nsp12 itself may form a transient GMP adduct in the NiRAN active site that has eluted detection in these initial studies due to instability of the covalent attachment. Our results demonstrate new strategies for detecting GMP-peptide linkages that can be adapted for higher throughput screening using mass spectrometric technologies. These data are expected to be important for a rapid and timely characterization of a new enzymatic activity in SARS-CoV-2 that may be an attractive drug target aimed at limiting viral replication in infected patients. [**note: lots of biochemical modifications go on during SARS-CoV-2 infection. Perhaps some of these are just incidental and unimportant but we don't have a full understanding right now. Here is a way to detect attachment of nucleotides to the RNA polymerase.**]
https://www.biorxiv.org/content/10.1101/2020.10.07.330324v1

- During the COVID-19 pandemic, structural biologists have rushed to solve the structures of the 28 proteins encoded by the SARS-CoV-2 genome in order to understand the viral life cycle and enable structure-based drug design. In addition to the 200 structures from SARS-CoV previously solved, 367 structures covering 16 of the viral proteins have been released in the span of only 6 months. These structural models serve as basis for research worldwide to understand how the virus hijacks human cells, for structure-based drug design and to aid in the development of vaccines. However, errors often occur in even the most careful structure determination - and are even more common among these structures, which were solved under immense pressure. From the beginning of the pandemic, the Coronavirus Structural Taskforce has categorized, evaluated and reviewed all of these experimental protein structures in order to help downstream users and original authors. Our website also offers improved models for many key structures, which have been used by Folding@Home, OpenPandemics, the EU JEDI COVID-19 challenge, and others. Here, we describe our work for the first time, give an overview of common problems, and describe a few of these structures that have since acquired better versions in the worldwide Protein Data Bank, either from new data or as depositor re-versions using our suggested changes. [**note: this is a good effort to establish a public site to aggregate structural information on the viral proteins. It is updated with new data coming in so that errors (normal in the scientific process) can be corrected and disseminated.**]
https://www.biorxiv.org/content/10.1101/2020.10.07.307546v1
- COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which spread worldwide in 2019, is an urgent problem to be overcome. The ORF8 of SARS-CoV-2 has been suggested to be associated with the symptoms of COVID-19, according to reports of clinical studies. However, little is known about the function of ORF8. As one of the ways to advance the functional analysis of ORF8, mass production of ORF8 with the correct three-dimensional structure is necessary. In this study, we attempted to produce ORF8 protein by chemical-inducible protein production system using tobacco BY-2 cells. An ORF8-producing line was generated by the Agrobacterium method. As a result, the production of ORF8 of 8.8 ± 1.4 mg/L of culture medium was confirmed. SDS-PAGE and Nuclear magnetic resonance (NMR) analysis confirmed that the ORF8 produced by this system is a dimeric form with a three-dimensional structure, unlike that produced in *Escherichia coli*. Furthermore, it was suggested that the ORF8 produced by this system was *N*-glycosylated. Through this study, we succeeded in producing ORF8 with the correct three-dimensional structure in a chemical-inducible protein

production system using tobacco BY2 cells. It is expected that the functional analysis of ORF8 will be advanced using the ORF8 produced by this system and that it will greatly contribute to the development of vaccines and antibodies against ORF8. [**note: here is work from Japan on the cloning and expression of the ORF8 protein from SARS-CoV-2. Little is known about this protein at present.**] https://www.biorxiv.org/content/10.1101/2020.10.07.325910v1

- Genomic epidemiology has become an increasingly common tool for epidemic response. Recent technological advances have made it possible to sequence genomes rapidly enough to inform outbreak response, and cheaply enough to justify dense sampling of even large epidemics. With increased availability of sequencing it is possible for agile networks of sequencing facilities to collaborate on the sequencing and analysis of epidemic genomic data. In response to the ongoing SARS-CoV-2 pandemic in the United Kingdom, the COVID-19 Genomics UK (COG-UK) consortium was formed with the aim of rapidly sequencing SARS-CoV-2 genomes as part of a national-scale genomic surveillance strategy. The network consists of universities, academic institutes, regional sequencing centres and the four UK Public Health Agencies. We describe the development and deployment of Majora, an encompassing digital infrastructure to address the challenge of collecting and integrating both genomic sequencing data and sample-associated metadata produced across the COG-UK network. The system was designed and implemented pragmatically to stand up capacity rapidly in a pandemic caused by a novel virus. This approach has underpinned the success of COG-UK, which has rapidly become the leading contributor of SARS-CoV-2 genomes to international databases and has generated over 60,000 sequences to date. [**note: this paper presents a UK effort to support SARS-CoV-2 genomic surveillance.**] https://www.biorxiv.org/content/10.1101/2020.10.06.328328v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-10-09

One more day of country to round out the week. I have had some clips of Emmylou Harris in the past but here is a full set from a 1981 Austin City Limits show: https://www.youtube.com/watch?v=YMCHDgR4NEo Over the years Harris has played and sung with an amazing number of musicians and is always pitch perfect. As a special treat here is Emmylou with The Band from the Scorsese documentary The Last Waltz: https://www.youtube.com/watch?v=hqDqzGG8ba0

The Washington Post has a tale of two Alabama universities. One controlled COVID-19 and the other did not. China is joining the global COVAX initiative to distribute COVID-19 vaccines. The European Commission and Gilead have reached agreement for remdesivir supplies. Hong Kong will test a dual COVID-19/flu vaccine. Here is how your kids or grandkids can safely visit playgrounds. This story points out how complicated track and trace can be as those who attended the White House ceremony on the new Supreme Court nominee came from a variety of geographical regions.

