2020-06-15

Welcome to Week 13 of what hope is not going to be an endless wandering in the wilderness.

Of the 32 Beethoven piano sonatas, #31 is my favorite. Here we have Vladimir Ashkenazy playing at a live recital at University of Essex in 1972: https://www.youtube.com/watch?v=QI1viKPG3TI I saw him about five years later when I was a post doc at Cornell and he gave a mesmerizing performance of this sonata. I had a special thrill this morning when my YouTube feed showed that Joan Chamorro had given me a ❤️ for my comment to the video of 'Love Your Spell is Everywhere' featuring the young vocalists Alaba Armengou and Élia Bastida: https://www.youtube.com/watch?v=JRtH41FP3nI As soon as it's safe to travel, I am going back to Barcelona to see them live!

I totally forgot to mention that four astute readers had the correct answer to Saturday's contest. Van Cliburn was the winner of the first Tchaikovsky Piano Competition held in Moscow in 1958. I received a note from a loyal reader yesterday in response to my music choice of Bach's Goldberg Variations., asking if I had read Richard Powers fine novel, 'The Goldbug Variations.' OMG!! Powers is my favorite American author, and this is for me, his finest book. To all my loyal readers I give this one a big 👍

I don't know if this was from the larger survey of epidemiologists that The New York Times did a couple of weeks ago, but here is their response to the question, '…when would you send your children back to school.' A significant number said this fall!!

Will the US fail at contact tracing? It's not clear but this Washington Post story does not paint an optimistic picture. However, this New Yorker story about the implementation of contact tracing in Massachusetts makes me think it is possible.

Interesting story on a remote town in Michigan and how they are approaching COVID-19 (I *think* the author is a former NY Times reporter)

Here is a good summary article on who is dying from serious COVID-19 infections. Social factors need to be looked at and there are some good reference links embedded in this article from STAT.

Victor Fuchs writes about health care policy after the COVID-19 pandemic. For those not familiar with Fuchs, he is one of the preeminent health care economists and together with Zeke Emanuel proposed a voucher system for providing ALL Americans with health insurance. When I was at PhRMA I had Zeke come down and give a lunch time talk to the staff on the proposal and why it was better than the patchwork system of plans that were then (and still are) prevalent. I continue to believe this approach to be viable even with the political difficulty of getting a VAT through Congress. Perhaps with the current crisis of people losing health insurance will give this added impetus. Recovered COVID-19 patients are getting some very hefty hospital bills!

China is undertaking another mass testing effort, this time in Beijing, following an outbreak of SARS-CoV-2 infection at a large fruit and vegetable market. Several states in the US are seeing upticks in confirmed cases and hospitalization. It appears these are resulting from people not practicing good public health practices. Put the damn masks on when you go out to shop!

Thankfully, it is Monday which means a short day for abstract and paper reading!!

MODELING

- A few animals have been suspected to be intermediate hosts of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, a large-scale single-cell screening of SARS-CoV-2 target cells on a wide variety of animals is missing. Here, we constructed the single-cell atlas for 11 representative species in pets, livestock, poultry, and wildlife. Notably, the proportion of SARS-CoV-2 target cells in cat was found considerably higher than other species we investigated and SARS-CoV-2 target cells were detected in multiple cell types of domestic pig, implying the necessity to carefully evaluate the risk of cats during the current COVID-19 pandemic and keep pigs under surveillance for the possibility of becoming intermediate hosts in future coronavirus outbreak. Furthermore, we screened the expression patterns of receptors for 144 viruses, resulting in a comprehensive atlas of virus target cells. Taken together, our work provides a novel and fundamental strategy to screen virus target cells and susceptible species, based on single-cell transcriptomes we generated for domesticated animals and wildlife, which could function as a valuable resource for controlling current pandemics and serve as an early warning system for coping with future infectious disease threats. [**note: from China, a study of SARS-CoV-2 target cells in a variety of animals. The cat data is confirmatory of the finding of the virus in that species. This does not imply animal to human transmission.**] https://www.biorxiv.org/content/10.1101/2020.06.13.149690v1

NEWLY REGISTERED CLINICAL TRIALS

- The purpose of this randomized, double blinded, placebo controlled study is to assess the efficacy and safety of tofacitinib in hospitalized adult (18-65 years old) patients with SARS-CoV-2 and pneumonia who require supplemental oxygen and have serologic markers of inflammation but do not need mechanical ventilation. [**note: this is a JAK inhibitor that is used to treat rheumatoid and psoriatic arthritis. Trial is at Yale.**] NCT04415151
- The purpose of this study is to evaluate safety, tolerability, and pharmacodynamics of MK-5475 after administration of multiple doses to participants with COVID-19 pneumonia. The primary hypothesis is that MK-5475 when administered to participants with COVID-19 pneumonia and hypoxemia improves arterial oxygenation as measured by the ratio of blood oxygen saturation to fraction of inspired oxygen (SpO2/FiO2 ratio) compared to placebo. [**note: this is a Merck experimental drug that has been studied for pulmonary hypertension. I cannot find a structure.**] NCT04425733

CLINICAL TRIAL RESULTS

- We studied plasma antibody responses of 35 patients about 1 month after SARS-CoV-2 infection. Titers of antibodies binding to the viral nucleocapsid and spike proteins were significantly higher in patients with severe disease. Likewise, mean antibody neutralization titers against SARS-CoV-2 pseudovirus and live virus were higher in the sicker patients, by ~5-fold and ~7-fold, respectively. These findings have important implications for those pursuing plasma therapy, isolation of neutralizing monoclonal antibodies, and determinants of immunity. [**note: similar to other researcher's findings, high titer antibody production is more robust in patients with severe disease.**] https://www.biorxiv.org/content/10.1101/2020.06.13.150250v1

DRUG DEVELOPMENT

- To circumvent time-consuming clinical trials, testing whether existing drugs are effective inhibitors of SARS-CoV-2, has led to the discovery of Remdesivir. We decided to follow this path and screened approved medications off-label against SARS-CoV-2. In these screenings, Fluoxetine inhibited SARS-CoV-2 at a concentration of 0.8µ/ml significantly, and the EC50 was determined with 2.5ng/ml. Fluoxetine is a racemate consisting of both stereoisomers, while the S-form is the dominant serotonin reuptake inhibitor. We found that both isomers show similar activity on the virus. Fluoxetine treatment resulted in a decrease in viral protein expression. Furthermore, Fluoxetine inhibited neither Rabies virus, human respiratory syncytial virus replication nor the Human Herpesvirus 8 or Herpes simplex virus type 1 gene expression, indicating that it acts virus-specific. We see the role of Fluoxetine in the early treatment of SARS-CoV-2 infected patients of risk groups. [**note: WOW – solve the SARS-CoV-2 infection problem and level out everyone's mood at the same time. Actually, someone ought to do datamining to see if there is some protection to be offered with this drug.** https://www.biorxiv.org/content/10.1101/2020.06.14.150490v1
- An effective prophylactic vaccine is urgently needed to protect against SARS-CoV-2 infection. The viral spike, which mediates entry into cells by interacting with the host angiotensin-converting enzyme 2, is the primary target of most vaccines in development. These vaccines aim to elicit protective immunity against the glycoprotein by use of inactivated virus, vector-mediated delivery of the antigen in vivo, or by direct immunization with the purified antigen following expression in a heterologous system. These approaches are mostly dependent on the growth of mammalian or insect cells, which requires a sophisticated infrastructure that is not generally available in developing countries due to the incumbent costs which are prohibitive. Plant-based subunit vaccine production has long been considered as a cheaper alternative, although low expression yields and differences along the secretory pathway to mammalian cells have posed a challenge to producing certain viral glycoproteins. Recent advances that have enabled many of these constraints to be addressed include expressing the requisite human proteins in plants to support the maturation of the protein of interest. In this study we investigated these approaches to support the production of a soluble and putatively trimeric SARS-CoV-2 spike mimetic in Nicotiana benthamiana via transient Agrobacterium-mediated expression. The co-expression of human calreticulin dramatically improved the accumulation of the viral spike, which was barely detectable in the absence of the co-expressed accessory protein. The viral antigen was efficiently processed even in the absence of co-expressed furin, suggesting that processing may have occurred at the secondary cleavage site and was mediated by an endogenous plant protease. In contrast, the spike was not efficiently processed when expressed in mammalian cells as a control, although the co-expression of furin improved processing considerably. This study demonstrates the feasibility of molecular engineering to improve the production of viral glycoproteins in plants, and supports plant-based production of SARS-CoV-2 spike-based vaccines and reagents for serological assays. [**note: this South African group expresses the viral spike protein in a tobacco related species. This type of technology has been around for thirty years, yet no commercial vaccine or therapeutic protein has been licensed via this approach.**] https://www.biorxiv.org/content/10.1101/2020.06.14.150458v1
- Pandemic spread of emerging human pathogenic viruses such as the current SARS-CoV2, poses both an immediate and future challenge to human health and society. Currently, effective treatment of infection with SARS-CoV2 is limited and broad spectrum antiviral therapies to meet

other emerging pandemics are absent leaving the World population largely unprotected. Identification of compounds with antiviral properties is thus highly desirable. Here, we have identified distinct members of the family of polyether ionophore antibiotics with potent ability to inhibit SARS-CoV2 replication and cytopathogenicity in cells. Several compounds from this class displayed more than 100-fold selectivity between viral-induced cytopathogenicity and inhibition of cell viability, however the compound X-206 displayed >500-fold selectivity and was furthermore able to inhibit viral replication even at sub-nM levels. The antiviral mechanism of the polyether ionophores is currently not understood in detail. We demonstrate, through unbiased bioactivity profiling, that their effects on the host cells differ from those of cationic amphiphiles such as hydroxychloroquine. Collectively, our data suggest that polyether ionophore antibiotics should be subject to further investigations as potential broad-spectrum antiviral agents. [**note: this finding comes from a screening project of polyether ionophores some of which are approved for pharmaceutical use. The most potent is an experimental drug X-206 and I don't have any clue whether this has ever been looked at clinically in humans.**] https://www.biorxiv.org/content/10.1101/2020.06.14.149153v1

DIAGNOSTIC DEVELOPMENT

- There is an urgent need for methods that can rapidly detect and sequence SARS-CoV-2 simultaneously. Here we describe a method for multiplex isothermal amplification of the SARS-CoV-2 genome in 20 minutes. Based on this, we developed NIRVANA (Nanopore sequencing of Isothermal Rapid Viral Amplification for Near real-time Analysis) to detect viral sequences and monitor mutations in multiple regions of SARS-CoV-2 genome for up to 96 patients at a time. NIRVANA uses a newly developed algorithm for on-the-fly data analysis during Nanopore sequencing. The whole workflow can be completed in as short as 3.5 hours, and all reactions can be done in a simple heating block. NIRVANA provides a rapid field-deployable solution of SARS-CoV-2 detection and surveillance of pandemic strains. [**note: cool new approach to rapid detection and mutation surveillance from Saudi Arabia.**] https://www.medrxiv.org/content/10.1101/2020.06.12.20129247v1

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2020-06-16

Two of the great ladies of American song. Does it get any better than this? Jo Stafford and Ella Fitzgerald having a great time with classic songs about love: https://www.youtube.com/watch?v=ctyT-9o4Vl0 There wasn't any description about when this was recorded but judging by some other clips, I suspect this took place in the early 1960s. Stafford and her husband did a number of music spoof recording under the names: Darlene and Jonathan Edwards. Most of the recordings were sung off key and with terrible rhythm which made them all the more fun. As a special treat, here are Darlene and Jonathan Edwards on the Jack Benny show from 1958! How I wish we had television as witty as this right now.

Contest Alert!!! Today is famous in literary circles (and I know we have some very able and literate readers who will get this one right away). It's a two part question: 1) what is today famous for and 2)

who spoke this famous line (greatly excerpted – hint, hint) ***"….the sea crimson sometimes like fire and the glorious sunsets and the fig trees in the Alameda gardens yes and all the queer little streets and the pink and blue and yellow houses and the rose gardens and the jessamine and geraniums and cactuses and Gibraltar as a girl where I was a Flower of the mountain…"?*** **A prize awaits the correct answers!**

As you all know, I have worked hard to make this newsletter apolitical and focus on the science and research. I reached the breaking point yesterday when senior political leaders said that the only reason the US is seeing so many COVID-19 cases is because "…if we stop testing right now, we'd have very few cases, if any…" I hate to be the bearer of bad tidings, but testing has nothing to do with the number of viral infections. Were this so, nobody would need to go to the hospital and be admitted to the ICU for treatment. *Enough of this, speak candidly to the American public or don't speak at all.* Rant over.

Derek Lowe comments on drug and vaccine safety. Too often the focus is on efficacy with safety a distant thought.

The big news yesterday was FDA's revocation of the Emergency Use Authorization for chloroquine and hydroxychloroquine in the treatment of SARS-CoV-2. "We've made clear throughout the public health emergency that our actions will be guided by science and that our decisions may evolve as we learn more about the SARS-CoV-2 virus, review the latest data, and consider the balance of risks versus benefits of treatments for COVID-19," said FDA Deputy Commissioner for Medical and Scientific Affairs Anand Shah, M.D. "The FDA always underpins its decision-making with the most trustworthy, high-quality, up-to-date evidence available. We will continue to examine all of the emergency use authorizations the FDA has issued and make changes, as appropriate, based on emerging evidence." It was never clear to me that the original decision was based on science but that's just one person's opinion. One wonders whether this decision will have any impact on the ongoing clinical trials here in the US, particularly the large Duke healthcare provider prevention trial. I'll leave it up to you, my loyal readers, to find your favorite popular press discussions of this issue. I moved on several weeks ago but I will give this STAT piece the final say.

It's science Tuesday for many newspapers and here are some pertinent articles related to COVID-19. Here is an overview of the CDC dataset on COVID-19 infections in the US sowing patients with underlying conditions were 12 times likely to die than otherwise health people. CDC dataset is here. Pricing of COVID-19 tests varies greatly and perhaps there is some gouging going on. Inside a vaccine clinical trial and a nice graphic that tracks vaccine development. A good article on the links between Vitamin D and COVID-19 resistance.

JAMA have a viewpoint article on the use of monoclonal antibodies and convalescent plasma.

Finally, a clinical trial result with some really good news. The UK RECOVERY trial looked at whether dexamethasone had any impact on mortality. I only have a news report link. Death in severe COVID-19 case was reduced by about a third. "This is a result that shows that if patients who have COVID-19 and are on ventilators or are on oxygen are given dexamethasone, it will save lives, and it will do so at a remarkably low cost," said Martin Landray, an Oxford University professor who is co-leading the trial.

And really finally, the science behind some claims made on the classic TV comedy Seinfeld!!!!

MODELING

- Models and rethinking risk are the subject of this Lancet commentary.
- We used viral genomics to deeply analyze the first SARS-CoV-2 infection clusters in the metropolitan region of Hamburg, Germany. Epidemiological analysis and contact tracing together with a thorough investigation of virus variant patterns revealed low and high infection dose transmissions to be involved in transmission events. Methods: Infection control measures were applied to follow up contract tracing. Metagenomic RNA- and SARS-CoV-2 amplicon sequencing was performed from 25 clinical samples for sequence analysis and variant calling. Results: The index patient acquired SARS-CoV-2 in Italy and after his return to Hamburg transmitted it to 2 out of 132 contacts. Virus genomics and variant pattern clearly confirms the initial local cluster. We identify frequent single nucleotide polymorphisms at positions 241, 3037, 14408, 23403 and 28881 previously described in Italian sequences and now considered as one major genotype in Europe. While the index patient showed a single nucleotide polymorphism only one variant was transmitted to the recipients. Different to the initial cluster, we observed in household clusters occurring at the time in Hamburg also intra-host viral species transmission events. Conclusions: SARS-CoV-2 variant tracing highlights both, low infection dose transmissions suggestive of fomites as route of infection in the initial cluster and high and low infection dose transmissions in family clusters indicative of fomites and droplets as infection routes. This suggests (1) single viral particle infection can be sufficient to initiate SARS-CoV-2 infection and (2) household/family members are exposed to high virus loads and therefore have a high risk to acquire SARS-CoV-2. [**note: keep the Clorox spray ready to go at your kitchen and other environmental surfaces. However, I'm not convinced about the single viral particle theory.**] https://www.medrxiv.org/content/10.1101/2020.06.11.20127332v1
- To support public health policymakers in Connecticut as they begin phased lifting of social distancing restrictions, we developed a county-structured compartmental SEIR-type model of SARS-CoV-2 transmission and COVID-19 disease progression. We calibrated this model to the local dynamics of deaths and hospitalizations and the exact timing of state interventions, including school closures and stay-at-home order. In this technical report, we describe the details of the model design, implementation and calibration, and show projections of epidemic development through the Summer of 2020 under different assumptions about the increase in contact rates following partial state reopening. Our model results are consistent with high effectiveness of state lockdown measures, but changes in human interaction patterns during the coming months are unknown. In addition, a lot of uncertainty remains with respect to several key epidemiological parameters and the effectiveness of increased testing and contact tracing capacity. As more information becomes available, we will update the projections presented in this report. Reports in this series are posted to https://crawford-lab.github.io/covid19_ct/. [**note: this model is for all my loyal readers in the Nutmeg State!**] https://www.medrxiv.org/content/10.1101/2020.06.12.20126391v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Other than the dexamethasone report above, I saw nothing else which was somewhat surprising.

DRUG DEVELOPMENT

- Recently, a novel isolate of the SARS-CoV-2 virus carrying a point mutation in the Spike protein (D614G) has emerged and rapidly surpassed others in prevalence, including the original SARS-CoV-2 isolate from Wuhan, China. This Spike variant is a defining feature of the most prevalent clade (A2a) of SARS-CoV-2 genomes worldwide. Using phylogenomic data, several groups have proposed that the D614G variant may confer increased transmissibility leading to positive selection, while others have claimed that currently available evidence does not support positive selection. Furthermore, in the A2a clade, this mutation is in linkage disequilibrium with a ORF1b protein variant (P314L), making it difficult to discern the functional significance of the Spike D614G mutation from population genetics alone. Here, we perform site-directed mutagenesis on a human codon-optimized spike protein to introduce the D614G variant and produce SARS-CoV-2-pseudotyped lentiviral particles (S-Virus) with this variant and with D614 Spike. We show that in multiple cell lines, including human lung epithelial cells, that S-Virus carrying the D614G mutation is up to 8-fold more effective at transducing cells than wild-type S-Virus. This provides functional evidence that the D614G mutation in the Spike protein increases transduction of human cells. Further we show that the G614 variant is more resistant to cleavage in vitro and in human cells, which may suggest a possible mechanism for the increased transduction. Given that several vaccines in development and in clinical trials are based on the initial (D614) Spike sequence, this result has important implications for the efficacy of these vaccines in protecting against this recent and highly-prevalent SARS-CoV-2 isolate. [**note: another paper that could go into a several different categories. We still don't fully understand the impact of mutations in SARS-CoV-2 and a lot of what is being said in preprints is conjecture. Even so, researchers need to take these studies seriously. Whether vaccine development is impacted by this particular mutation is still unknown, another example of the Rumsfeld Paradigm.**] https://www.biorxiv.org/content/10.1101/2020.06.14.151357v1
- The causative agent of the coronavirus disease 2019 (COVID-19) pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is steadily mutating during continuous transmission among humans. Such mutations can occur in the spike (S) protein that binds to the angiotensin-converting enzyme-2 (ACE2) receptor and is cleaved by transmembrane protease serine 2 (TMPRSS2). However, whether S mutations affect SARS-CoV-2 infectivity remains unknown. Here, we show that naturally occurring S mutations can reduce or enhance cell entry via ACE2 and TMPRSS2. A SARS-CoV-2 S-pseudotyped lentivirus exhibits substantially lower entry than SARS-CoV S. Among S variants, the D614G mutant shows the highest viral entry, as supported by structural observations. *Nevertheless, the D614G mutant remains susceptible to neutralization by antisera against prototypic viruses. Taken together, these data indicate that the D614G mutation enhances viral infectivity while maintaining neutralization susceptibility*. [**note: this Japanese group is looking at the same mutation as the prior paper from NYU. Note the conclusion!**] https://www.biorxiv.org/content/10.1101/2020.06.15.151779v1
- Several techniques to detect SARS-CoV-2 infection have been established, mainly based on counting infected cells by staining plaques or foci, or by quantifying the viral genome by PCR. These methods are laborious, time-consuming and expensive and therefore not suitable for a

high sample throughput or rapid diagnostics. We here report a novel enzyme-based immunodetection assay that directly quantifies the amount of de novo synthesized viral spike protein within fixed and permeabilized cells. This in-cell ELISA enables a rapid and quantitative detection of SARS-CoV-2 infection in microtiter format, regardless of the virus isolate or target cell culture. It follows the established method of performing ELISA assays and does not require expensive instrumentation. Utilization of the in-cell ELISA allows to e.g. determine TCID50 of virus stocks, antiviral efficiencies (IC50 values) of drugs or neutralizing activity of sera. Thus, the in-cell spike ELISA represents a promising alternative to study SARS-CoV-2 infection and inhibition and may facilitate future research. [**note: this is an interesting German paper that may provide an easier way to do drug development.**]
https://www.biorxiv.org/content/10.1101/2020.06.14.150862v1

- We have developed a new class of tetravalent, biparatopic therapy, 89C8-ACE2. It combines the specificity of a monoclonal antibody (89C8) that recognizes the relatively conserved N-terminal domain (NTD) of the viral S glycoprotein, and the ectodomain of ACE2, which binds to the receptor-binding domain (RBD) of S. This molecule shows exceptional performance in vitro, inhibiting the interaction of recombinant S1 to ACE2 and transduction of ACE2-overexpressing cells by S-pseudotyped lentivirus with IC50s substantially below 100 pM, and with potency approximately 100-fold greater than ACE2-Fc itself. Moreover, 89C8-ACE2 was able to neutralize authentic virus infection in a standard assay at low nanomolar concentrations, making this class of molecule a promising lead for therapeutic applications. [**note: this is a way cool technological approach and if it works, offers a whole new way of designing immunotherapy drugs. They created a biapartopic drug with two different binding domains. Good outside the box thinking but I wonder how amenable it is to scale up. There is a trial or two going on with soluble ACE2.**] **.**https://www.biorxiv.org/content/10.1101/2020.06.14.147868v1
- SARS-CoV-2, the etiologic agent of COVID-19, uses ACE2 as a cell entry receptor. Soluble ACE2 has been shown to have neutralizing antiviral activity but has a short half-life and no active transport mechanism from the circulation into the alveolar spaces of the lung. To overcome this, we constructed an ACE2-human IgG1 fusion protein with mutations in the catalytic domain of ACE2. This fusion protein contained a LALA mutation that abrogates Fcrgamma binding, but retains FcRN binding to prolong the half-life, as well as achieve therapeutic concentrations in the lung lavage. Interestingly, a mutation in the catalytic domain of ACE2, MDR504, completely abrogated catalytic activity, but significantly increased binding to SARS-CoV-2 spike protein in vitro. This feature correlated with more potent viral neutralization in a plaque assay. *Parental administration of the protein showed stable serum concentrations with a serum half-life of approximately 152 hours with excellent bioavailability in the epithelial lining fluid of the lung. These data support that the MDR504 mutant is an excellent candidate for pre- or post-exposure prophylaxis or treatment of COVID-19.* [**note: wow, the very next paper up is a riff on the one above.**] https://www.biorxiv.org/content/10.1101/2020.06.15.152157v1

DIAGNOSTIC DEVELOPMENT

- Rapid, scalable, point-of-need, COVID-19 diagnostic testing is necessary to safely re-open economies and prevent future outbreaks. We developed an assay that detects single copies of SARS-CoV-2 virus directly from saliva and swab samples in 30 min using a simple, one-step protocol that utilizes only a heat block and microcentrifuge tube prefilled with a mixture

containing the necessary reagents and has a sensitivity and specificity of 97% and 100%, respectively. [**note: is this one too good to be true? Only development and outside validation by other labs will say for sure. Columbia University have applied for a patent on the approach. Will it be scaled up in time to be of use?**]
https://www.medrxiv.org/content/10.1101/2020.06.13.20129841v1

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2020-06-17

Stacey Kent has been one of my favorite contemporary vocalists. She floats between a number of different jazz influenced genres as well as the classic American songbook. Along with other artists, she has been doing isolation videos. Here she is with 'A Time for Love' something that there is too little of these days: https://www.youtube.com/watch?v=iU-S2ScUKLk

The answer to yesterday's contest was Ulysses by James Joyce where the action of the book takes place on June 16th which is now referred to in literary circles as 'Bloomsday'. The quotation was from the lengthy soliloquy delivered by Molly Bloom in the book's final chapter. Here is Angeline Ball from the film Bloom doing the last fifty lines, well worth watching, yes, yes, yes!

Here is the press release on the UK dexamethasone trial. I was a little too obtuse yesterday. This is part of a large randomized control trial of several possible COVID-19 therapies. From the trial register we see: https://clinicaltrials.gov/ct2/show/NCT04381936 this is just one part of a large trial with several treatment arms. I would assume the randomization arm is being used as the comparator for the other drugs, "RECOVERY is a randomised trial investigating whether treatment with either Lopinavir-Ritonavir, Hydroxychloroquine, Corticosteroids, Azithromycin, Convalescent plasma or Tocilizumab prevents death in patients with COVID-19." The end point for the trial is pretty clear and non-arguable. 😊 As is noted in the press release the dexamethasone arm of the trial was stopped as there was sufficient enrollment to show efficacy. This is effective only in serious COVID-19 cases where oxygen or ventilation is required. We still need a good oral drug that can treat mild to moderate COVID-19.

Operation Warp Speed, the Trump Administration's effort to accelerate a COVID-19 vaccine, may be taking a political turn. This is not the way to do responsible product development. What will happen to the 66 million doses of stockpiled chloroquine and hydroxychloroquine. This New York Times article discusses but doesn't answer the question.

What to do when the toilet in a public bathroom does not have a lid to put down before you flush? Certainly a plume of infectious SARS-CoV-2 must be avoided. These Chinese scientists do the necessary research! I need to get out my old college physics text and read up on fluid dynamics; in the meantime I should add to my signature line "*always put the toilet lid down before you flush!*" I can see the smiles on all my reader's faces, but this is not a laughing matter (actually it is funny in a bizarre way).

STAT offers an overview of the road ahead as COVID-19 cases and hospitalizations rise in certain parts of the US. Whether the road is long or short is highly dependent on the behavior of the American public. Here is a nice frontline physician's account of cough and pneumonia in the time of coronavirus.

Finally, a cautionary tale from Florida courtesy of the Washington Post Live Update page, "A night of partying on the weekend that bars in Florida reopened resulted in a group of 16 friends becoming infected with the novel coronavirus and regretting the decision to go out, they said. On June 6, Erika Crisp and her friends visited a crowded Lynch's Irish Pub in Jacksonville Beach to celebrate a friend's birthday. The pub was packed with other celebrators who weren't wearing masks, she told CNN's Chris Cuomo on "Cuomo Prime Time" on Tuesday. "At the time it was more out of sight out of mind. We hadn't known anybody who had it personally. Governor, mayor, everybody says it's fine," she said, adding that her friends showed symptoms within days of the outing. "It was a mistake. I feel foolish. It's too soon."

Lots of stuff to read today, especially in the drug development section!

MODELING

- All 4125 staff were invited and 3056 (74%) participated (306 physicians, 1266 nurses, 292 paramedical staff, 555 technical staff, 445 administrative staff, and 192 others, including students and volunteers). At least one-third of those not tested were individuals not at work during the period. Overall, 197 staff (6.4% [95% CI, 5.5%-7.3%]) had IgG antibodies for SARS-CoV-2. Age and sex were not statistically significantly different among staff with or without antibodies (mean age, 39.5 [SD, 13.1] vs 41.3 [SD, 12.4] years; 38/197 [19%] vs 614/2859 [21%] men). Being involved in clinical care, having worked during the lockdown phase, being involved in care for patients with COVID-19, and exposure to COVID-19–positive coworkers were not statistically significantly associated with seroprevalence (Figure 2A). In contrast, having a household contact with suspected or confirmed COVID-19 was associated with antibody positivity (81/593 [13.7%] with household contacts vs 116/2435 [4.8%] without household exposure; *P* < .001), with an odds ratio of 3.15 (95% CI, 2.33-4.25). [**note: this is a serology and viral PCR testing of hospital staff in a hard hit area of Belgium. They observed a low level of infection that they attribute high availability of PPE, high standards of infection prevention, and polymerase chain reaction screening in symptomatic staff, coupled with contact tracing and quarantine**.] https://jamanetwork.com/journals/jama/fullarticle/2767382
- During the 2020 COVID-19 pandemic, an outbreak occurred following attendance of a symptomatic index case at a regular weekly rehearsal on 10 March of the Skagit Valley Chorale (SVC). After that rehearsal, 53 members of the SVC among 61 in attendance were confirmed or strongly suspected to have contracted COVID-19 and two died. Transmission by the airborne route is likely. It is vital to identify features of cases such as this so as to better understand the factors that promote superspreading events. Based on a conditional assumption that transmission during this outbreak was by inhalation of respiratory aerosol, we use the available evidence to infer the emission rate of airborne infectious quanta from the primary source. We also explore how the risk of infection would vary with several influential factors: the rates of removal of respiratory aerosol by ventilation; deposition onto surfaces; and viral decay. The results indicate an emission rate of the order of a thousand quanta per hour (mean [interquartile range] for this event = 970 [680-1190] quanta per hour) and demonstrate that the

risk of infection is modulated by ventilation conditions, occupant density, and duration of shared presence with an infectious individual. [**note: this is a model of the now well known viral spreading event during a choral practice. It's useful for choral singers to read as there are some sound comments on ventilation and HVAC systems. I do miss choir rehearsals and probably won't be going back any time soon** ☹]
https://www.medrxiv.org/content/10.1101/2020.06.15.20132027v1

- We characterized SARS-CoV-2 infections in a densely-populated, majority Latinx San Francisco community six-weeks into the city's shelter-in-place order. Methods: We offered SARS-CoV-2 reverse transcription-PCR and antibody (Abbott ARCHITECT IgG) testing, regardless of symptoms, to all residents (>=4 years) and workers in a San Francisco census tract (population: 5,174) at outdoor, community-mobilized events over four days. We estimated SARS-CoV-2 point prevalence (PCR-positive) and cumulative incidence (antibody or PCR-positive) in the census tract and evaluated risk factors for recent (PCR-positive/antibody-negative) versus prior infection (antibody-positive/PCR-negative). SARS-CoV-2 genome recovery and phylogenetics were used to measure viral strain diversity, establish viral lineages present, and estimate number of introductions. Results: We tested 3,953 persons: 40% Latinx; 41% White; 9% Asian/Pacific Islander; and 2% Black. Overall, 2.1% (83/3,871) tested PCR-positive: 95% were Latinx and 52% asymptomatic when tested. 1.7% of residents and 6.0% of workers (non-census tract residents) were PCR-positive. Among 2,598 census tract residents, estimated point prevalence of PCR-positives was 2.3% (95%CI: 1.2-3.8%): 3.9% (95%CI: 2.0-6.4%) among Latinx vs. 0.2% (95%CI: 0.0-0.4%) among non-Latinx persons. Estimated cumulative incidence among residents was 6.1% (95%CI: 4.0-8.6%). Prior infections were 67% Latinx, 16% White, and 17% other ethnicities. Among recent infections, 96% were Latinx. Risk factors for recent infection were Latinx ethnicity, inability to shelter-in-place and maintain income, frontline service work, unemployment, and household income <$50,000/year. Five SARS-CoV-2 phylogenetic lineages were detected. Conclusion: SARS-CoV-2 infections from diverse lineages continued circulating among low-income, Latinx persons unable to work from home and maintain income during San Francisco's shelter-in-place ordinance. [**note: here is a thorough study of one minority census tract in San Francisco. Infections in Latinx individuals was markedly higher and reasons for this are discussed.**] https://www.medrxiv.org/content/10.1101/2020.06.15.20132233v1
- Coronavirus disease 2019 (COVID-19) pandemic has affected the aviation industry. Existing protocols have relied on scientifically questionable evidence and might not lead to the optimal balance between public health safety and airlines' financial viability. Objective: To explore the implementation feasibility of Thai Airways International protocol from the perspectives of passengers and aircrews. Design: An online questionnaire survey of passengers and an in-depth interview with aircrews. Setting: Two randomly selected repatriation flights operated by Thai Airways International using Boeing 777 aircraft (TG476 from Sydney and TG492 from Auckland to Bangkok) Participants: 377 Thai passengers and 35 aircrews. Results: The mean age of passengers was 28.14 (95%CI 26.72 to 29.55) years old; 57.03% were female. TG492 passengers were mostly students and significantly younger than that of TG476 (p<0.0001) with comparable flying experience (p=0.1192). The average body temperature was 36.52 (95%CI 36.48 to 36.55) degrees Celsius. Passengers estimated average physical distances of 1.59 (95%CI 1.48 to 1.70), 1.41 (95%CI 1.29 to 1.53), and 1.26 (95%CI 1.12 to 1.41) meters at check-in, boarding, and in-flight, respectively. Passengers were checked for body temperature during the flight 1.97 (95%CI