The New York Times reports on a possible second wave of COVID-19 in the Northeast. Here is an interesting article on bats and the need for research on these mammals.

STAT have 7 good questions about the rollout of a COVID-19 vaccine. Here is a largely *apolitical* article on the 'compassionate use' of the Regeneron's monoclonal antibody duo by President Trump.

Science have an article that compares Ebola and SARS-CoV-2 entry into cells. Here is a report on SARS-CoV-2 antibody response in saliva. "This study confirms that serum and saliva IgG antibodies to SARS-CoV-2 are maintained in the majority of COVID-19 patients for at least 3 months PSO. IgG responses in saliva may serve as a surrogate measure of systemic immunity to SARS-CoV-2 based on their correlation with serum IgG responses." This is a useful finding in terms of using saliva-based diagnostic tests.

MODELING

- Introduction: SARS-CoV-2 is the beta-coronavirus responsible for COVID-19. Facemask use has been qualitatively associated with reduced COVID-19 cases, but no study has quantitatively assessed the impact of government mask mandates (MM) on new COVID-19 cases across multiple US States. Data and Methods: We utilized a non-parametric machine-learning algorithm to test the *a priori* hypothesis that MM were associated with reductions in new COVID-19 cases. Publicly available data were used to analyze new COVID-19 cases from 37 States and the District of Columbia (i.e., "38 States"). We conducted confirmatory All-States and State-Wise analyses, validity analyses [e.g., leave-one-out (LOO) and bootstrap resampling], and covariate analyses. Results: No statistically significant difference in the daily number of new COVID-19 infections was discernible in the All-States analysis. In State-Wise LOO validity analysis, 11 States exhibited reductions in new COVID-19 and the reductions in four of these States (AK, MA, MN, VA) were significant in bootstrap resampling. Only the Social Capital Index predicted MM success (training $p<0.028$ and LOO $p<0.013$). Conclusion: Results obtained when studying the impact of MM on COVID-19 cases varies as a function of the heterogeneity of the sample being considered, providing clear evidence of Simpson's Paradox and thus of confounded findings. As such, studies of MM effectiveness should be conducted on disaggregated data. Since transmissions occur at the individual rather than at the collective level, additional work is needed to identify optimal social, psychological, environmental, and educational factors which will reduce the spread of SARS-CoV-2 and facilitate MM effectiveness across diverse settings. [**note: here is an assessment of mask mandates in the US. I confess to not knowing about Simpson's Paradox until reading this paper!]**
https://www.medrxiv.org/content/10.1101/2020.10.06.20208033v1
- Coronavirus disease 2019 (COVID-19) is a pandemic. To characterize the disease transmissibility, we propose a Bayesian change point detection model using daily actively infectious cases. Our model is built upon a Bayesian Poisson segmented regression model that can 1) capture the epidemiological dynamics under the changing conditions caused by external or internal factors; 2) provide uncertainty estimates of both the number and locations of change points; 3) adjust any explanatory time-varying covariates. Our model can be used to evaluate public health interventions, identify latent events associated with spreading rates, and yield better short-term forecasts. [**note: I love the acronym for this approach, "BayesSMILES" which reminds me to brush up on my Bayesian statistics.**]
https://www.medrxiv.org/content/10.1101/2020.10.06.20208132v2

NEWLY REGISTERED CLINICAL TRIALS

- I told you once a week!! You received the update yesterday.