1.77 to 2.18) times on average which is significantly more frequent in longer than shorter flight (p<0.0001). Passengers moved around or went to the toilet during the flight 2.00 (95%CI 1.63 to 2.37) and 2.08 (95%CI 1.73 to 2.43) times which are significantly more frequent in longer than shorter flight (p=0.0186 and 0.0049, respectively). The aircrews were satisfied with the protocol and provided several practical suggestions. Conclusion: The protocol was well received by the passengers and aircrews of the repatriation flights with some suggestions for improvement. [**note: for those of us who want to safely board a plane and fly for business or tourism should take a look at this paper. I think the airlines are going to have a tough time building public confidence and have to be very transparent about their practices and outbreaks if any as a result of flying.**] https://www.medrxiv.org/content/10.1101/2020.06.15.20132183v1

- SARS-CoV-2 is straining healthcare systems globally. The burden on hospitals during the pandemic could be reduced by implementing prediction models that can discriminate between patients requiring hospitalization and those who do not. The COVID-19 vulnerability (C-19) index, a model that predicts which patients will be admitted to hospital for treatment of pneumonia or pneumonia proxies, has been developed and proposed as a valuable tool for decision making during the pandemic. However, the model is at high risk of bias according to the Prediction model Risk Of Bias ASsessment Tool and has not been externally validated. Methods: We followed the OHDSI framework for external validation to assess the reliability of the C-19 model. We evaluated the model on two different target populations: i) 41,381 patients that have SARS-CoV-2 at an outpatient or emergency room visit and ii) 9,429,285 patients that have influenza or related symptoms during an outpatient or emergency room visit, to predict their risk of hospitalization with pneumonia during the following 0 to 30 days. In total we validated the model across a network of 14 databases spanning the US, Europe, Australia and Asia. Findings: The internal validation performance of the C-19 index was a c-statistic of 0.73 and calibration was not reported by the authors. When we externally validated it by transporting it to SARS-CoV-2 data the model obtained c-statistics of 0.36, 0.53 (0.473-0.584) and 0.56 (0.488-0.636) on Spanish, US and South Korean datasets respectively. The calibration was poor with the model under-estimating risk. When validated on 12 datasets containing influenza patients across the OHDSI network the c-statistics ranged between 0.40-0.68. Interpretation: The results show that the discriminative performance of the C-19 model is low for influenza cohorts, and even worse amongst COVID-19 patients in the US, Spain and South Korea. These results suggest that C-19 should not be used to aid decision making during the COVID-19 pandemic. *Our findings highlight the importance of performing external validation across a range of settings, especially when a prediction model is being extrapolated to a different population. In the field of prediction, extensive validation is required to create appropriate trust in a model*. [**note: another fine piece of work from the OHDSI group!**]
  https://www.medrxiv.org/content/10.1101/2020.06.15.20130328v1
- Airborne transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) via air-conditioning systems poses a significant threat for the continued escalation of the current coronavirus disease (COVID-19) pandemic. Considering that SARS-CoV-2 cannot tolerate temperatures above 70 degree C, here we designed and fabricated efficient air disinfection systems based on heated nickel (Ni) foam to catch and kill SARS-CoV-2. Virus test results revealed that 99.8% of the aerosolized SARS-CoV-2 was caught and killed by a single pass through a Ni-foam-based filter when heated up to 200 degree C. Additionally, the same filter

was also used to catch and kill 99.9% of Bacillus anthracis, an airborne spore. This study paves the way for preventing transmission of SARS-CoV-2 and other highly infectious airborne agents in closed environments. [**note: I don't have a category for this one. It's a nice engineering approach to cleaning HVAC air via recirculation through a heated filter. I'm unsure if this is a practical solution.**] https://www.biorxiv.org/content/10.1101/2020.06.13.150243v1

NEWLY REGISTERED CLINICAL TRIALS

- We hypothesize that early institution of TNFα inhibitor therapy in patients with severe COVID-19 infections will prevent further clinical deterioration and reduce the need for advanced cardiorespiratory support and early mortality. To address this hypothesis, a prospective, single center, phase 2 trial is proposed to assess the efficacy of infliximab or infliximab-abda in hospitalized adult patients with severe or critical COVID-19. Observations from this study will inform the conduct of prospective randomized controlled studies to follow. [**note: I assume this trial was planned before the dexamethasone announcement. Certainly this is not a low cost alternative. We'll see if they get anyone enrolled.**] NCT04425538
- Thymalfasin (thymosin alpha 1 or Ta1), the active pharmaceutical ingredient in ZADAXIN® injection, is a 28-amino acid synthetic peptide, identical to natural Ta1 produced by the thymus gland. Ta1 is a biological response modifier which activates various cells of the immune system, and is therefore expected to have clinical benefits in disorders where immune responses are impaired or ineffective, including acute and chronic viral and bacterial infections, cancers, and vaccine non-responsiveness. Patients with end-stage renal disease (ESRD) on hemodialysis, in addition to their intrinsic kidney disease and frequent burden of comorbidities, also have increased risk of exposure to communicable diseases as they are treated several times each week at hemodialysis centers with several other patients and clinic staff in attendance. The majority of patients are over 60 years of age and many are receiving immunosuppressive medications. Accordingly, ESRD patients are particularly susceptible to COVID-19 infection. Ta1 has been shown to be safely administered to hemodialysis patients. It is our hypothesis that a course of Ta1 administered to individuals with ESRD will reduce the rate and severity of infection with COVID-19. [**note: maybe it works for the subset of patients they propose to try it on.**] NCT04428008
- Recently the researchers have shown that the use of icatibant in COVID-19 results in a potent decrease in oxygen use. Yet the effect of the three dosages as according to the label dose was insufficient to maintain the clinical improvement in a small group of patients. The researchers argue that with the use of lanadelumab a more lasting effect can be reached due to its longer half life. [**note: both of the mentioned drugs are for the treatment of hereditary angioedema. Whether they work for COVID-19 is not known. I have not seen any data on icatibant, though there were some investigators studying it.**] NCT04422509
- This is a multicenter, randomized, double-blind, parallel group study to investigate the efficacy of PB1046 by improving the clinical outcomes and increasing days alive and free of respiratory failure in hospitalized COVID-19 patients at high risk for rapid clinical deterioration, acute respiratory distress syndrome (ARDS) and death. [**note: this trial is sponsored by PhaseBio The drug is a sustained release human vasoactive intestinal peptide and that is the extent of my knowledge on this one.**] NCT04433546

CLINICAL TRIAL RESULTS

- We aimed to investigate which specific responses from either cellular or humoral immunity associate to severity and progression of COVID-19. Methods: A cohort of 276 patients classified in mild, moderate and severe, was studied. Peripheral blood lymphocyte subpopulations were quantified by flow cytometry, and immunoglobulins and complement proteins by nephelometry. Results: At admission, dramatic lymphopenia of T, B and NK cells associated to severity. However, only the proportion of B cells increased, while T and NK cells appeared unaffected. Accordingly, the number of plasma cells and circulating follicular helper T cells (cTfh) increased, but levels of IgM, IgA and IgG were unaffected. When degrees of severity were considered, IgG was lower in severe patients, suggesting an IgG consumption by complement activation or antibody-dependent cellular cytotoxicity (ADCC). Activated CD56-CD16+ NK-cells, which mediate ADCC, were increased. Regarding complement, C3 and C4 protein levels were higher in mild and moderate, but not in severe patients, compared to healthy donors. Moreover, IgG and C4 decreased from day 0 to day 10 in patients who were hospitalized for more than two weeks, but not in patients who were discharged earlier. Conclusion: Our study provides important clues to understand the immune response observed in COVID-19 patients, which is probably related to viral clearance, but also underlies its pathogenesis and severity. This study associates for the first time COVID-19 severity with an imbalanced humoral immune response characterized by excessive consumption of IgG and C4, identifying new targets for therapeutic intervention. [**note: from Spain a study of the immune response in disease progression. We still don't know why things go out of control in some patients.**]
  https://www.medrxiv.org/content/10.1101/2020.06.15.20131706v1
- Patients with coronavirus disease 19 (COVID-19) are at high risk for thrombotic arterial and venous occlusions. At the same time, lung histopathology often reveals fibrin-based occlusion of small vessels in patients who succumb to the disease. Antiphospholipid syndrome (APS) is an acquired and potentially life-threatening thrombophilia in which patients develop pathogenic autoantibodies (aPL) targeting phospholipids and phospholipid-binding proteins. Small case series have recently detected aPL in patients with COVID-19. Here, we measured eight types of aPL (anticardiolipin IgG/IgM/IgA, anti-beta-2 glycoprotein I IgG/IgM/IgA, and anti-phosphatidylserine/prothrombin (PS/PT) IgG/IgM) in the sera of 172 patients hospitalized with COVID-19. We detected anticardiolipin IgM antibodies in 23%, anti-PS/PT IgG in 24%, and anti-PS/PT IgM in 18%. Any aPL was present in 52% of patients using the manufacturer's threshold and in 30% using a more stringent cutoff (>40 units). Higher levels of aPL were associated with neutrophil hyperactivity (including the release of neutrophil extracellular traps/NETs), higher platelet count, more severe respiratory disease, and lower glomerular filtration rates. Similar to patients with known and longstanding APS, IgG fractions isolated from patients with COVID-19 promoted NET release from control neutrophils. Furthermore, injection of these COVID-19 IgG fractions into mice accelerated venous thrombosis. Taken together, these studies suggest that a significant percentage of patients with COVID-19 become at least transiently positive for aPL and that these aPL are potentially pathogenic. [**note: here is a finding that I've not seen before. As an old lipid biochemist, I find the generation of transient antibodies to phospholipids interesting. It would be good to look at a larger cross section of patients to see how common this is.**] https://www.medrxiv.org/content/10.1101/2020.06.15.20131607v1

- Infected individuals exhibit variable severity, with no relation between the number of cases and mortality, suggesting the involvement of the populational genetic constitution and previous cross-reactive immune contacts in the individuals' disease outcome. Methods: A clustering approach was conducted to investigate the involvement of human MHC alleles with individuals' outcomes. HLA frequencies from affected countries were used to fuel the Hierarchical Clusterization Analysis. The formed groups were compared regarding their death rates. To prospect the T cell targets in SARS-CoV-2, and by consequence, the epitopes that are conferring cross-protection in the current pandemic, we modeled 3D structures of HLA-A*02:01 presenting immunogenic epitopes from SAR-CoV-1, recovered from Immune Epitope Database. These pMHC structures were also compared with models containing the corresponding SARS-CoV-2 epitope, with alphacoronavirus sequences, and with a panel of immunogenic pMHC structures contained in CrossTope. Findings: The combined use of HLA-B*07, HLA-B*44, HLA-DRB1*03, and HLADRB1*04 allowed the clustering of affected countries presenting similar death rates, based only on their allele frequencies. SARS-CoV HLA-A*02:01 epitopes were structurally investigated. It reveals molecular conservation between SARS-CoV-1 and SARS-CoV-2 peptides, enabling the use of formerly SARS-CoV-1 experimental epitopes to inspect actual targets that are conferring cross-protection. Alpha-CoVs and, impressively, viruses involved in human infections share fingerprints of immunogenicity with SARS-CoV peptides. Interpretation: Wide-scale HLA genotyping in COVID-19 patients shall improve prognosis prediction. Structural identification of previous triggers paves the way for herd immunity examination and wide spectrum vaccine development. [**note: from hard hit Brazil, more information on the possible genetic linkage to severe disease. Again, more information is needed in this area.**] https://www.medrxiv.org/content/10.1101/2020.06.15.20131987v1
- Some COVID-19 cases re-tested positive for SARS-CoV-2 RNA after discharge raising the public concern on their infectivity. Characterization of re-positive cases are urgently needed for designing intervention strategies. Methods Clinical data were obtained through Guangdong COVID-19 surveillance network. Neutralization antibody titre was determined using a microneutralization assay. Potential infectivity of clinical samples was evaluated after the cell inoculation. SARS-CoV-2 RNA was detected using three different RT-PCR kits and multiplex PCR with nanopore sequencing. Results Among 619 discharged COVID-19 cases, 87 were re-tested as SARS-CoV-2 positive in circumstance of social isolation. All re-positive cases had mild or moderate symptoms in initial diagnosis and a younger age distribution (mean, 30.4). Re-positive cases (n=59) exhibited similar neutralization antibodies (NAbs) titre distributions to other COVID-19 cases (n=150) parallel-tested in this study. No infective viral strain could be obtained by culture and none full-length viral genomes could be sequenced for all re-positive cases. Conclusions Re-positive SARS-CoV-2 was not caused by the secondary infection and was identified in around 14% of discharged cases. A robust Nabs response and a potential virus genome degradation were detected from nearly all re-positive cases suggesting a lower transmission risk, especially through a respiratory route. [**note: this is an interesting study on discharged patients who tested re-positive. Apparently is was not caused by a secondary infection and patients had a robust antibody titer and the viral test showed degraded genome.**] https://www.medrxiv.org/content/10.1101/2020.06.15.20131748v1

DRUG DEVELOPMENT

- Broadly protective vaccines against known and pre-emergent human coronaviruses (HCoVs) are urgently needed. To gain a deeper understanding of cross-neutralizing antibody responses, we mined the memory B cell repertoire of a convalescent SARS donor and identified 200 SARS-CoV-2 binding antibodies that target multiple conserved sites on the spike (S) protein. A large proportion of the non-neutralizing antibodies display high levels of somatic hypermutation and cross-react with circulating HCoVs, suggesting recall of pre-existing memory B cells (MBCs) elicited by prior HCoV infections. Several antibodies potently cross-neutralize SARS-CoV, SARS-CoV-2, and the bat SARS-like virus WIV1 by blocking receptor attachment and inducing S1 shedding. These antibodies represent promising candidates for therapeutic intervention and reveal a target for the rational design of pan-sarbecovirus vaccines. [**note: current issue of Science has several papers on mAb development of which this is one.]**
  https://science.sciencemag.org/content/early/2020/06/15/science.abc7424
- Countermeasures to prevent and treat COVID-19 are a global health priority. We enrolled a cohort of SARS-CoV-2-recovered participants, developed neutralization assays to interrogate antibody responses, adapted our high-throughput antibody generation pipeline to rapidly screen over 1800 antibodies, and established an animal model to test protection. We isolated potent neutralizing antibodies (nAbs) to two epitopes on the receptor binding domain (RBD) and to distinct non-RBD epitopes on the spike (S) protein. We showed that passive transfer of a nAb provides protection against disease in high-dose SARS-CoV-2 challenge in Syrian hamsters, as revealed by maintained weight and low lung viral titers in treated animals. The study suggests a role for nAbs in prophylaxis, and potentially therapy, of COVID-19. The nAbs define protective epitopes to guide vaccine design. [**note: paper #2 from Science**]
  https://science.sciencemag.org/content/early/2020/06/15/science.abc7520
- Neutralizing antibodies have become an important tool in treating infectious diseases. Recently, two separate approaches yielded successful antibody treatments for Ebola – one from genetically-humanized mice, and the other from a human survivor. Here, we describe parallel efforts using both humanized mice and convalescent patients to generate antibodies against the SARS-CoV-2 spike protein, yielding a large collection of fully-human antibodies that were characterized for binding, neutralization and three dimensional structure. Based on these criteria, we selected pairs of highly-potent individual antibodies that simultaneously bind the receptor-binding domain of the spike protein, providing ideal partners for a therapeutic antibody cocktail that aims to decrease the potential for virus escape mutants that might arise in response to selective pressure from a single antibody treatment. [**note: paper #3 from Science; this one is from the Regeneron group who have just started some clinical trials.**]
  https://science.sciencemag.org/content/early/2020/06/15/science.abd0827
- The rapid spread of SARS-CoV-2 has a significant impact on global health, travel and economy. Therefore, preventative and therapeutic measures are urgently needed. Here, we isolated monoclonal antibodies from three convalescent COVID-19 patients using a SARS-CoV-2 stabilized prefusion spike protein. These antibodies had low levels of somatic hypermutation and showed a strong enrichment in VH1-69, VH3-30-3 and VH1-24 gene usage. A subset of the antibodies were able to potently inhibit authentic SARS-CoV-2 infection as low as 0.007 µg/mL. Competition and electron microscopy studies illustrate that the SARS-CoV-2 spike protein contains multiple distinct antigenic sites, including several receptor-binding domain (RBD) epitopes as well as non-RBD epitopes. In addition to providing guidance for vaccine design, the

antibodies described here are promising candidates for COVID-19 treatment and prevention. [**note: paper #4 from Science**]
https://science.sciencemag.org/content/early/2020/06/15/science.abc5902

- Antibodies targeting the spike protein of SARS-CoV-2 present a promising approach to combat the COVID19 pandemic; however, concerns remain that mutations can yield antibody resistance. We investigate the development of resistance against four antibodies to the spike protein that potently neutralize SARS-CoV-2, individually as well as when combined into cocktails. These antibodies remain effective against spike variants that have arisen in the human population. However, novel spike mutants rapidly appeared following in vitro passaging in the presence of individual antibodies, resulting in loss of neutralization; such escape also occurred with combinations of antibodies binding diverse but overlapping regions of the spike protein. Importantly, escape mutants were not generated following treatment with a non-competing antibody cocktail. [**note: paper #5 from Science, also from the Regeneron group highlighting their approach of developing a cocktail therapy to minimize mutational escape of SARS-CoV-2 which can be an important barrier to treatment.**]
https://science.sciencemag.org/content/early/2020/06/15/science.abd0831
- Host cell invasion is initiated through direct binding of the viral spike protein to the host receptor angiotensin-converting enzyme 2 (ACE2). Disrupting the spike-ACE2 interaction is a potential therapeutic target for treating COVID-19. We have developed a proximity-based AlphaLISA assay to measure binding of SARS-CoV-2 spike protein Receptor Binding Domain (RBD) to ACE2. Utilizing this assay platform, a drug-repurposing screen against 3,384 small molecule drugs and pre-clinical compounds was performed, yielding 25 high-quality, small-molecule hits that can be evaluated in cell-based models. This established AlphaLISA RBD-ACE2 platform can facilitate evaluation of biologics or small molecules that can perturb this essential viral-host interaction to further the development of interventions to address the global health pandemic. [**note: another model for drug development, this time looking disrupting the spike protein interaction with ACE2. I'll leave it to interested readers to look at the table of small molecule hits this assay gave. It is a heterogeneous group of compounds and thankfully HCQ did not come up!**] https://www.biorxiv.org/content/10.1101/2020.06.16.154708v1
- A high-throughput, high-content imaging assay of human HeLa cells expressing the SARS-CoV-2 receptor ACE2 was used to screen ReFRAME, a best-in-class drug repurposing library. From nearly 12,000 compounds, we identified 66 compounds capable of selectively inhibiting SARS-CoV-2 replication in human cells. Twenty-four of these drugs show additive activity in combination with the RNA-dependent RNA polymerase inhibitor remdesivir and may afford increased in vivo efficacy. We also identified synergistic interaction of the nucleoside analog riboprine and a folate antagonist 10-deazaaminopterin with remdesivir. Overall, seven clinically approved drugs (halofantrine, amiodarone, nelfinavir, simeprevir, manidipine, ozanimod, osimertinib) and 19 compounds in other stages of development may have the potential to be repurposed as SARS-CoV-2 oral therapeutics based on their potency, pharmacokinetic and human safety profiles. [**note: this is from the Scripps group who do good work in this area. Some possible drugs that may work synergistically with remdesivir. It is going to be difficult to do clinical trials on such a large group of comounds.**]
https://www.biorxiv.org/content/10.1101/2020.06.16.153403v1

- Although clinically approved drugs have been repurposed to treat individuals with 2019 Coronavirus disease (COVID-19), the lack of safety studies and limited efficiency as well jeopardize clinical benefits. Daclatasvir and sofosbuvir (SFV) are clinically approved direct-acting antivirals (DAA) against hepatitis C virus (HCV), with satisfactory safety profile. In the HCV replicative cycle, daclatasvir and SFV target the viral enzymes NS5A and NS5B, respectively. NS5A is endowed with pleotropic activities, which overlap with several proteins from SARS-CoV-2. HCV NS5B and SARS-CoV-2 nsp12 are RNA polymerases that share homology in the nucleotide uptake channel. These characteristics of the HCV and SARS-CoV-2 motivated us to further study the activity of daclatasvir and SFV against the new coronavirus. Daclatasvir consistently inhibited the production of infectious SARS-CoV-2 virus particles in Vero cells, in the hepatoma cell line HuH-7 and in type II pneumocytes (Calu-3), with potencies of 0.8, 0.6 and 1.1 μM, respectively. Daclatasvir targeted early events during SARS-CoV-2 replication cycle and prevented the induction of IL-6 and TNF-α, inflammatory mediators associated with the cytokine storm typical of SARS-CoV-2 infection. Sofosbuvir, although inactive in Vero cells, displayed EC50 values of 6.2 and 9.5 μM in HuH-7 and Calu-3 cells, respectively. Our data point to additional antiviral candidates, in especial daclatasvir, among drugs overlooked for COVID-19, that could immediately enter clinical trials. [**note: the big day for drug development papers continues! This is from some Brazilian investigators.**]
  https://www.biorxiv.org/content/10.1101/2020.06.15.153411v1

DIAGNOSTIC DEVELOPMENT

- Serological survey for COVID-19 antibodies is crucial in area with low prevalence as well as epidemic area when addressing health and social issues caused by COVID-19. Rapid, accurate and easy-to-use antibody tests as well laboratory-based antibody tests are necessary for confirming immunity in a given community. Methods:Serum samples from healthcare workers (n = 1,000, mean 40 ± 11 years) of Iwate Prefectural Central Hospital, Iwate, Japan were tested for the prevalence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies. Two laboratory-based quantitative tests (Abbott Architect SARS-CoV-2 IgG and Roche Elecsys Anti-SARS-CoV-2 assays) and one point-of-care (POC) qualitative test (Alfa Instant-view plus COVID-19 Test) were performed simultaneously. Sensitivity and specificity were 100%, 99.6% in Abbott assay; 100%, 99.8% in Roche assay; 97.8%, 94.6% in Alfa POC test, respectively. Results:The laboratory-based quantitative tests showed positive in 4 of 1,000 samples (0.4%) (95% CI: 0.01 to 0.79): 4/1,000 (0.4%) (95% CI: 0.01 to 0.79) in Abbott; 0/1,000 (0%) in Roche. Positive samples were not detected for both Abbott and Roche assays. The POC qualitative test showed positive in 33 of 1,000 samples (3.3%) (95% CI: 2.19 to 4.41), showing higher rates than those of the laboratory-based quantitative tests. There were no samples with simultaneous positive reaction for two quantitative tests and a POC test. Conclusions: Infected COVID-2 cases were not confirmed by a retrospective serological study in healthcare workers of our hospital. The POC qualitative tests with lower specificity have the potential for higher false positive reactions than the laboratory-based quantitative tests in areas with very low prevalence of COVID-19. [**note: serology testing in a Japanese region where there were no confirmed COVID-19 cases. It highlights the difference between test platforms and that some lateral flow tests are not reliable (nothing new here).**]
  https://www.medrxiv.org/content/10.1101/2020.06.15.20132316v1

- Despite widespread concern for cytokine storms leading to severe morbidity in COVID-19, rapid cytokine assays are not routinely available for monitoring critically ill patients. We report the clinical application of a machine learning-based digital protein microarray platform for rapid multiplex quantification of cytokines from critically ill COVID-19 patients admitted to the intensive care unit (ICU) at the University of Michigan Hospital. The platform comprises two low-cost modules: (i) a semi-automated fluidic dispensing/mixing module that can be operated inside a biosafety cabinet to minimize the exposure of technician to the virus infection and (ii) a 12-12-15 inch compact fluorescence optical scanner for the potential near-bedside readout. The platform enabled daily cytokine analysis in clinical practice with high sensitivity (<0.4pg/mL), inter-assay repeatability (~10% CV), and near-real-time operation with a 10 min assay incubation. A cytokine profiling test with the platform allowed us to observe clear interleukin-6 (IL-6) elevations after receiving tocilizumab (IL-6 inhibitor) while significant cytokine profile variability exists across all critically ill COVID-19 patients and to discover a weak correlation between IL-6 to clinical biomarkers, such as Ferritin and CRP. Our data revealed large subject-to-subject variability in a patient's response to anti-inflammatory treatment for COVID-19, reaffirming the need for a personalized strategy guided by rapid cytokine assays. [**note: these Michigan researchers come up with a good way to quantify cytokines in critically ill patients. This is important for both diagnostic and treatment.**]
  https://www.medrxiv.org/content/10.1101/2020.06.15.20131870v1

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2020-06-18

In response to my selection of Stacey Kent yesterday, a loyal reader asked whether I had ever listened to Nancy LaMott. This was new name to me. She was a fine singer who was taken from us way to early at the age of 44 from uterine cancer. Here she is with an original song written by David Friedman, 'I'll be Here with You' that aired on a Valentine's Day episode of Good Morning America: https://www.youtube.com/watch?v=zb40nYUshTE There are a number of clips and albums on YouTube.

Let us all take a minute to mourn the death of Vera Lynn, the English singer, whose song 'We'll Meet Again' became an anthem of hope during WW-II.

One of the nice things about the Washington Post is the two pages of comic strips that can help take one's mind off of the swirling torrents of politics and the pandemic. Here is today's Non Sequitur:

In my never-ending quest to get people to mask up, here is a great story in the Washington Post on how an outbreak of COVID-19 infection was averted in Springfield MO. This is the famous hair salon where two workers were diagnosed with COVID-19 and potentially exposed scores of women. The two stylists and all the customers were wearing masks!!! No further viral transmission was noted.

Oh dear, restaurants that have reopened are closing because of employee viral infections. As with a lot of things there seem to be no standards here. Personally, I would expect all wait staff to wear masks and gloves but I'm also not in any hurry to rush back into a restaurant. Our county is opening for indoor service this Friday.

Carolyn Chen of ProPublica on vaccine prospects.

JAMA have an opinion piece and small trial study report on the effect of prone positioning in hypoxemic patients. The trial is small but the patients oxygen saturation improved. There are some larger clinical trials registered. I have not linked them as this is outside my field of expertise.

Here is the paper on genomewide association study of severe COVID-19 with respiratory failure. It is a large study of Spanish and Italian patients and I linked to the preprint in an earlier newsletter. They identified a 3p21.31 gene cluster as a genetic susceptibility locus in patients with Covid-19 with respiratory failure and confirmed a potential involvement of the ABO blood-group system.

STAT has an overview on the likelihood that children will get COVID-19. It is a 'huge puzzle' without easy answers.

Derek Lowe on the dexamethasone study.

MODELING

- Nothing surprising on the preprint front.

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- There is currently a paucity of data describing bacterial coinfections, related antibiotic prescribing patterns, and the potential role of antimicrobial stewardship in the care of patients infected with SARS-CoV-2. Methods: This prospective, observational study was conducted from March 10, 2020 to April 21, 2020 in admitted patients with confirmed COVID-19. Patients were included if ≥ 18 years old and admitted to the hospital for further treatment. Data was collected via chart review from the enterprise electronic health record database. Data collected include factors driving antibiotic choice, indication, and duration of therapy as well as microbiological data. Findings: Antibiotics were initiated on admission in 87/147 (59%) patients. Of these, 85/87 (98%) prescriptions were empiric. The most common indication for empiric antibiotics was concern for community-acquired pneumonia (76/85, 89%) with the most prescribed antibiotics being ceftriaxone and azithromycin. The median duration of antibiotic therapy was two days (interquartile range 1-5). No patients had a community-acquired bacterial respiratory coinfection, but 10/147 (7%) of patients were found to have concurrent bacterial infections from a non-respiratory source, and one patient was diagnosed with active pulmonary tuberculosis at the time of admission for COVID-19. Interpretation: Bacterial coinfection in patients with COVID-19 was infrequent. Antibiotics are likely unnecessary in patients with mild symptoms. There is little role for broad-spectrum antibiotics to empirically treat multidrug resistant organisms in patients with COVID-19, regardless of disease severity. Antimicrobial stewardship remains important in patients infected with SARS-CoV-2. [**note: this is a useful cohort study. While azithromycin was used in conjunction with HCQ, the role of antimicrobial therapy was not clearly known. From this Dallas report, bacterial coinfection was infrequent and broad-spectrum antibiotic therapy not warranted as standard of care.**]
  https://www.medrxiv.org/content/10.1101/2020.06.16.20133181v1
- Blacks/African-Americans are overrepresented in the number of COVID-19 infections, hospitalizations and deaths. Reasons for this disparity have not been well-characterized but may be due to underlying comorbidities or sociodemographic factors. Objective: To systematically determine patient characteristics associated with racial/ethnic disparities in COVID-19 outcomes. Design: A retrospective cohort study with comparative control groups. Setting: Patients tested for COVID-19 at University of Michigan Medicine from March 10, 2020 to April 22, 2020. Participants: 5,698 tested patients and two sets of comparison groups who were not tested for COVID-19: randomly selected unmatched controls (n = 7,211) and frequency-matched controls by race, age, and sex (n = 13,351). Main Outcomes and Measures: We identified factors associated with testing and testing positive for COVID-19, being hospitalized, requiring intensive care unit (ICU) admission, and mortality (in/out-patient during the time frame). Factors included race/ethnicity, age, smoking, alcohol consumption, healthcare utilization, and residential-level socioeconomic characteristics (SES; i.e., education, unemployment, population density, and poverty rate). Medical comorbidities were defined from the International Classification of Diseases (ICD) codes, and were aggregated into a comorbidity score. Results: Of 5,698 patients, (median age, 47 years; 38% male; mean BMI, 30.1), the majority were non-Hispanic Whites (NHW, 59.2%) and non-Hispanic Black/African-Americans (NHAA, 17.2%). Among 1,119 diagnosed, there were 41.2% NHW and 37.4% NHAA; 44.8% hospitalized, 20.6% admitted to ICU, and 3.8% died. Adjusting for age, sex, and SES, NHAA were 1.66 times more likely to be hospitalized (95% CI, 1.09-2.52; P=.02), 1.52 times more likely to enter ICU (95% CI, 0.92-2.52; P=.10). In addition to older age, male sex and obesity, high population density neighborhood

(OR, 1.27 associated with one SD change [95% CI, 1.20-1.76]; P=.02) was associated with hospitalization. Pre-existing kidney disease led to 2.55 times higher risk of hospitalization (95% CI, 1.62-4.02; P<.001) in the overall population and 11.9 times higher mortality risk in NHAA (95% CI, 2.2-64.7, P=.004). Conclusions and Relevance: *Pre-existing type II diabetes/kidney diseases and living in high population density areas were associated with high risk for COVID-19 susceptibility and poor prognosis. Association of risk factors with COVID-19 outcomes differed by race. NHAA patients were disproportionately affected by obesity and kidney disease*. [**note: I have previously posted some large cohort studies from the UK NHS, but this is the first large one from the US that looks at racial demographics.**]
https://www.medrxiv.org/content/10.1101/2020.06.16.20133140v1

- We aimed to determine whether a 6-day course of intravenous methylprednisolone (MP) improves outcome in patients with SARS CoV-2 infection at risk of developing Acute Respiratory Distress Syndrome (ARDS). Methods. Multicentric, partially randomized, preference, open-label trial, including adults with COVID-19 pneumonia, impaired gas exchange and biochemical evidence of hyper-inflammation. Patients were assigned to standard of care (SOC), or SOC plus intravenous MP [40mg/12h 3 days, then 20mg/12h 3 days]. The primary endpoint was a composite of death, admission to the intensive care unit (ICU) or requirement of non-invasive ventilation (NIV). Results. We analyzed 85 patients (34, randomized to MP; 22, assigned to MP by clinician preference; 29, control group). Patient age (mean 68±yr) was related to outcome. The use of MP was associated with a reduced risk of the composite endpoint in the intention-to-treat, age-stratified analysis (combined risk ratio -RR- 0.55 [95% CI 0.33-0.91]; p=0.024). In the per-protocol analysis, RR was 0.11 (0.01-0.83) in patients aged 72 yr or less, 0.61 (0.32-1.17) in those over 72 yr, and 0.37 (0.19-0.74, p=0.0037) in the whole group after age-adjustment by stratification. The decrease in C-reactive protein levels was more pronounced in the MP group (p=0.0003). Hyperglycemia was more frequent in the MP group. Conclusions A short course of MP had a beneficial effect on the clinical outcome of severe COVID-19 pneumonia, decreasing the risk of the composite end point of admission to ICU, NIV or death. [**note: here is a steroid study from Spain looking at the use of methylprednisolone. It's much smaller than the UK RECOVERY study with only 34 on treatment. It is also a bit messy with patients in both arms sometimes given tocilizumab or anakinra. I would not give this as much weight as the UK study.**] https://www.medrxiv.org/content/10.1101/2020.06.17.20133579v1