CLINICAL TRIAL RESULTS

- COVID-19 causes persistent endothelial inflammation, lung and cardiovascular complications. SARS-CoV-2 utilises the catalytic site of full-length membrane-bound angiotensin converting enzyme 2 (ACE2) for cell entry causing downregulation of tissue ACE2. We reported downregulation of cardiac ACE2 is associated with increased plasma ACE2 activity. In this prospective observational study in recovered COVID-19 patients, we hypothesised that SARS-CoV-2 infection would be associated with shedding of ACE2 from cell membranes and increased plasma ACE2 activity. Methods We measured plasma ACE2 catalytic activity using a validated, sensitive quenched fluorescent substrate-based assay in a cohort of Australians aged 18 years and over who had recovered from mild, moderate or severe SARS-CoV-2 infection (positive result by PCR testing) (n=66) and age and gender matched uninfected controls (n=70). Serial samples were available in 23 recovered SARS-CoV-2 patients. Results Plasma ACE2 activity at a median of 35 days post-infection [interquartile range 30-38 days] was 97-fold higher in recovered SARS-CoV-2 patients compared to controls (5.8 [2-11.3] vs. 0.06 [0.02-2.2] pmol/min/ml, p<0.0001). There was a significant difference in plasma ACE2 activity according to disease severity (p=0.033), with severe COVID-19 associated with higher ACE2 activity compared to mild disease (p=0.027). Men (n=39) who were SARS-CoV-2 positive had higher median plasma ACE2 levels compared to women (n=27) (p<0.0001). We next analysed whether an elevated plasma ACE2 activity level persisted following SARS-CoV-2 infection in subjects with blood samples at 63 [56-65] and 114 [111-125] days post infection. Plasma ACE2 activity remained persistently elevated in almost all subjects, with no significant differences between timepoints in post-hoc comparisons (p>0.05). *Discussion This is the first description that plasma ACE2 activity is elevated after COVID-19 infection, and the first with longitudinal data indicating plasma ACE2 activity remains elevated out to a median of 114 days post- infection. Larger studies are now needed to determine if persistent elevated plasma ACE2 activity identifies people at risk of prolonged illness following COVID-19*. [**note: Here is an Australian study sowing that plasma ACE2 activity is persistently elevated following SARS-CoV-2 infection. Whether this is linked to prolonged illness still needs clarification.**]
https://www.medrxiv.org/content/10.1101/2020.10.06.20207514v2
- Abstract Introduction The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), created a pandemic in late 2019. Acute respiratory distress syndrome can occur in patients with COVID-19 due to viral replication and an uncontrolled immune reaction. Therefore, antiviral and anti-inflammatory treatments are of particular interest to clinicians. We compared the efficacy of methylprednisolone and dexamethasone in reducing inflammation and improving the partial pressure of arterial oxygen and fraction of inspired oxygen (PaO2/FiO2 or P/F) ratio in COVID-19 patients. Methods We selected 60 files for this retrospective quasi-experimental study using a convenient sampling technique and divided them into two groups of 30 patients each who had received either dexamethasone or methylprednisolone. The data were taken from the medical records of the treated patients. Group 1 patients were given dexamethasone 8 mg twice daily, and Group 2 patients were given methylprednisolone 40 mg twice daily for eight days during their stay in our

high dependency unit and our Intensive Care Unit. The remaining treatment was the same for both groups using antibiotics and anticoagulation. We reviewed C-reactive protein (CRP), serum ferritin level, and P/F ratio before and after the administration of both drugs for eight days. We used a paired t-test to assess the effectiveness of both drugs on the P/F ratio of participants. Results The initial mean CRP level of Group 1 was 110.34 mg/L, which decreased to 19.45 mg/L after administration of dexamethasone; similarly, the CRP of Group 2 was 108.65 mg/L, which decreased to 43.82 mg/L after administering methylprednisolone for eight days. *Both dexamethasone and methylprednisolone significantly improved the P/F ratio ($p<0.05$), and dexamethasone was significantly more effective than methylprednisolone ($p<0.05$).* Conclusion Steroids have ability to reduce inflammation and suppress the immune response make them an effective tool in the treatment of COVID-19. Steroid therapy is effective in controlling inflammation markers, and, specifically, dexamethasone is effective in improving the P/F ratio in COVID-19 patients. Physicians should consider the use of dexamethasone use in appropriate patients with COVID-19. [**note: this is a small sample Pakastani study on the difference ini treatment between dexamethasone and methylprednisolone in COVID-19 treatment. It appears that dexamethasone is the treatment of choice. This reminds me to brush up on my steroid pharmacology.**] https://www.medrxiv.org/content/10.1101/2020.10.06.20171579v1

- We performed a retrospective single-center study of consecutively admitted patients between March 1st and May 15th, 2020, with a definitive diagnosis of SARS-CoV-2 infection. The primary endpoint was to evaluate the association of lipid markers with 30-days all-cause mortality in COVID-19. Results: A total of 654 patients were enrolled, with an estimated 30-day mortality of 22.8% (149 patients). Non-survivors had lower total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-c) levels during the entire course of the disease with complete resolution among survivors. Both showed a significant inverse correlation with inflammatory markers and a positive correlation with lymphocyte count. In a multivariate analysis, LDL-c < 69 mg/dl (hazard ratio [HR] 1.94; 95% confidence interval [CI] 1.14-3.31), C-reactive protein > 88 mg/dl (HR 2.44; 95% CI, 1.41-4.23) and lymphopenia < 1000 cells/ml (HR 2.68; 95% CI, 1.91-3.78) at admission were independently associated with 30-day mortality. This association was maintained 7 days after admission. Conclusion: *Hypolipidemia in SARS-CoV-2 infection may be secondary to an immune-inflammatory response, with complete recovery in survivors. Low LDL-c serum levels are independently associated with higher 30-day mortality in COVID-19 patients*. [**note: yet another marker for poor COVID-19 prognosis from Spain. This time it is hypolipidemia.**] https://www.medrxiv.org/content/10.1101/2020.10.06.20207092v1
- Introduction: In Latin America, Peru is the most impacted country due to COVID-19 pandemic. Given the authorized nationwide use of hydroxychloroquine, azithromycin, and ivermectin in COVID-19 patients, we aimed to evaluate their effectiveness alone or combined to reduce 30-day mortality among COVID-19 hospitalized patients without life-threatening illness. Methods: Design. Retrospective cohort study using electronic health records to emulate a target trial. Settings. Nationwide data of mid- and high-level complexity hospitals from the Peruvian Social Health Insurance (EsSalud) between April 1 and July 19, 2020. Participants. Patients 18 years old and above with confirmed SARS-CoV-2 by PCR, and no diagnosis of severe disease at admission. Interventions. Five treatment groups, hydroxychloroquine/chloroquine alone (HCQ), ivermectin alone (IVM), azithromycin alone (AZIT), HCQ + AZIT group, and IVM + AZIT within 48 hours of admission at doses recommended by the Peruvian Ministry of Health. Comparison. Standard of