DRUG DEVELOPMENT

- Main protease (Mpro, also known as 3CLpro) has a major role in the replication of coronavirus life cycle and is one of the most important drug targets for anticoronavirus agents. Here we report the crystal structure of main protease of SARS-CoV-2 bound to a previously identified Chinese herb inhibitor shikonin at 2.45 angstrom resolution. Although the structure revealed here shares similar overall structure with other published structures, there are several key differences which highlight potential features that could be exploited. The catalytic dyad His41-Cys145 undergoes dramatic conformational changes, and the structure reveals an unusual arrangement of oxyanion loop stabilized by the substrate. Binding to shikonin and binding of covalent inhibitors show different binding modes, suggesting a diversity in inhibitor binding. As we learn more about different binding modes and their structure-function relationships, it is probable that we can design more effective and specific drugs with high potency that can serve

as effect SARS-CoV-2 anti-viral agents. [**note: this compound was first extracted from borage. The Wikipedia link goes into more detail. Perhaps the crystal structure information will help in drug design.**] https://www.biorxiv.org/content/10.1101/2020.06.16.155812v1

- Identification of host genes essential for SARS-CoV-2 infection may reveal novel therapeutic targets and inform our understanding of COVID-19 pathogenesis. Here we performed a genome-wide CRISPR screen with SARS-CoV-2 and identified known SARS-CoV-2 host factors including the receptor ACE2 and protease Cathepsin L. We additionally discovered novel pro-viral genes and pathways including the SWI/SNF chromatin remodeling complex and key components of the TGF-β signaling pathway. Small molecule inhibitors of these pathways prevented SARS-CoV-2-induced cell death. We also revealed that the alarmin HMGB1 is critical for SARS-CoV-2 replication. In contrast, loss of the histone H3.3 chaperone complex sensitized cells to virus-induced death. Together this study reveals potential therapeutic targets for SARS-CoV-2 and highlights host genes that may regulate COVID-19 pathogenesis. [**note: more unravelling of the SARS-CoV-2 genome using CRISPR. It offers some new targets for drug therapy.**] https://www.biorxiv.org/content/10.1101/2020.06.16.155101v1

DIAGNOSTIC DEVELOPMENT

- Identifying SARS-CoV-2 infections through aggressive diagnostic testing remains critical in tracking and curbing the spread of the COVID-19 pandemic. Collection of nasopharyngeal swabs (NPS), the preferred sample type for SARS-CoV-2 detection, has become difficult due to the dramatic increase in testing and consequential supply strain. Therefore, alternative specimen types have been investigated, that provide similar detection sensitivity with reduced health care exposure and potential for self-collection. In this study, the detection sensitivity of SARS-CoV-2 in nasal swabs (NS) and saliva was compared to that of NPS, using matched specimens from two outpatient cohorts in New York State (total n = 463). The first cohort showed only a 5.4% positivity but the second cohort (n=227) had a positivity rate of 41%, with sensitivity in NPS, NS and saliva of 97.9%, 87.1%, and 87.1%, respectively. Whether the reduced sensitivity of NS or saliva is acceptable must be assessed in the settings where they are used. However, we sought to improve on it by validating a method to mix the two sample types, as the combination of nasal swab and saliva resulted in 94.6% SARS-CoV-2 detection sensitivity. Spiking experiments showed that combining them did not adversely affect the detection sensitivity in either. Virus stability in saliva was also investigated, with and without the addition of commercially available stabilizing solutions. The virus was stable in saliva at both 4C and room temperature for up to 7 days. The addition of stabilizing solutions did not enhance stability and in some situations reduced detectable virus levels. [**note: this is a very useful paper and the abstract does not do it justice. The New York state researchers take a careful look at the quality of personal collected saliva and nasal swab samples and compare virus detection to the gold standard nasopharangyl specimen. Combining the nasal swab and saliva samples gives higher sensitivity. They also discuss the presence of mucous in saliva samples and note that patients should not cough prior to collecting the saliva sample.**] https://www.medrxiv.org/content/10.1101/2020.06.16.20133041v1
- Background Rapid COVID-19 diagnosis in hospital is essential for patient management and identification of infectious patients to limit the potential for nosocomial transmission. The diagnosis is complicated by 30-50% of COVID-19 hospital admissions with negative nose/throat

swabs negative for SARS-CoV-2 nucleic acid, frequently after the first week of illness when SARS-CoV-2 antibody responses become detectable. We assessed the diagnostic accuracy of combined rapid antibody point of care (POC) and nucleic acid assays for suspected COVID-19 disease in the emergency department. Methods We developed (i) an in vitro neutralization assay using a lentivirus expressing a genome encoding luciferase and pseudotyped with spike protein and (ii) an ELISA test to detect IgG antibodies to nucleocapsid (N) and spike (S) proteins from SARS-CoV-2. We tested two promising candidate lateral flow rapid fingerprick test with bands for IgG and IgM. We then prospectively recruited participants with suspected moderate to severe COVID-19 and tested for SARS-CoV-2 nucleic acid in a combined nasal/throat swab using the standard laboratory RT-PCR and a validated rapid nucleic acid test. Additionally, serum collected at admission was retrospectively tested by in vitro neutralization, ELISA and the candidate POC antibody tests. We determined the sensitivity and specificity of the individual and combined rapid POC diagnostic tests against a composite gold standard of neutralisation and the standard laboratory RT-PCR. Results 45 participants had specimens tested for nucleic acid in nose/throat swabs as well as stored sera for antibodies. Serum neutralisation assay, SARS-CoV-2 Spike IgG ELISA and the POC antibody test results were concordant. Using the composite gold standard, prevalence of COVID-19 disease was 53.3% (24/45). Median age was 73.5 (IQR 54.0-86.5) years in those with COVID-19 disease by our gold standard and 63.0 (IQR 41.0-72.0) years in those without disease. Median duration of symptoms was 7 days (IQR 1-8) in those with infection. The overall sensitivity of rapid NAAT diagnosis was 79.2% (95CI 57.8-92.9%) and 50.0% (11.8-88.2) at days 8-28. *Sensitivity and specificity of the combined rapid POC diagnostic tests reached 100% (95CI 85.8-100) and 94.7% (95CI 74.0-99.0) overall. Conclusions Dual point of care SARS-CoV-2 testing can significantly improve diagnostic sensitivity, whilst maintaining high specificity. Rapid combined tests have the potential to transform our management of COVID-19, including inflammatory manifestations where nucleic acid test results are negative. A rapid combined approach will also aid recruitment into clinical trials and in prescribing therapeutics, particularly where potentially harmful immune modulators (including steroids) are used.* [**note: here is an interesting approach from a large UK group who use two point of care lateral flow antibody tests. They see increased sensitivity approaching 100%.**] https://www.medrxiv.org/content/10.1101/2020.06.16.20133157v1

- Detecting antibody responses during and after SARS-CoV-2 infection is essential in determining the seroepidemiology of the virus and the potential role of antibody in disease. Scalable, sensitive and specific serological assays are essential to this process. The detection of antibody in hospitalized patients with severe disease has proven straightforward; detecting responses in subjects with mild disease and asymptomatic infections has proven less reliable. We hypothesized that the suboptimal sensitivity of antibody assays and the compartmentalization of the antibody response may contribute to this effect. Methods: We systemically developed an ELISA assay, optimising different antigens and amplification steps, in serum and saliva from symptomatic and asymptomatic SARS-CoV-2-infected subjects. Results: Using trimeric spike glycoprotein, rather than nucleocapsid enabled detection of responses in individuals with low antibody responses. IgG1 and IgG3 predominate to both antigens, but more anti-spike IgG1 than IgG3 was detectable. All antigens were effective for detecting responses in hospitalized patients. *Anti-spike, but not nucleocapsid, IgG, IgA and IgM antibody responses were readily detectable in saliva from non-hospitalized symptomatic and asymptomatic individuals. Antibody responses in*

saliva and serum were largely independent of each other and symptom reporting. Conclusions. Detecting antibody responses in both saliva and serum is optimal for determining virus exposure and understanding immune responses after SARS-CoV-2 infection. [**note: UK researchers have been very busy! Here is a second report of a combined antibody assay system using sera and saliva.**] https://www.medrxiv.org/content/10.1101/2020.06.16.20133025v1

- Highly accurate detection of antibodies for COVID-19 is an indispensable part of the effort to combat the pandemic1,2. Here we developed two-plex antibody detection against SARS-CoV-2 spike proteins3 (the S1 subunit and receptor binding domain RBD) in human serum and saliva on a near-infrared nano-plasmonic gold (pGOLD) platform4-8. By testing nearly 600 serum samples, pGOLD COVID-19 assay achieved ~ 99.78 % specificity for detecting both IgG and IgM with 100 % sensitivity in sera collected > 14 days post disease symptom onset, with zero cross-reactivity to other diseases. Two-plex correlation analysis revealed higher binding of serum IgM to RBD than to S1. IgG antibody avidity toward multiple antigens were measured, shedding light on antibody maturation in COVID-19 patients and affording a powerful tool for differentiating recent from remote infections and identifying re-infection by SARS-CoV-2. Just as important, due to high analytical sensitivity, the pGOLD COVID-19 assay detected minute amounts of antibodies in human saliva, offering the first non-invasive detection of SARS-CoV-2 antibodies. [**note: a different type of duplex antibody assay from these Palo Alto researchers. They look at the spike protein and receptor binding domains and get improved sensitivity. They also show it to be useful in antibody testing of saliva samples.**]
  https://www.biorxiv.org/content/10.1101/2020.06.16.155580v1

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2020-06-19

Let us stay with cabaret performers again today. Andrea Marcovicci got her start in movies and television (I remember her well from an old Kojak show in the early 1970s). In the 1980s she turned to cabaret and is an arresting singer. She celebrates her 65th birthday in this clip from a stint at Joe's Pub singing the Cole Porter song 'Don't Look at Me That Way':
https://www.youtube.com/watch?v=TM34ABlP1_8 and paying homage to the great chanteuse, Julie Wilson.

Here is a good broad overview of the issues related to returning to work during the COVID-19 pandemic courtesy of the New England Journal of Medicine. Let's put all political differences aside and agree that the pandemic is still going on.

Terry Gross of NPR's 'Fresh Air' (a fitting name for a show in the pandemic era) has a very good interview with Michael Osterholm of University of Minnesota. There is a lot of very good information here. For you beach goers, Osterholm said that might be one of the safest activities!! This is well worth listening to, plain talk and common sense.

I am unsure where Richard Horton, editor of The Lancet, is going with this commentary. There have been proposals for some time about exposing those under 40 to community prevalent SARS-CoV-2 to assist in the building of herd immunity. I have seen a proposal for deliberate variolation. If this were a

viable concept, why would not the US military who are most interested in maintaining the health of servicemen and women have implemented this? They could have used the USS Theodore Roosevelt as a variolation facility bringing as the large flight deck makes it easy to supply and move people in and out. One thing we know for sure is that there is no age group wholly immune from severe COVID-19. Former FDA Commissioner, Scott Gottlieb noted yesterday that ¼ of those hospitalized in Texas are under the age of 30. Perhaps they don't progress to severe COVID-19 but they do occupy hospital beds, complicating emergency healthcare.

Here is an editorial arguing for a global COVID-19 vaccine in The Lancet.

From The Washington Post a good summary of where things stand and some comments from Tony Fauci (caution: some politics in this article) and a summary of some of the recent genetics research.

I have not seen a direct link to the paper, but researchers in Italy found SARS-CoV-2 in wastewater collected in December 2019 from two large cities in the northern part of the country. The results, confirmed in two different laboratories by two different methods, showed the presence of SARS-CoV-2 in samples taken in Milan and Turin on December 18, 2019 and in Bologna on January 29, 2020.

We continue to await the results of some of the large clinical trials. There are some good abstracts today particularly on new characteristics of the SARS-CoV-2 virus. I continue to be baffled by people not wanting to wear masks. A protesting passenger was kicked off an American Airlines flight for refusing to comply with that airline's requirement. Go figure!

MODELING

- Closure of schools and the statewide "Stay Safe, Stay Home" order have effectively reduced COVID-19 transmission in Connecticut, with model projections estimating incidence at about 1,300 new infections per day. If close interpersonal contact increases quickly in Connecticut following reopening on May 20, the state is at risk of a substantial increase of COVID-19 infections, hospitalizations, and deaths by late Summer 2020. Real-time metrics including case counts, hospitalizations, and deaths may fail to provide enough advance warning to avoid resurgence. Substantial uncertainty remains in our knowledge of cumulative COVID-19 incidence, the proportion of infected individuals who are asymptomatic, infectiousness of children, the effects of testing and contact tracing on isolation of infected individuals, and how contact patterns may change following reopening. [**note: I continue to track happenings in Connecticut for my significant readership in the Nutmeg state.**]
  https://www.medrxiv.org/content/10.1101/2020.06.16.20126425v1

NEWLY REGISTERED CLINICAL TRIALS

- This is a randomized, double-blind, placebo-controlled phase 2 study to evaluate the efficacy and safety of intravenous pamrevlumab, a monoclonal antibody against connective-tissue growth factor (CTGF), in hospitalized subjects with acute COVID-19 disease. [**note: the sponsor is FibroGen.**] NCT04432298

- The purpose of this trial is to test the efficacy and safety of crizanlizumab in patients hospitalized with COVID-19. Crizanlizumab is a monoclonal antibody that targets P-selectin. Crizanlizumab can decrease inflammation by binding to P-selectin, blocking leucocyte and platelet adherence to the vessel wall. [**note: this trial is a Novartis drug.**] NCT04435184
- the purpose of this study: to evaluate the safety, tolerability and immunogenicity of the drug "Gam-COVID-Vac Lyo", a lyofilizate for preparing solution for intramuscular administration, at various times after vaccination in healthy adult volunteers. [**note: another entrant into the vaccine race, this time from Russia. It's an adenoviral based vaccine, probably similar to those already in trials.**] NCT04437875
- This is a Phase 2, multicentre, randomized, double blind, 2 arm placebo-controlled study in adults with moderate COVID-19 with gastrointestinal signs and symptoms. Continued SOC therapy together with Niclosamide tablets for 14 days. [**note: sponsor is First Wave Bio and the drug is for tapeworm infestations.**] NCT04436458
- The primary aim of this study is to test whether Doxycycline or Dihydropyridine can benefit patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections by inhibiting the replication of the virus while at the same time blocking the development of cytokine storms or inhibiting cytokine-associated coagulopathy respectively. The investigators hypothesize that either Doxycycline or Dipyridamole (or both) will improve survival and reduce morbidity in SARS-CoV-2 infected patients. [**note: this is a study at Temple Univ. I think doxycycline is being studied in conjunction with HCQ but not this drug.**] NCT04433078

CLINICAL TRIAL RESULTS

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes a range of extra-respiratory signs and symptoms. One such manifestation is delirium, an acute confusional state occurring in 60-70% of severe SARS-CoV-2 cases. Delirium is also a common clinical syndrome following planned orthopedic surgery. This investigation initially explored the underlying role of metabolism in delirium-susceptibility in this setting. Metabolomics profiles of cerebrospinal fluid (CSF) and blood taken prior to surgery found significant concentration differences of several amino acids, acylcarnitines and polyamines were observed in delirium-prone patients. Phenethylamine (PEA) concentrations in delirium-prone patients was significantly lower in CSF than in blood, whilst in age- and gender-matched controls the opposite was observed (adjusted p values: 1.8x10-6 (control) and 1.788x10-10 (delirium)). PEA is metabolised by monoamine oxidase B (MAOB), a putative enzyme target for the treatment of Alzheimers disease, Parkinsons disease and depression. Our computational structural comparisons of MAOB and angiotensin converting enzyme (ACE) 2 found high similarity, specifically within the SARS-CoV-2 spike protein. MAOB structural alignment to ACE2 was 51% overall, but this was over 95% in the ACE2-spike protein binding region. Thus, it is possible that the spike protein interacts with MAOB on a molecular level. A previously published metabolomic dataset of control subjects and patients with either mild or severe COVID-19 was then analysed. Major concentration differences in some metabolites attributed to altered MAO activity were detected. Therefore, our hypothesis is that the SARS-CoV-2 influences MAOB activity, which is one potential cause for the many observed neurological and platelet based complications of SARS-CoV-2 infection. [**note: this is an interesting hypothesis about how SARS-CoV-2 infection may cause a state of delirium through interaction with monoamine oxidase B. However, it still doesn't explain**

COVID-19 nightmares in non-infected individuals 😊 I do note in my small trial of one, that COVID nightmares have all but disappeared.]
https://www.medrxiv.org/content/10.1101/2020.06.16.20128660v1

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the current coronavirus disease 2019 (COVID-19) pandemic. Understanding both the immunological processes providing specific immunity and potential immunopathology underlying the pathogenesis of this disease may provide valuable insights for potential therapeutic interventions. Here, we quantified SARS-CoV-2 specific immune responses in patients with different clinical courses. Compared to individuals with a mild clinical presentation, CD4+ T cell responses were qualitatively impaired in critically ill patients. Strikingly, however, in these patients the specific IgG antibody response was remarkably strong. The observed disparate T and B cell responses could be indicative of a deregulated immune response in critically ill COVID-19 patients. [**note: more information on the immune response to COVID-19 and how things go out of whack in severe disease progression.**]
https://www.biorxiv.org/content/10.1101/2020.06.18.159202v1
- Here is a very large Mayo Clinic study on the safety of convalescent plasma for treating severe COVID-19. It shows that transfusion of convalescent plasma is safe in hospitalized patients and support the notion that earlier administration of plasma within the clinical course of COVID-19 is more likely to reduce mortality.
https://mayoclinicproceedings.org/pb/assets/raw/Health%20Advance/journals/jmcp/jmcp_ft95_6_8.pdf

DRUG DEVELOPMENT

- Enveloped viruses infect the host cell by cellular membrane fusion, a crucial mechanism required for virus replication. The SARS-CoV-2 spike glycoprotein, due to its primary interaction with the human angiotensin-converting enzyme 2 (ACE2) cell-surface receptor, is considered as a potential target for drug development. Based on in silico screening followed by in vitro studies, here we report that the existing FDA-approved Bcr-Abl tyrosine kinase inhibitor, imatinib, inhibits SARS-CoV-2 with an IC50 of 130 nM. We provide initial evidence that inhibition of virus fusion may explain the antiviral action of imatinib. This finding is significant since pinpointing the mode of action allows evaluating the drug's affinity to the SARS-CoV-2-specific target protein, and in turn, helps make inferences on the potency of the drug and evidence-based recommendations on its dosage. To this end, we provide evidence that imatinib binds to the receptor-binding domain (RBD) of SARS-CoV-2 spike protein with an affinity at micromolar, i.e., 2.32 ± 0.9 μM, levels. We also show that imatinib inhibits other coronaviruses, SARS-CoV and MERS-CoV, possibly via fusion inhibition. Based on promising in vitro results, we propose the Abl tyrosine kinase inhibitor (ATKI), imatinib, to be a viable repurposable drug candidate for further clinical validation against COVID-19. [**note: I don't know when this paper was written but the first trial for imatinib was registered by Spanish researchers in early April, followed by a French group and then one at University of Maryland.**]
https://www.biorxiv.org/content/10.1101/2020.06.18.158196v1
- SARS-CoV-2 encodes three putative ion channels: E, 8a, and 3a. In related SARS-CoV-1, 3a is implicated in viral release, inflammasome activation, and cell death and its deletion reduces viral titer and morbidity in animal models, suggesting 3a-targeted therapeutics could treat SARS

and COVID-19. However, the structural basis for the function of 3a is unknown. Here, we show that SARS-CoV-2 forms large conductance cation channels and present cryo-EM structures of dimeric and tetrameric SARS-CoV-2 3a in lipid nanodiscs. 3a adopts a novel fold and is captured in a closed or inactivated state. A narrow bifurcated exterior pore precludes conduction and leads to a large polar cavity open to the cytosol. 3a function is conserved in a common variant among circulating SARS-CoV-2 that alters the channel pore. We identify 3a-like proteins in *Alpha-* and *Beta-coronaviruses* that infect bats and humans, suggesting therapeutics targeting 3a could treat a range of coronaviral diseases. [**note: the more I read about various proteins encoded by the virus, the more fascinating the story becomes. I really wonder about the evolutionary pressures on the virus and why all these different functions came about. This might be an interesting therapeutic approach as we are seeing lots of new possibilities.**] https://www.biorxiv.org/content/10.1101/2020.06.17.156554v1

- COVID-19, caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), represents a global crisis. Key to SARS-CoV-2 therapeutic development is unraveling the mechanisms driving high infectivity, broad tissue tropism and severe pathology. Our cryo-EM structure of SARS-CoV-2 spike (S) glycoprotein reveals that the receptor binding domains (RBDs) tightly and specifically bind the essential free fatty acid (FFA) linoleic acid (LA) in three composite binding pockets. The pocket also appears to be present in the highly pathogenic coronaviruses SARS-CoV and MERS-CoV. Lipid metabolome remodeling is a key feature of coronavirus infection, with LA at its core. LA metabolic pathways are central to inflammation, immune modulation and membrane fluidity. Our structure directly links LA and S, setting the stage for interventions targeting LA binding and metabolic remodeling by SARS-CoV-2. [**note: just another fascinating aspect of the virus and yet another therapeutic target!**] https://www.biorxiv.org/content/10.1101/2020.06.18.158584v1
- Given its sequence similarity to SARS-CoV, as well as related coronaviruses circulating in bats, SARS-CoV-2 is thought to have originated in Chiroptera species in China. However, whether the virus spread directly to humans or through an intermediate host is currently unclear, as is the potential for this virus to infect companion animals, livestock and wildlife that could act as viral reservoirs. Using a combination of surrogate entry assays and live virus we demonstrate that, in addition to human ACE2, the Spike glycoprotein of SARS-CoV-2 has a broad host tropism for mammalian ACE2 receptors, despite divergence in the amino acids at the Spike receptor binding site on these proteins. Of the twenty-two different hosts we investigated, ACE2 proteins from dog, cat and rabbit were the most permissive to SARS-CoV-2, while bat and bird ACE2 proteins were the least efficiently used receptors. The absence of a significant tropism for any of the three genetically distinct bat ACE2 proteins we examined indicates that SARS-CoV-2 receptor usage likely shifted during zoonotic transmission from bats into people, possibly in an intermediate reservoir. Interestingly, while SARS-CoV-2 pseudoparticle entry was inefficient in cells bearing the ACE2 receptor from bats or birds the live virus was still able to enter these cells, albeit with markedly lower efficiency. The apparently broad tropism of SARS-CoV-2 at the point of viral entry confirms the potential risk of infection to a wide range of companion animals, livestock and wildlife. [**note: here is a paper showing broad species tropism with ACE2 receptors.**] https://www.biorxiv.org/content/10.1101/2020.06.17.156471v1
- Here we report the isolation of 61 SARS-CoV-2-neutralizing monoclonal antibodies from 5 infected patients hospitalized with severe disease. Among these are 19 antibodies that potently

neutralized the authentic SARS-CoV-2 in vitro, 9 of which exhibited exquisite potency, with 50% virus-inhibitory concentrations of 1 to 9 ng/mL. Epitope mapping showed this collection of 19 antibodies to be about equally divided between those directed to the receptor-binding domain (RBD) and those to the N-terminal domain (NTD), indicating that both of these regions at the top of the viral spike are quite immunogenic. In addition, two other powerful neutralizing antibodies recognized quaternary epitopes that are overlapping with the domains at the top of the spike. Cyro-electron microscopy structures of one antibody targeting RBD, a second targeting NTD, and a third bridging RBD and NTD revealed recognition of the closed, "all RBD-down" conformation of the spike. Several of these monoclonal antibodies are promising candidates for clinical development as potential therapeutic and/or prophylactic agents against SARS-CoV-2. [**note: more potential mAbs this time from Columbia University.**]
https://www.biorxiv.org/content/10.1101/2020.06.17.153486v1

DIAGNOSTIC DEVELOPMENT

- COVID-19 testing as sufficient as needed is essential for healthcare workers, patients, and authorities to make informed decisions to confront and eventually defeat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Currently, diagnosis of COVID-19 relies on quantitative reverse-transcription PCR, which is low-throughput, laborious, and often false-negative, making it overwhelmingly challenging to meet testing needs even in industrialized countries. Here we propose a new strategy, which employs a modified loop-mediated isothermal amplification (LAMP) assay, a simple procedure requiring no sophisticated instruments, to index and amplify viral genes from individual specimens, of which the products are readily available for construction of multiplexed libraries for next-generation sequencing. Our strategy would allow precise diagnosis of thousands of specimens in 1-2 days with significantly lower operating expenses. Furthermore, this strategy will make it possible for patients to collect, process, and mail their own samples to facilities for a quick, reliable diagnosis at a population scale. [**note: another approach to high throughput screening. FDA has finally issued some guidance on sample pooling which addresses this matter in part.**]
https://www.medrxiv.org/content/10.1101/2020.06.12.20129718v1
- Unfortunately, standard SARS-CoV-2 testing protocols are invasive and rely on numerous items that can be subject to supply chain bottlenecks, and as such are not suitable for frequent repeat testing. Specifically, personal protective equipment (PPE), nasopharyngeal (NP) swabs, the associated viral transport media (VTM), and kits for RNA isolation and purification have all been in short supply at various times during the COVID-19 pandemic. Moreover, SARS-CoV-2 is spread through droplets and aerosols transmitted through person-to-person contact, and thus saliva may be a relevant medium for diagnosing SARS-CoV-2 infection status. Here we describe a saliva-based testing method that bypasses the need for RNA isolation/purification. In experiments with inactivated SARS-CoV-2 virus spiked into saliva, this method has a limit of detection of 500-1000 viral particles per mL, rivalling the standard NP swab method, and initial studies also show excellent performance with 100 clinical samples. This saliva-based process is operationally simple, utilizes readily available materials, and can be easily implemented by existing testing sites, thus allowing for high-throughput, rapid, and repeat testing of large populations. [**note: another approach to saliva testing.**]
https://www.biorxiv.org/content/10.1101/2020.06.18.159434v1

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2020-06-20

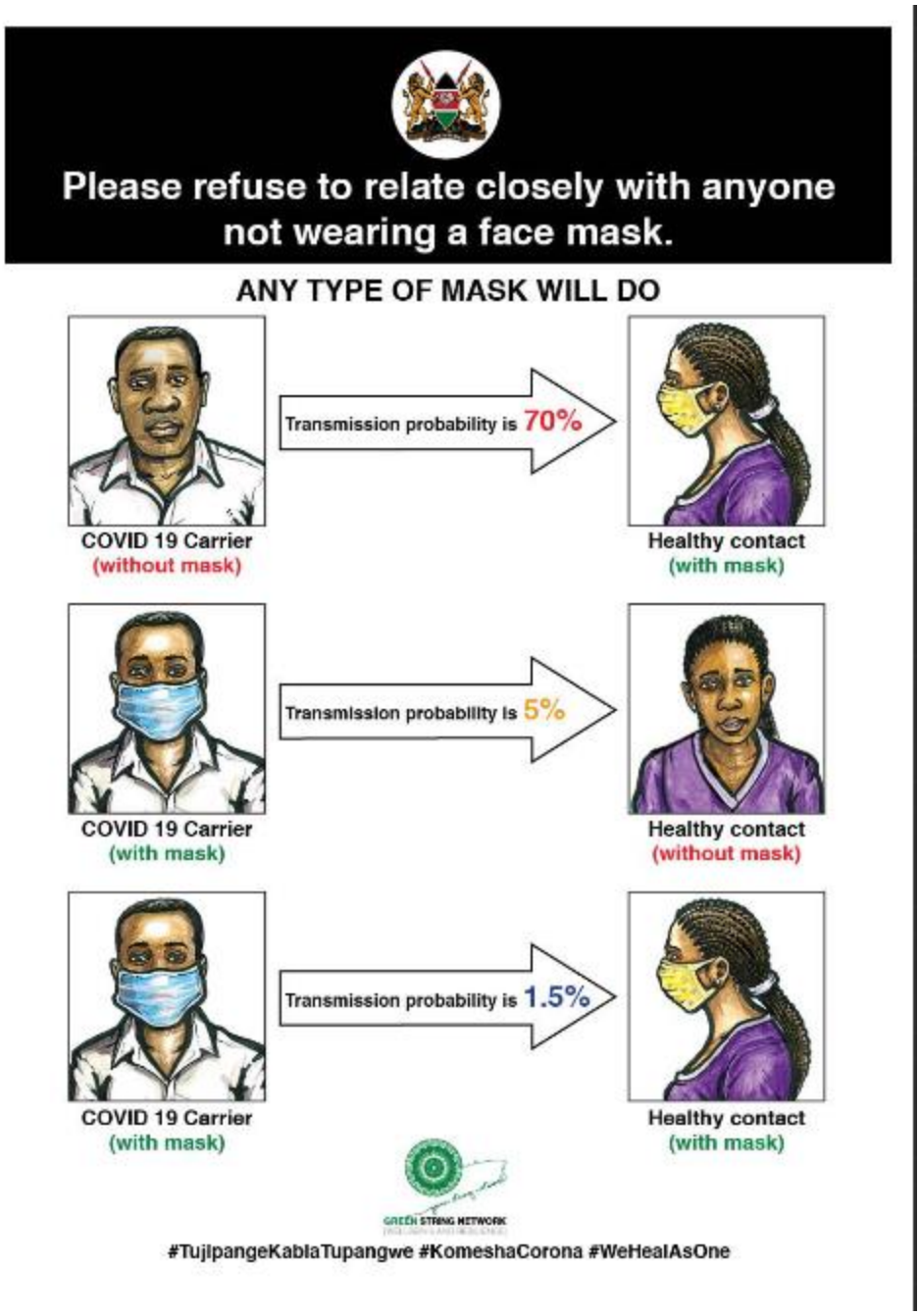
Cabaret again today, this time featuring Diana Krall. YouTube has a number of full concert performances that you can seek out. Here is a music video of 'Just the Way You Are', a fine Billy Joel composition: https://www.youtube.com/watch?v=d8RYUZT57XA and as a double treat Saturday here is a fun conversation and performance of 'You Can't Take That Away from Me' with Tony Bennett!!!: https://www.youtube.com/watch?v=ZogFMxidChM this song by George and Ira Gershwin was first sung by Fred Astaire way back in 1937: https://www.youtube.com/watch?v=fuufFgAMkGE why not three music selections and a bonus dance video with Fred and Ginger?

Derek Lowe on viral mutations in SARS-CoV-2. I linked to the paper the other day. As Derek notes, this type of research was not possible until we had modern tools of molecular biology.

This Washington Post story has some caustic comments from foreign observers about how the US has managed the SARS-CoV-2 outbreak.

Host range and inter-species transmission of SARS-CoV-2 is still something that needs to be resolved. A commentary in The Lancet and a Washington Post article point to some interesting public health issues. I previously linked to a preprint about the Dutch finding of virus infected mink.

The wearing of masks continues to be fraught with political controversy. As loyal reader sent me an email yesterday with a graphic that provides precise information about how the wearing of masks reduces the risk of viral transmission. There are a number of these graphics floating around the Internet these days. Here is one from a Kenyan source:



I have seen a similar graphic from India and China. The internet debunking website snopes.com has an April 21 entry on this particular graphic saying the claims are mostly false: https://www.snopes.com/fact-check/covid-19-mask-efficacy-chart/ ("Trust but Verify" should be the watchword for all of us these days). There have been preprints since that date on the efficacy of masks. The Lancet offered this overview of physical distancing and mask wearing. Graphics such as the one above can convey useful information to the public but they should not be misleading with transmission probabilities that are not science based. WHO has some good information on masks as does the CDC The Washington Post weights in on Why are people protesting the wearing of masks? Finally, there is yet another opinion piece on the impact of intervention delays on COVID-19 mortality and some of the

papers cited were previously referenced in this newsletter. I'll leave it at that and just continue to be baffled. Let's get back to research progress.

Some new drug repurposing studies and an Israeli vaccine effort are the highlights today. There are even a couple of hydroxychloroquine reports!