care treatment without receiving any of the mentioned drugs within 48 of admission. Outcomes: Primary outcome was all-cause mortality rate, and secondary outcomes were survival without death or ICU transfer, and survival without death or oxygen prescription. Analysis. Analysis were adjusted for confounding factors using inverse probability of treatment weighting. Propensity scores were estimated using machine learning boosting models. Weighted hazard ratios (wHR) were calculated using Cox regression Results: Among 5683 patients, 200 received HCT, 203 IVM, 1600 AZIT, 692 HCQ + AZIT, 358 IVM + AZIT, and 2630 received standard of care. The AZIT + HCQ group was associated with 84% higher all-cause mortality hazard rate compared to standard care (wHR=1.84, 95%CI 1.12-3.02). Consistently, AZIT + HCQ treatment was associated with deaths or ICU transfer ICU (wHR=1.49, 95%CI 1.01-2.19), and deaths or oxygen prescription (wHR=1.70, 95%CI 1.07-2.69). HCQ treatment was only associated with death or oxygen prescription (wHR=1.77, 95% CI 1.01-3.11), and IVM was only associated with death or ICU transfer (wHR=1.58, 95%CI 1.11-2.25). No effect was found for AZIT or AZIT + IVM. Conclusion: *Our study reported no beneficial effects of hydroxychloroquine, ivermectin, azithromycin, or their combinations. The AZIT+HCQ treatment reported increased risk of all-cause mortality*. [**note: here is an observational study from hard hit Peru showing that HCQ, azithromycin, and ivermectin in various combinations does not have any beneficial effects. Time for all clinicians to move on.**] https://www.medrxiv.org/content/10.1101/2020.10.06.20208066v2

- Objectives: to undertake a multidisciplinary follow-up at 12 weeks after an acute episode of COVID-19 to assess the functional status, persistence of symptoms and immunoserological situation. Methods: this prospective, observational, single-centre study included outpatients reviewed 12 weeks after an acute infection with SARS-CoV-2. The clinical evaluation included data about the acute episode and epidemiological and clinical variables. The patients were classified as symptomatic or asymptomatic depending on the persistence or otherwise of symptoms. All the patients underwent a full blood test and serology for SARS-CoV-2, as well as imaging tests and spirometry if needed. Results: The mean age of the 108 patients was 55.5 (SD: 15.4) years and 27.8% were health-care workers; 75.9% presented some type of symptoms, with dyspnoea being the most common. A D-dimer >500 ng/mL was detected in 32 (31.4%) patients. All the patients had antibodies against SARS-CoV-2. Being a health-care worker was associated with symptom persistence, with age ≥65 years being a protective factor. Conclusions*: The persistence of symptoms in patents with COVID is usual 12 weeks after the acute episode, especially in patients <65 years and health-care workers. All our patients had developed antibodies by 12 weeks*. [**note: here is a Spanish study of serologica status of COVID-19 patients at the 12 week mark. Some patients can exhibit symptoms at this point following acute episodes.**] https://www.medrxiv.org/content/10.1101/2020.10.06.20206060v1

DRUG DEVELOPMENT

- The COVID-19 pandemic caused by SARS-CoV-2 is in immediate need of an effective antidote. Although the Spike glycoprotein (SgP) of SARS-CoV-2 has been shown to bind to heparins, the structural features of this interaction, the role of a plausible heparan sulfate proteoglycan (HSPG) receptor, and the antagonism of this pathway through small molecules remain unaddressed. Using an in vitro cellular assay, we demonstrate HSPGs modified by the 3-O-sulfotransferase isoform-3, but not isoform-5, preferentially increased SgP-mediated cell-to-cell fusion in comparison to control, unmodified, wild-type HSPGs. Computational studies support