MODELING

- The hand of molecular mimicry in shaping SARS-CoV-2 evolution and immune evasion remains to be deciphered. Here, we identify 33 distinct 8-mer/9-mer peptides that are identical between SARS-CoV-2 and human proteomes, including 20 novel peptides not observed in any previous human coronavirus (HCoV) strains. Four of these mimicked 8-mers/9-mers map onto HLA-B*40:01, HLA-B*40:02, and HLA-B*35:01 binding peptides from human PAM, ANXA7, PGD, and ALOX5AP proteins. This striking mimicry of multiple human proteins by SARS-CoV-2 is made more salient by the targeted genes being focally expressed in arteries, lungs, esophagus, pancreas, and macrophages. Further, HLA-A*03 restricted 8-mer peptides are shared broadly by human and coronaviridae helicases with primary expression of the mimicked human proteins in the neurons and immune cells. These findings highlight molecular mimicry as a shared strategy adopted by evolutionary titans -- the virus in its quest for escaping herd immune surveillance, and the host immune systems that are constantly learning the patterns of 'self' and 'non-self'. [**note: this is molecular/genomic modelling and is intriguing in its concept.**] https://www.biorxiv.org/content/10.1101/2020.06.19.161620v1
- Trade in wildlife likely played a role in the origin of COVID-19, and viruses closely related to SARS-CoV-2 have been identified in bats and pangolins, both traded widely. To investigate the possible role of pangolins as a source of potential zoonoses, we collected throat and rectal swabs from 334 Sunda pangolins (Manis javanica) confiscated in Peninsular Malaysia and Sabah between August 2009 and March 2019. Total nucleic acid was extracted for viral molecular screening using conventional PCR protocols used to routinely identify known and novel viruses in extensive prior sampling (>50,000 mammals). No sample yielded a positive PCR result for any of the targeted viral families: Coronaviridae, Filoviridae, Flaviviridae, Orthomyxoviridae and Paramyxoviridae. In light of recent reports of coronaviruses including a SARS-CoV-2 related virus in Sunda pangolins in China, the lack of any coronavirus detection in our "upstream" market chain samples suggests that these detections in "downstream" animals more plausibly reflect exposure to infected humans, wildlife or other animals within the wildlife trade network. While confirmatory serologic studies are needed, it is likely that Sunda pangolins are incidental hosts of coronaviruses. Our findings further support the importance of ending the trade in wildlife globally. [**note: good ecological research on one of the animals linked to the spread of SARS-CoV-2**] https://www.biorxiv.org/content/10.1101/2020.06.19.158717v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- To examine outcomes among patients with COVID-19, a retrospective cohort study using data from Danish national administrative registries was conducted. Patients with COVID-19 from February 22 to May 4, 2020, were identified using *ICD-10* codes and followed up from day of diagnosis to outcome or end of study period (May 4, 2020). To examine susceptibility to COVID-19, a Cox regression model with a nested case-control framework was used to examine the association between use of ACEI/ARBs vs other antihypertensive drugs and the incidence rate of a COVID-19 diagnosis in a cohort of patients with hypertension from February 1 to May 4, 2020. In the retrospective cohort study, 4480 patients with COVID-19 were included (median age, 54.7 years [interquartile range, 40.9-72.0]; 47.9% men). There were 895 users (20.0%) of ACEI/ARBs and 3585 nonusers (80.0%). In the ACEI/ARB group, 18.1% died within 30 days vs 7.3% in the nonuser group, but this association was not significant after adjustment for age, sex, and medical history (adjusted hazard ratio [HR], 0.83 [95% CI, 0.67-1.03]). Death or severe COVID-19 occurred in 31.9% of ACEI/ARB users vs 14.2% of nonusers by 30 days (adjusted HR, 1.04 [95% CI, 0.89-1.23]). In the nested case-control analysis of COVID-19 susceptibility, 571 patients with COVID-19 and prior hypertension (median age, 73.9 years; 54.3% men) were compared with 5710 age- and sex-matched controls with prior hypertension but not COVID-19. Among those with COVID-19, 86.5% used ACEI/ARBs vs 85.4% of controls; ACEI/ARB use compared with other antihypertensive drugs was not significantly associated with higher incidence of COVID-19 (adjusted HR, 1.05 [95% CI, 0.80-1.36]). Prior use of ACEI/ARBs was not significantly associated with COVID-19 diagnosis among patients with hypertension or with mortality or severe disease among patients diagnosed as having COVID-19. These findings do not support discontinuation of ACEI/ARB medications that are clinically indicated in the context of the COVID-19 pandemic. [**note: further confirmation on the ACEI/ARB drugs not leading to enhanced incidence of COVID-19 We still don't know whether some of these are protective and we will need to see either a good observational analysis or clinical trial to show this. JAMA also have an editorial on this paper.]** https://jamanetwork.com/journals/jama/fullarticle/2767669
- Objective To assess the clinical effectiveness of oral hydroxychloroquine (HCQ) with or without azithromycin (AZI) in preventing death or leading to hospital discharge. Design Retrospective cohort study. Setting An analysis of data from electronic medical records and administrative claim data from the French Assistance Publique - Hopitaux de Paris (AP-HP) data warehouse, in 39 public hospitals, Ile-de-France, France. Participants All adult inpatients with at least one PCR-documented SARS-CoV-2 RNA from a nasopharyngeal sample between February 1st, 2020 and April 6th, 2020 were eligible for analysis. The study population was restricted to patients who did not receive COVID-19 treatments assessed in ongoing trials, including antivirals and immunosuppressive drugs. End of follow-up was defined as the date of death, discharge home, day 28 after admission, whichever occurred first, or administrative censoring on May 4, 2020. Intervention Patients were further classified into 3 groups: (i) receiving HCQ alone, (ii) receiving HCQ together with AZI, and (iii) receiving neither HCQ nor AZI. Exposure to a HCQ/AZI combination was defined as a simultaneous prescription of the 2 treatments (more or less one day). Main outcome measures The primary outcome was all-cause 28-day mortality as a time-to-event endpoint under a competing risks survival analysis framework. The secondary outcome was 28-day discharge home. Augmented inverse probability of treatment weighted (AIPTW) estimates of the average treatment effect (ATE) were computed to account for confounding. Results A total of 4,642 patients (mean age: 66.1 +/- 18; males: 2,738 (59%)) were included, of

whom 623 (13.4%) received HCQ alone, 227 (5.9%) received HCQ plus AZI, and 3,792 (81.7%) neither drug. Patients receiving "HCQ alone" or "HCQ plus AZI" were more likely younger, males, current smokers and overall presented with slightly more co-morbidities (obesity, diabetes, any chronic pulmonary diseases, liver diseases), while no major difference was apparent in biological parameters. After accounting for confounding, no statistically significant difference was observed between the "HCQ" and "Neither drug" groups for 28-day mortality: AIPTW absolute difference in ATE was +1.24% (-5.63 to 8.12), ratio in ATE 1.05 (0.77 to 1.33). 28-day discharge rates were statistically significantly higher in the "HCQ" group: AIPTW absolute difference in ATE (+11.1% [3.30 to 18.9]), ratio in ATE (1.25 [1.07 to 1.42]). As for the "HCQ+AZI" vs neither drug, trends for significant differences and ratios in AIPTW ATE were found suggesting higher mortality rates in the former group (difference in ATE +9.83% [-0.51 to 20.17], ratio in ATE 1.40 [0.98 to 1.81];p=0.062). Conclusions Using a large non-selected population of inpatients hospitalized for COVID-19 infection in 39 hospitals in France and robust methodological approaches, we found no evidence for efficacy of HCQ or HCQ combined with AZI on 28-day mortality. Our results suggested a possible excess risk of mortality associated with HCQ combined with AZI, but not with HCQ alone. Significantly higher rates of discharge home were observed in patients treated by HCQ, a novel finding warranting further confirmation in replicative studies. Altogether, our findings further support the need to complete currently undergoing randomized clinical trials. [**note: more confounding data on HCQ. There appeared to be no effect on 28 day mortality and giving the drug with azithromycin suggest excess mortality. However, they saw higher discharge rates for patients on HCQ. Whether this was drug related or not is unknown.**]
https://www.medrxiv.org/content/10.1101/2020.06.16.20132597v1 and here is a Brazilian study that shows no difference in viral load in patients being treated with HCQ
https://www.medrxiv.org/content/10.1101/2020.06.16.20133066v1

- Immune responses in lungs of Coronavirus Disease 2019 (COVID-19) are poorly characterized. We conducted transcriptomic, histologic and cellular profiling of post mortem COVID-19 and normal lung tissues. Two distinct immunopathological reaction patterns were identified. One pattern showed high expression of interferon stimulated genes (ISGs) and cytokines, high viral loads and limited pulmonary damage, the other pattern showed severely damaged lungs, low ISGs, low viral loads and abundant immune infiltrates. Distinct patterns of pulmonary COVID-19 immune responses correlated to hospitalization time and may guide treatment and vaccination approaches. [**note: this one will be of interest to the pathologists and I am not one.]**
https://www.medrxiv.org/content/10.1101/2020.06.17.20133637v1

DRUG DEVELOPMENT

- Among those persons with serious COVID-19 disease, acute respiratory distress syndrome (ARDS) is a common and often fatal presentation. SARS-CoV-2-induced ARDS is difficult to treat clinically, and new therapeutic strategies are needed. In order to evaluate such therapeutic strategies, animal models of SARS-CoV-2 infection that manifest severe disease are needed. Here we report fatal ARDS in two African green monkeys (AGMs) infected with SARS-CoV-2 that demonstrated pathological lesions and disease similar to severe COVID-19 in humans. Moreover, we report the observation of cytokine release (cytokine storm) in three of four infected AGMs. All four animals showed increased levels of IL-6 in plasma, a predictive marker

and presumptive therapeutic target in humans infected with SARS-CoV-2 infection. Our results suggest the AGM is a useful model to study disease pathogenesis of SARS-CoV-2, and for the evaluation of therapeutic interventions designed to combat serious pulmonary disease associated with this infection. [**note: another animal model for studying SARS-CoV-2 infection. These monkeys have ARDS and cytokine storm upon infection.**]
https://www.biorxiv.org/content/10.1101/2020.06.18.157933v1

- Screening in Vero cells found few antivirals, while screening in human Huh7.5 cells validated 23 diverse antiviral drugs. Extending our studies to lung epithelial cells, we found that there are major differences in drug sensitivity and entry pathways used by SARS-CoV-2 in these cells. Entry in lung epithelial Calu-3 cells is pH-independent and requires TMPRSS2, while entry in Vero and Huh7.5 cells requires low pH and triggering by acid-dependent endosomal proteases. Moreover, we found 9 drugs are antiviral in lung cells, 7 of which have been tested in humans, and 3 are FDA approved including Cyclosporine which we found is targeting Cyclophilin rather than Calcineurin for its antiviral activity. These antivirals reveal essential host targets and have the potential for rapid clinical implementation. [**note: this is an interesting new model for testing potential drugs using lung epithelial cells. Cyclosporine came up as a good inhibitor. There are ongoing clinical trials of this drug.**]
https://www.biorxiv.org/content/10.1101/2020.06.19.161042v1
- Here, we generated a replication competent recombinant VSV-ΔG-spike vaccine, in which the glycoprotein of VSV was replaced by the spike protein of the SARS-CoV-2. In vitro characterization of the recombinant VSV-ΔG-spike indicated expression and presentation of the spike protein on the viral membrane with antigenic similarity to SARS-CoV-2. A golden Syrian hamster in vivo model for COVID-19 was implemented. We show that vaccination of hamsters with recombinant VSV-ΔG-spike results in rapid and potent induction of neutralizing antibodies against SARS-CoV-2. Importantly, single-dose vaccination was able to protect hamsters against SARS-CoV-2 challenge, as demonstrated by the abrogation of body weight loss of the immunized hamsters compared to unvaccinated hamsters. Furthermore, whereas lungs of infected hamsters displayed extensive tissue damage and high viral titers, immunized hamsters lungs showed only minor lung pathology, and no viral load. Taken together, we suggest recombinant VSV-ΔG-spike as a safe, efficacious and protective vaccine against SARS-CoV-2 infection. [**note: this one was below my radar screen as an Israeli group looks to be in the vaccine race. However, this technology looks to be the same one that the IAVI/Merck group is pursuing. Still the animal study is good confirmation that the approach is viable.**]
https://www.biorxiv.org/content/10.1101/2020.06.18.160655v1
- The papain-like protease PLpro cleaves the viral polyprotein and reverses inflammatory ubiquitin and anti-viral ubiquitin-like ISG15 protein modifications. Drugs that target SARS-CoV-2 PLpro (hereafter, SARS2 PLpro) may hence be effective as treatments or prophylaxis for COVID-19, reducing viral load and reinstating innate immune responses. We here characterise SARS2 PLpro in molecular and biochemical detail. SARS2 PLpro cleaves Lys48-linked polyubiquitin and ISG15 modifications with high activity. Structures of PLpro bound to ubiquitin and ISG15 reveal that the S1 ubiquitin binding site is responsible for high ISG15 activity, while the S2 binding site provides Lys48 chain specificity and cleavage efficiency. We further exploit two strategies to target PLpro. A repurposing approach, screening 3727 unique approved drugs and clinical compounds against SARS2 PLpro, identified no compounds that inhibited PLpro consistently or

that could be validated in counterscreens. More promisingly, non-covalent small molecule SARS PLpro inhibitors were able to inhibit SARS2 PLpro with high potency and excellent antiviral activity in SARS-CoV-2 infection models. [**note: this is an exceedingly dense but interesting paper. Most of the drug repurposing work that I have read focuses on the Mpro enzyme and not this one. These Australian do some nice work showing that virtually all approved drugs do not inhibit the PLpro but do find some experimental compounds that do. Perhaps this is another target to look at for a 'drug cocktail.'**
https://www.biorxiv.org/content/10.1101/2020.06.18.160614v1

DIAGNOSTIC DEVELOPMENT

- Alternative methodologies to PCR and immunodiagnostic that would not require specific reagents are worth to investigate not only for fighting the COVID-19 pandemic, but also to detect other emergent pathogenic threats. Here, we propose the use of tandem mass spectrometry to detect SARS-CoV-2 marker peptides in nasopharyngeal swabs. We documented that the signal from the microbiota present in such samples is low and can be overlooked when interpreting shotgun proteomic data acquired on a restricted window of the peptidome landscape. Simili nasopharyngeal swabs spiked with different quantities of purified SARS-CoV-2 viral material were used to develop a nanoLC-MS/MS acquisition method, which was then successfully applied on COVID-19 clinical samples. We argue that peptides ADETQALPQR and GFYAQGSR from the nucleocapsid protein are of utmost interest as their signal is intense and their elution can be obtained within a 3 min window in the tested conditions. These results pave the way for the development of time-efficient viral diagnostic tests based on mass spectrometry. [**note: this is interesting but I am unsure about the viability of employment. Mass spec instrumentation is not particularly cheap.**]
https://www.biorxiv.org/content/10.1101/2020.06.19.161000v1

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2020-06-21

This week's reflection Sunday piece is the magical Schubert Symphony #8. While it is unfinished by the composer it still has a lot going for it Here is the Vienna Philharmonic under the direction of Phillipe Jordan: https://www.youtube.com/watch?v=TgZj4Vd3HHk&t=479s

The Data Safety Monitoring Board for the HCQ study funded by NIH has stopped the trial. This was a multicenter trial and here is the trial link: https://clinicaltrials.gov/ct2/show/NCT04332991 I wonder what this means for the very large prevention study that Duke University is running.

A group of scientists have formally called for a retraction of a recent paper on the efficacy of masks to present COVID-19. The paper, referenced in this newsletter on June 14, appeared in the Proceedings of the National Academy of Sciences. Here is the link to the letter and I will only say that this spat/fight won't change my opinion on the value of wearing masks. I'll let this play out on its own.

Surprisingly, lots of abstracts in all the categories today.