preferential recognition of the receptor-binding domain of SgP by 3-O-sulfated HS sequences. Competition with either fondaparinux, a 3-O-sulfated HS-binding oligopeptide, or a synthetic, non-sugar small molecule, blocked SgP-mediated cell-to-cell fusion. Finally, the synthetic, sulfated molecule inhibited fusion of GFP-tagged pseudo SARS-CoV-2 with human 293T cells with sub-micromolar potency. Overall, overexpression of 3-O-sulfated HSPGs contribute to fusion of SARS-CoV-2, which could be effectively antagonized by a synthetic, small molecule. [**note: here is another approach to drug development via Spike protein binding to heparan**] https://www.biorxiv.org/content/10.1101/2020.10.08.331751v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The COVID-19 pandemic has claimed the lives of more than one million people worldwide. The causative agent, SARS-CoV-2, is a member of the Coronaviridae family, which are viruses that cause respiratory infections of varying severity. The cellular host factors and pathways co-opted by SARS-CoV-2 and other coronaviruses in the execution of their life cycles remain ill-defined. To develop an extensive compendium of host factors required for infection by SARS-CoV-2 and three seasonal coronaviruses (HCoV-OC43, HCoV-NL63, and HCoV-229E), we performed parallel genome-scale CRISPR knockout screens. These screens uncovered multiple host factors and pathways with pan-coronavirus and virus-specific functional roles, including major dependency on glycosaminoglycan biosynthesis, SREBP signaling, and glycosylphosphatidylinositol biosynthesis, as well as an unexpected requirement for several poorly characterized proteins. We identified an absolute requirement for the VTT-domain containing protein TMEM41B for infection by SARS-CoV-2 and all other coronaviruses. *This human Coronaviridae host factor compendium represents a rich resource to develop new therapeutic strategies for acute COVID-19 and potential future coronavirus spillover events*. [**note: from The Rockefeller University here is a genome-scale identification of SARS-CoV-2 and other coronavirus host factor networks.**] https://www.biorxiv.org/content/10.1101/2020.10.07.326462v1
- Characterization of the T cell response in individuals who recover from SARS-CoV-2 infection is critical to understanding its contribution to protective immunity. A multiplexed peptide-MHC tetramer approach was used to screen 408 SARS-CoV-2 candidate epitopes for CD8+ T cell recognition in a cross-sectional sample of 30 COVID-19 convalescent individuals. T cells were evaluated using a 28-marker phenotypic panel, and findings were modelled against time from diagnosis, humoral and inflammatory responses. 132 distinct SARS-CoV-2-specific CD8+ T cell epitope responses across six different HLAs were detected, corresponding to 52 unique reactivities. T cell responses were directed against several structural and non-structural virus proteins. *Modelling demonstrated a coordinated and dynamic immune response characterized by a decrease in inflammation, increase in neutralizing antibody titer, and differentiation of a specific CD8+ T cell response. Overall, T cells exhibited distinct differentiation into stem-cell and transitional memory states, subsets, which may be key to developing durable protection*.[**note: here is a good report on CD8+ T cell differentiation in convalescent COVID-19 individuals.}** https://www.biorxiv.org/content/10.1101/2020.10.08.330688v1
- Knowledge of viral load is essential for formulating strategies for antiviral treatment, vaccination, and epidemiological control of COVID-19. Moreover, patients identification with high viral load could also be useful to understand risk factors such as age, comorbidities, severity of symptoms and hypoxia to decide the need for hospitalization. Several studies are evaluating

the importance of analyzing viral load in different types of samples, clinical outcomes and viral transmission pathways. However, in a great number of emerging studies cycle threshold (Ct) values by itself is often used as a viral load indicator, which may be a mistake. In this study, we compared tracheal aspirate with nasopharyngeal samples obtained from critically ill COVID-19 patients and demonstrate how the raw Ct could lead to misinterpretation of results. Further, we analyzed nasopharyngeal swabs positive samples and propose a method to reduce evaluation error that could occur from using raw Ct. Based on these findings, we show the impact that normalization of Ct values has on interpretation of viral load data from different biological samples from patients with COVID-19, transmission and lastly in relations with clinical outcomes. [**note: this is from Brazil and propose a method to better evaluate viral load which may be important is figuring out disease progression.**]
https://www.medrxiv.org/content/10.1101/2020.10.06.20208009v1

- Microglia, the resident brain immune cells, play a critical role in normal brain development, and are impacted by the intrauterine environment, including maternal immune activation and inflammatory exposures. The COVID-19 pandemic presents a potential developmental immune challenge to the fetal brain, in the setting of maternal SARS-CoV-2 infection with its attendant potential for cytokine production and, in severe cases, cytokine storming. There is currently no biomarker or model for in utero microglial priming and function that might aid in identifying the neonates and children most vulnerable to neurodevelopmental morbidity, as microglia remain inaccessible in fetal life and after birth. This study aimed to generate patient-derived microglial-like cell models unique to each neonate from reprogrammed umbilical cord blood mononuclear cells, adapting and extending a novel methodology previously validated for adult peripheral blood mononuclear cells. We demonstrate that umbilical cord blood mononuclear cells can be used to create microglial-like cell models morphologically and functionally similar to microglia observed in vivo. We illustrate the application of this approach by generating microglia from cells exposed and unexposed to maternal SARS-CoV-2 infection. Our ability to create personalized neonatal models of fetal brain immune programming enables non-invasive insights into fetal brain development and potential childhood neurodevelopmental vulnerabilities for a range of maternal exposures, including COVID-19. [**note: there is a lot we don't know about fetal development if there is SARS-CoV-2 exposure. These researchers may have a model using cord blood derived cells that can help inform this.**]
https://www.biorxiv.org/content/10.1101/2020.10.07.329748v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-10-10

Did Felix Mendelssohn ever write a piece of music that was not lyrical? I've not found any piece yet that I did not enjoy on multiple levels. Here are two of my favorites. The first one is the Violin Concerto in E minor and here we have the Dutch violinist Janine Jansen with the Munich Philharmonic under the direction of tiny baton wielding Valery Gergiev: https://www.youtube.com/watch?v=Wzhk3frB1ao and here is one of my favorite songs, 'Auf Flügeln des Gesanges' sung by Susanna Phillips with Craig Terry on

the piano: https://www.youtube.com/watch?v=LzKZS3OGUw8 the song was also transcribed by Franz Liszt into a piano only version and here is Stéphanie Elbaz in a nice performance with all the Lisztian accoutrements: https://www.youtube.com/watch?v=SeJ_uvgWrK4

One of the most interesting stories from The Washington Post is that mortality rates from COVID-19 appear to be declining in many areas worldwide. This may result from patients having a lower viral load. This is a good trend and it will be important to understand the underlying reason. Here is a report on life in Russia's North Caucasus region under the pandemic. Iran's hospitals are no longer accepting non-emergency patients. Also experiencing a surge in COVID-19 cases, the province of Ontario is implementing new restrictions. According to a new study, some pregnant women are experiencing prolonged symptoms. Tony Fauci noted that there was a superspreader event at the White House. 12,000 minks in Utah and Wisconsin have died from COVID-19. Why are mink farms still in existence? Maybe Sweden's approach is not as good as some people think. Cases are on the rise such that 'herd immunity' is still poorly understood.

The New York Times talks about some COVID-19 patients who require a long recovery after hospitalization. Pandemic fatigue is affecting Europe.

STAT have a video showing how COVID-19 ripped across the US.

The Lancet have a viewpoint article on whether interleukin-33 is the Achilles heel of COVID-19. This is a useful paper to read.

Medscape's editor Eric Topol interviews FDA Commissioner Stephen Hahn.

MODELING

- Angiotensin-converting enzyme 2 (ACE2) is the cellular receptor for the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that is engendering the severe coronavirus disease 2019 (COVID-19) pandemic. The spike (S) protein receptor-binding domain (RBD) of SARS-CoV-2 binds to the three sub-domains viz. amino acids (aa) 22-42, aa 79-84, and aa 330-393 of ACE2 on human cells to initiate entry. It was reported earlier that the receptor utilization capacity of ACE2 proteins from different species, such as cats, chimpanzees, dogs, and cattle, are different. A comprehensive analysis of ACE2 receptors of nineteen species was carried out in this study, and the findings propose a possible SARS-CoV-2 transmission flow across these nineteen species. [**note: here is a study from a multi-national group looking at ACE2 receptors from a number of different animal species.**]
  https://www.biorxiv.org/content/10.1101/2020.10.08.332452v1
- Epidemiological data on the spread of SARS-CoV-2 in the absence and presence of various non-pharmaceutical interventions indicate that the virus is not transmitted uniformly in the population. Transmission tends to be more effective in select settings that involve exposure to relatively high viral dose, such as in crowded indoor settings, assisted living facilities, prisons, or food processing plants. To explore the effect on infection dynamics, we describe a new mathematical model where transmission can occur (i) in the community at large, characterized by low dose exposure and mostly mild disease, and (ii) in so called transmission hot zones, characterized by high dose exposure that can be associated with more severe disease. Interestingly, *we find that successful infection spread can hinge upon high-dose hot zone*

transmission, yet the majority of infections are predicted to occur in the community at large with mild disease. This gives rise to the prediction that targeted interventions that specifically reduce virus transmission in the hot zones (but not in the community at large) have the potential to suppress overall infection spread, including in the community at large. The model can further reconcile seemingly contradicting epidemiological observations. While in some locations like California, strict stay-home orders failed to significantly reduce infection prevalence, in other locations, such as New York and several European countries, stay-home orders lead to a pronounced fall in infection levels, which remained suppressed for some months after re-opening of society. Differences in hot zone transmission levels during and after social distancing interventions can account for these diverging infection patterns. These modeling results warrant further epidemiological investigations into the role of high dose hot zone transmission for the maintenance of SARS-CoV-2 spread. [**note: this is a useful mathematical model that looks at infection spread and non-pharmaceutical interventions. Community at-large transmission can maintain infection spread and needs to be addressed.**]
https://www.medrxiv.org/content/10.1101/2020.10.07.20208231v1

- Human infection with SARS-CoV-2, the causative agent of COVID-19, leads to a remarkably diverse spectrum of outcomes, ranging from asymptomatic to fatal. Recent reports suggest that both clinical and genetic risk factors may contribute to COVID-19 susceptibility and severity. To investigate genetic risk factors, we collected over 500,000 COVID-19 survey responses between April and May 2020 with accompanying genetic data from the AncestryDNA database. We conducted sex-stratified and meta-analyzed genome-wide association studies (GWAS) for COVID-19 susceptibility (positive nasopharyngeal swab test, ncases=2,407) and severity (hospitalization, ncases=250). *The severity GWAS replicated associations with severe COVID-19 near ABO and SLC6A20 (P<0.05). Furthermore, we identified three novel loci with P<5x10-8. The strongest association was near IVNS1ABP, a gene involved in influenza virus replication, and was associated only in males. The other two novel loci harbor genes with established roles in viral replication or immunity: SRRM1 and the immunoglobulin lambda locus. We thus present new evidence that host genetic variation likely contributes to COVID-19 outcomes and demonstrate the value of large-scale, self-reported data as a mechanism to rapidly address a health crisis.* [**note: this is an interesting genetic study from the company AncestryDNA that has a very large database. Several genome wide associations are identified.**
https://www.medrxiv.org/content/10.1101/2020.10.06.20205864v1

NEWLY REGISTERED CLINICAL TRIALS

- You need to be patient and wait until next week to find out what new trial are registered.

CLINICAL TRIAL RESULTS

- Nothing today.

DRUG DEVELOPMENT

- Nothing today.

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The emergence of a SARS-CoV-2 variant with a point mutation in the spike (S) protein, D614G, has taken precedence over the original Wuhan isolate by May 2020. With an increased infection and transmission rate, it is imperative to determine whether antibodies induced against the D614 isolate may cross-neutralize against the G614 variant. *In this report, profiling of the anti-SARS-CoV-2 humoral immunity reveals similar neutralization profiles against both S protein variants, albeit waning neutralizing antibody capacity at the later phase of infection*. These findings provide further insights towards the validity of current immune-based interventions. [**note: neutralizing antibodies from early cases of SARS-CoV-2 offer cross protection against the D614G variant.**] https://www.biorxiv.org/content/10.1101/2020.10.08.332544v1
- Characterization of the humoral response to SARS-CoV-2, the etiological agent of Covid-19, is essential to help control the infection. In this regard, we and others recently reported that the neutralization activity of plasma from COVID-19 patients decreases rapidly during the first weeks after recovery. However, the specific role of each immunoglobulin isotype in the overall neutralizing capacity is still not well understood. In this study, we selected plasma from a cohort of Covid-19 convalescent patients and selectively depleted immunoglobulin A, M or G before testing the remaining neutralizing capacity of the depleted plasma. We found that depletion of immunoglobulin M was associated with the most substantial loss of virus neutralization, followed by immunoglobulin G. This observation may help design efficient antibody-based COVID-19 therapies and may also explain the increased susceptibility to SARS-CoV-2 of autoimmune patients receiving therapies that impair the production of IgM. [**note: this study from Montreal discusses the major role of IgM in convalescent plasma.** https://www.biorxiv.org/content/10.1101/2020.10.09.333278v1
- COVID-19 is associated with a wide spectrum of disease severity, ranging from asymptomatic to acute respiratory distress syndrome (ARDS). Paradoxically, a direct relationship has been suggested between COVID-19 disease severity, and the levels of circulating SARS-CoV-2-specific antibodies, including virus neutralizing titers. Through a serological analysis of serum samples from 536 convalescent healthcare workers, we found that SARS-CoV-2-specific and virus-neutralizing antibody levels were indeed elevated in individuals that experienced severe disease. The severity-associated increase in SARS-CoV-2-specific antibody was dominated by IgG, with an IgG subclass ratio skewed towards elevated receptor binding domain (RBD)- and S1-specific IgG3. However, RBD- and S1-specific IgG1, rather than IgG3 were best correlated with virus-neutralizing titers. We propose that Spike-specific IgG3 subclass utilization contributes to COVID-19 disease severity through potent Fc-mediated effector functions. These results have significant implications for SARS-CoV-2 vaccine design, and convalescent plasma therapy. [**note: this serological analysis reveals an imbalanced IgG subclass composition associated with COVID-19 severity.**] https://www.medrxiv.org/content/10.1101/2020.10.07.20208603v1
- Serological testing in the COVID-19 pandemic is mainly implemented to gain sero-epidemiological data, but can also retrospectively inform about suspected SARS-CoV-2 infection. We verified and applied a two-tiered testing strategy combining a SARS-CoV-2 receptor-binding domain (RBD)-specific lateral flow assay (LFA) with a nucleocapsid protein (NCP) IgG ELISA to assess seroconversion in n=7241 individuals. The majority had experienced symptoms consistent with COVID-19, but had no access to RT-PCR testing. Longitudinal follow-up in n=97 LFA+ individuals was performed up to 20 weeks after initial infection using NCP and spike protein S1 domain (S1) IgG ELISAs and a surrogate virus neutralization test (sVNT). Individuals reporting

symptoms from January 2020 onwards showed seroconversion, as did a considerable proportion of asymptomatic individuals. Seroconversion for symptomatic and asymptomatic individuals was higher in an area with a known infection cluster compared to a low incidence area. Overall, 94% of individuals with a positive IgG result by LFA were confirmed by NCP ELISA. The proportion of ELISA-confirmed LFA results declined over time, in line with contracting NCP IgG titers during longitudinal follow-up. Neutralizing antibody activity was considerably more stable than S1 and NCP IgG titers, and both reach a plateau after approximately 100 days. The sVNT proved to be not only highly specific, but also more sensitive than the specificity-focussed two-tiered serology approach. Our results demonstrate the high specificity of two-tiered serology testing and highlight the sVNT used as a valuable tool to support modelling of SARS-CoV-2 transmission dynamics, complement molecular testing and provide relevant information to individuals. [**note: this is a Dutch seroconversion study looking at the stability of nucleocapsid, spike protein domain I and neutralizing antibodies.**]
https://www.medrxiv.org/content/10.1101/2020.10.07.20187641v1

DIAGNOSTIC DEVELOPMENT

- Novel technologies are needed to facilitate large-scale detection and quantification of SARS-CoV-2 specific antibodies in human blood samples. Such technologies are essential to support seroprevalence studies, vaccine clinical trials, and to monitor quality and duration of immunity. We developed a microfluidic nano-immunnoassay for the detection of anti-SARS-CoV-2 IgG antibodies in 1024 samples per device. The method achieved a specificity of 100% and a sensitivity of 98% based on the analysis of 289 human serum samples. To eliminate the need for venipuncture, we developed low-cost, ultra-low volume whole blood sampling methods based on two commercial devices and repurposed a blood glucose test strip. The glucose test strip permits the collection, shipment, and analysis of 0.6 µL whole blood easily obtainable from a simple fingerprick. The nano-immunoassay platform achieves high-throughput, high sensitivity and specificity, negligible reagent consumption, and a decentralized and simple approach to blood sample collection. We expect this technology to be immediately applicable to current and future SARS-CoV-2 related serological studies and to protein biomarker diagnostics in general. [**note: this is a cool development of a fingerprick collection using a repurposed blood glucose test strip. It can be applied to a nano-immunoassay platform.**]
https://www.medrxiv.org/content/10.1101/2020.10.07.20208280v1

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2020-10-11

Here is a special treat for reflection Sunday. You will need to set aside a bit of time to take this one in. It is a stream of the current Vienna State Opera of Verdi's Don Carlos. I believe this performance was staged on October 4. Yes, it is the French version rather than the Italian one, complete with the ballet (required of all Parisian staged operas in that time). This is one of my favorite Verdi operas but until now I only have seen the Italian version. Some traditionalists may object to the staging. I like these kinds of productions! The cast and notes are at the Vienna Opera link above. Enjoy this one!!!
https://www.youtube.com/watch?v=xaE206ziAq8

The Washington Post writes about a Frederick MD choral group that is resuming singing with precautions (as a singer, I find it difficult to sing with a mask on). In the continuing 'Voices from the Pandemic', a cautionary tale about a COVID-19 denier. It sad that it takes contracting COVID-19 to reveal a public health truth. This group of women turned to activism following the loss of loved ones to COVID-19. Thinking about renting an Airbnb? This story might help assuage your fears. Here is an op-ed about possible weather impacts on COVID-19 case numbers as we enter fall.

Perhaps men are the root problem of COVID-19 spread according to The New York Times. Yes, I have COVID dreams that are sometimes bizarre; this story talks about dreams people are having.

The Guardian has an interesting article on the rise of Chinese medical research.

Science has a report on animal data for the Regeneron paired monoclonal antibody therapeutic.

Very short reading day but you have been gifted a wonderful opera to watch rather than read about COVID-19 research.

MODELING

- Nothing.

NEWLY REGISTERED CLINICAL TRIALS

- It is Sunday and I deserve a day off.

CLINICAL TRIAL RESULTS

- No results today.

DRUG DEVELOPMENT

- The unprecedented and rapid spread of SARS-CoV-2 has motivated the need for a rapidly producible and scalable vaccine. Here, we developed a synthetic soluble SARS-CoV-2 spike (S) DNA-based vaccine candidate, GX-19. In mice, immunization with GX-19 elicited not only S-specific systemic and pulmonary antibody responses but also Th1-biased T cell responses in a dose-dependent manner. GX-19 vaccinated nonhuman primate seroconverted rapidly and exhibited detectable neutralizing antibody response as well as multifunctional CD4+ and CD8+ T cell responses. Notably, when the immunized nonhuman primates were challenged at 10 weeks after the last vaccination with GX-19, they did not develop fever and reduced viral loads in contrast to non-vaccinated primates as a control. These findings indicate that GX-19 vaccination provides durable protective immune response and also support further development of GX-19 as a vaccine candidate for SARS-CoV-2 in human clinical trials. [**note: here is animal data for the Vaxigen COVID-19 vaccine from South Korea. It is in clinical trials there.**] https://www.biorxiv.org/content/10.1101/2020.10.09.334136v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- COVID-19 patients present high incidence of kidney abnormalities, which are associated with poor prognosis and high mortality. Identification of SARS-CoV-2 in kidney of COVID-19 patients suggests renal tropism and direct infection. Presently, it is generally recognized that SARS-CoV-2 initiates invasion through binding of receptor-binding domain (RBD) of spike protein to host cell-membrane receptor ACE2, however, whether there is additional target of SARS-CoV-2 in kidney remains unclear. Kidney injury molecule-1 (KIM1) is a transmembrane protein that drastically up-regulated after renal injury. Here, binding between SARS-CoV2-RBD and the extracellular Ig V domain of KIM1 was identified by molecular simulations and co-immunoprecipitation, which was comparable in affinity to that of ACE2 to SARS-CoV-2. Moreover, KIM1 facilitated cell entry of SARS-CoV2-RBD, which was potently blockaded by a rationally designed KIM1-derived polypeptide. Together, the findings suggest KIM1 may mediate and exacerbate SARS-CoV-2 infection in a vicious cycle, and KIM1 could be further explored as a therapeutic target. [**note: here is report from China about another viral entry point in kidneys.**] https://www.biorxiv.org/content/10.1101/2020.10.09.334052v1

DIAGNOSTIC DEVELOPMENT

- No results today.