MODELING

- Following the outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-2) in China, airborne water droplets (aerosols) have been identified as the main transmission route, although other transmission routes are likely to exist. We quantify SARS-CoV-2 virus survivability within water and the risk of infection posed by faecal contaminated water within 39 countries. We identify that the virus can remain stable within water for up to 25 days, and country specific relative risk of infection posed by faecal contaminated water is related to the environment. Faecal contaminated rivers, waterways and water systems within countries with high infection rates can provide infectious doses >100 copies within 100 ml of water. The implications for freshwater systems, the coastal marine environment and virus resurgence are discussed. [**note: I don't think it is time to start boiling tap water but this is a useful paper to read. SARS-CoV-2 can be disseminated via fecal discharge and find its way into the environment. Whether it can be transmitted via mollusks as some other human viruses are is still an open question. For those on untreated well water, this might be a risk but more research is needed.**]
  https://www.medrxiv.org/content/10.1101/2020.06.17.20133504v1
- Since the COVID-19 pandemic started, the public has been eager for news about promising treatments, and social media has played a large role in information dissemination. In this paper, our objectives are to characterize the public discussion of treatments on Twitter, and demonstrate the utility of these discussions for public health surveillance. We pulled tweets related to three promising COVID-19 treatments (hydroxychloroquine, remdevisir and convalescent plasma), between the dates of February 28th and May 22nd using the Twitter public API. We characterize treatment tweet trends over this time period. Most major tweet/retweet/sentiment trends correlated to public announcement made by the white house and/or to new clinical trial evidence about treatments. Most of the websites people shared in treatment-related tweets were non-scientific media sources that leaned conservative. Hydroxychloroquine was the most discussed treatment on Twitter, and over 10% of hydroxychloroquine tweets mentioned an adverse drug reaction. There is a gap between the public attention/discussion around COVID-19 treatments and their evidence. Twitter data can and should be used public health surveillance during this pandemic, as it is informative for monitoring adverse drug reactions, especially as many people avoid going to hospitals/doctors. [**note: despite my aversion to Twitter and reluctance to link to abstracts that do Twitter datamining this one is posted as some of my loyal readers are graduates of New York University and may be interested in what scientists at their alma mater are doing. I will let my readers determine the value of this research.**]
  https://www.medrxiv.org/content/10.1101/2020.06.18.20134668v1
- Adaptive Biotechnologies and Microsoft have recently partnered to release ImmuneCode, a database containing SARS-CoV-2 specific T-cell receptors derived through MIRA, a T-cell receptor (TCR) sequencing based sequencing approach to identify antigen-specific TCRs. Herein, we query the extent of cross reactivity between these derived SARS-CoV-2 specific TCRs and other known antigens present in McPas-TCR, a manually curated catalogue of pathology-associated TCRs. We reveal cross reactivity between SARS-CoV-2 specific TCRs and the immunodominant Influenza GILGFVFTL M1 epitope, suggesting the importance of further work in characterizing the implications of prior Influenza exposure or co-exposure to the pathology of SARS-CoV-2 illness. [**note: I was unaware of this database and offer kudos to the two**

companies that have released this. The revolution in IT makes this kind of stuff possible and to think we can do these analyses on desk top computers!!! I still have fond memories of the far past learning Fortran programing on an IBM mainframe with punch cards! Obviously more research is needed to figure out whether there is a linkage between the influenza and SARS-CoV-2 infection exposures.] https://www.biorxiv.org/content/10.1101/2020.06.20.160499v1

NEWLY REGISTERED CLINICAL TRIALS

- To evaluate the efficacy and safety of azvudine in treatment of COVID-19 [**note: this is a reverse transcriptase inhibitor developed for Hepatitis C synthesized by Roche in 2009. Study is sponsored by a Chinese drug company**] NCT04425772
- The CONTAIN (CiclesOnide cliNical TriAl covId-19 treatmeNt) is a randomized control study of ciclesonide vs placebo for mild covid-19 disease. The need for potential therapy for COVID-19 patients is urgent. Ciclesonide has shown encouraging in vitro results, is easy to be used and is readily available. It has a low rate of side effects and few interactions with other drugs. It is unusual to use an inhaled steroid drug for COVID-19 but there has been new data suggesting steroids may have an antiviral effect in addition to an anti-inflammatory effect. We propose to use inhaled and nasal ciclesonide to stop viral replication in the nose and airways. We hope this will accelerate recovery from COVID-19 illness in individuals who are not admitted to hospital at time of diagnosis of COVID-19. [**note: this glucocorticoid has been around for a while. There are some other trials of similar drugs. I guess I'll start taking my fluticasone more regularly to make sure I'm fully protected!**] NCT04435795
- Maraviroc, a C-C Chemokine Receptor 5 (CCR5) antagonist, is well-tolerated without significant side effects in its current use in patients with HIV. CCR5 antagonism prior to the 'second wave' of inflammatory mediator expression in SARS-CoV-2 may reverse lymphoid depletion and may alter cell trafficking of inflammatory cells, both increasing viral control capacity and dampening damage to lung tissue, respectively. Our study seeks to establish whether one week of treatment with Maraviroc, used at its approved dosage for HIV, is safe and tolerable in patients with SARS-CoV-2. [**note: this is an old Pfizer HIV drug**] NCT04435522

CLINICAL TRIAL RESULTS

- Here we report on an inpatient cohort of COVID-19 positive patients where plasma cytokines were tested for association with future need for mechanical ventilation. Hierarchical clustering, Kaplan-Meier curves, and odds ratios demonstrated that two cytokines, IL-13 (OR: 1.57) and IL-7 (OR: 1.04) and the growth factor bFGF (OR: 1.04), were predictive for intubation. [**note: more markers to predict COVID-19 progression. I wish someone would publish a summary of all this information; it certainly would help in the design of quick clinical assays.**] https://www.medrxiv.org/content/10.1101/2020.06.18.20134353v1 but here is a preprint that does this type of survey https://www.medrxiv.org/content/10.1101/2020.06.19.20134767v1 I'll leave it to clinicians to continue looking at these approaches to improving practice guidelines as it is not in my wheel house.
- Background: Several preclinical and clinical investigations have argued for nervous system involvement in SARS-CoV-2 infection. Some sparse case reports have described various forms of encephalitis in COVID-19 disease, but very few data have focused on clinical presentations,

clinical course, response to treatment and outcomes yet. Objective: to describe the clinical phenotype, laboratory and neuroimaging findings of encephalitis associated with SARS-CoV-2 infection, their relationship with respiratory function and inflammatory parameters and their clinical course and response to treatment. Design: The ENCOVID multicentre study was carried out in 13 centres in northern Italy between February 20th and May 31st, 2020. Only patients with altered mental status and at least two supportive criteria for encephalitis with full infectious screening, CSF, EEG, MRI data and a confirmed diagnosis of SARS-CoV-2 infection were included. Clinical presentation and laboratory markers, severity of COVID-19 disease, response to treatment and outcomes were recorded. Results: Out of 45 cases screened, twenty-five cases of encephalitis positive for SARS-CoV-2 infection with full available data were included. The most common symptoms at onset were delirium (68%), aphasia/dysarthria (24%) and seizures (24%). CSF showed hyperproteinorrachia and/or pleocytosis in 68% of cases whereas SARS-CoV-2 RNA by RT-PCR resulted negative. Based on MRI, cases were classified as ADEM (n=3), limbic encephalitis (LE, n=2), encephalitis with normal imaging (n=13) and encephalitis with MRI alterations (n=7). ADEM and LE cases showed a delayed onset compared to the other encephalitis (p=0.001) and were associated with previous more severe COVID-19 respiratory involvement. Patients with MRI alterations exhibited worse response to treatment and final outcomes compared to other encephalitis. Conclusions and relevance: We found a wide clinical spectrum of encephalitis associated with COVID19 infection, underlying different pathophysiological mechanisms. Response to treatment and final outcome strongly depended on specific CNS-manifestations. **[note: this study comes from Italy. The authors note that cases of COVID-19 encephalitis are small (58/100K) and in agreement with findings from the UK. However, the background rate may change as more cases are analyzed.**]
https://www.medrxiv.org/content/10.1101/2020.06.19.20133991v1

- Background In hospitalized patients with COVID-19 pneumonia, progression to acute respiratory failure requiring invasive mechanical ventilation (MV) is associated with significant morbidity and mortality. Severe dysregulated systemic inflammation is the putative mechanism. We hypothesize that early prolonged methylprednisolone (MP) treatment could accelerate disease resolution, decreasing the need for ICU and mortality. Methods We conducted a multicenter, observational study to explore the association between exposure to prolonged, low-dose, MP treatment and need for ICU referral, intubation or death within 28 days (composite primary endpoint) in patients with severe COVID-19 pneumonia admitted to Italian respiratory high-dependency units. Secondary outcomes were invasive MV-free days and changes in C-reactive protein (CRP) levels. Results Findings are reported as MP (n=83) vs. control (n=90). The composite primary endpoint was met by 19 vs. 40 [adjusted hazard ratio (HR) 0.41; 95% confidence interval (CI): 0.24-0.72]. Transfer to ICU and need for invasive MV was necessary in 15 vs. 27 (p=0.07) and 14 vs. 26 (p=0.10), respectively. By day 28, the MP group had fewer deaths (6 vs. 21, adjusted HR=0.29; 95% CI: 0.12-0.73) and more days off invasive MV (24.0 plus-or-minus sign 9.0 vs. 17.5 plus-or-minus sign 12.8; p=0.001). Study treatment was associated with rapid improvement in PaO2:FiO2 and CRP levels. The complication rate was similar for the two groups (p=0.84). Conclusion In patients with severe COVID-19 pneumonia, early administration of prolonged MP treatment was associated with a significantly lower hazard of death (71%) and decreased ventilator dependence. Randomized controlled studies are needed to confirm these findings. [**note: this is an observational study from Italy showing low dose**

methylprednisolone is effective in reducing mortality and need for ventilation. Of course TIWWDCT and that will confirm this finding.]
https://www.medrxiv.org/content/10.1101/2020.06.17.20134031v1

- Background Clozapine, an antipsychotic with unique efficacy in treatment resistant psychosis, is associated with increased susceptibility to infection, including pneumonia. Aims To investigate associations between clozapine treatment and increased risk of COVID-19 in patients with schizophrenia-spectrum disorders who are receiving antipsychotic medications, using electronic health records data, in a geographically defined population in London. Method Using information from South London and Maudsley NHS Foundation Trust (SLAM) clinical records, via the Clinical Record Interactive Search system, we identified 6,309 individuals who had an ICD-10 diagnosis of schizophrenia-spectrum disorders and were taking antipsychotics at the time on the COVID-19 pandemic onset in the UK. People who were on clozapine treatment were compared with those on any other antipsychotic treatment for risk of contracting COVID-19 between 1 March and 18 May 2020. We tested associations between clozapine treatment and COVID-19 infection, adjusting for gender, age, ethnicity, BMI, smoking status, and SLAM service use. Results Of 6,309 patients, 102 tested positive for COVID-19. Individuals who were on clozapine had increased risk of COVID-19 compared with those who were on other antipsychotic medication (unadjusted HR = 2.62 (95% CI 1.73 - 3.96), which was attenuated after adjusting for potential confounders, including clinical contact (adjusted hazard ratio HR=1.76, 95% CI 1.14 - 2.72). Conclusions These findings provide support for the hypothesis that clozapine treatment is associated with an increased risk of COVID-19. Further research will be needed in other samples to confirm this association. Potential clinical implications are discussed. [**note: more datamining, this time showing an association between clozapine therapy and increased risk of COVID-19. One wonders whether this cohort engage in behavior that might make them more susceptible to infection and I am unsure whether this potential confounder can be separated out.**] https://www.medrxiv.org/content/10.1101/2020.06.17.20133595v1
- Background Type 2 diabetes (T2DM) and obesity are significant risks for mortality in Covid19. Metformin has been hypothesized as a treatment for COVID19. Metformin has sex specific immunomodulatory effects which may elucidate treatment mechanisms in COVID-19. In this study we sought to identify whether metformin reduced mortality from Covid19 and if sex specific interactions exist. Methods De-identified claims data from UnitedHealth were used to identify persons with at least 6 months continuous coverage who were hospitalized with Covid-19. Persons in the metformin group had at least 90 days of metformin claims in the 12 months before hospitalization. Unadjusted and multivariate models were conducted to assess risk of mortality based on metformin as a home medication in individuals with T2DM and obesity, controlling for pre-morbid conditions, medications, demographics, and state. Heterogeneity of effect was assessed by sex. Results 6,256 persons were included; 52.8% female; mean age 75 years. Metformin was associated with decreased mortality in women by logistic regression, OR 0.792 (0.640, 0.979); mixed effects OR 0.780 (0.631, 0.965); Cox proportional-hazards: HR 0.785 (0.650, 0.951); and propensity matching, OR of 0.759 (0.601, 0.960). TNF-alpha inhibitors were associated with decreased mortality in the 38 persons taking them, by propensity matching, OR 0.19 (0.0378, 0.983). Conclusions Metformin was significantly associated with reduced mortality in women with obesity or T2DM in observational analyses of claims data from individuals hospitalized with Covid-19. This sex-specific finding is consistent with metformin reducing TNF-

alpha in females over males, and suggests that metformin conveys protection in Covid-19 through TNF-alpha effects. Prospective studies are needed to understand mechanism and causality. [**note: more observational datamining this time for metformin. I believe this drug came up in some screens as potentially useful in treating COVID-19 There does appear to be limited protection in this cohort but of course TIWWDCT to confirm these observations though larger data sets may prove useful.**]
https://www.medrxiv.org/content/10.1101/2020.06.19.20135095v1

- We previously reported that the ApoE e4e4 genotype was associated with COVID-19 test positivity (OR=2.31, 95% CI: 1.65 to 3.24, $p=1.19\times10^{-6}$) in the UK Biobank (UKB) cohort, during the epidemic peak in England, from March 16 to April 26, 2020. With more COVID-19 test results (March 16 to May 31, 2020) and mortality data (to April 26, 2020) linked to UKB, we re-evaluated the ApoE e4 allele association with COVID-19 test positivity, and with all-cause mortality following test-confirmed COVID-19. Logistic regression models compared ApoE e4e4 participants (or e3e4s) to e3e3s with adjustment for sex; age on April 26th or age at death; baseline UKB assessment center in England (accounting for geographical differences in viral exposures); genotyping array type; and the top five genetic principal components (accounting for possible population admixture). ApoE e4e4 genotype was associated with increased risks of test positivity (OR=2.24, 95% CI: 1.72 to 2.93, p=3.24×10-*9*) and of mortality with test-confirmed COVID-19 (OR=4.29, 95% CI: 2.38 to 7.72, p=1.22×10-*6*), compared to e3e3s. Independent replications are needed to confirm our findings and mechanistic work is needed to understand how ApoE e4e4 results in the marked increase in vulnerability, especially for COVID-19 mortality. These findings also demonstrate that risks for COVID-19 mortality are not simply related to advanced chronological age or the comorbidities commonly seen in aging. [**note: more good information from the UK Biobank related to the ApoE e4e4 genotype. This may be another marker for a vulnerable population.**]
https://www.medrxiv.org/content/10.1101/2020.06.19.20134908v1
- Background: Early descriptions of the coronavirus outbreak showed a lower prevalence of asthma and COPD than was expected for people diagnosed with COVID-19, leading to speculation that inhaled corticosteroids (ICS) may protect against infection with SARS-CoV-2, and development of serious sequelae. We evaluated the association between ICS and COVID-19 related death using linked electronic health records in the UK. Methods: We conducted cohort studies on two groups of people (COPD and asthma) using the OpenSAFELY platform to analyse data from primary care practices linked to national death registrations. People receiving an ICS were compared to those receiving alternative respiratory medications. Our primary outcome was COVID-19 related death. Findings: We identified 148,588 people with COPD and 817,973 people with asthma receiving relevant respiratory medications in the four months prior to 01 March 2020. People with COPD receiving ICS were at a greater risk of COVID-19 related death compared to those receiving a long-acting beta agonist (LABA) and a long-acting muscarinic antagonist (LAMA) (adjusted HR = 1.38, 95% CI = 1.08 - 1.75). People with asthma receiving high dose ICS were at an increased risk of death compared to those receiving a short-acting beta agonist (SABA) only (adjusted HR = 1.52, 95%CI = 1.08 - 2.14); the adjusted HR for those receiving low-medium dose ICS was 1.10 (95% CI = 0.82 - 1.49). Quantitative bias analyses indicated that an unmeasured confounder of only moderate strength of association with exposure and outcome could explain the observed associations in both populations.

Interpretation: These results do not support a major role of ICS in protecting against COVID-19 related deaths. Observed increased risks of COVID-19 related death among people with COPD and asthma receiving ICS can be plausibly explained by unmeasured confounding due to disease severity. [**note: this is a useful study because of the widespread use of inhaled corticosteroids to treat asthma and COPD. The researchers had a very large initial drug use database to mine (24 million patients). There was no evidence that inhaled corticosteroids had a protective effect and the small harmful association may have been a result of other factors. People taking these medications should continue to do so. There was always a question in my mind whether inhaled steroids for asthma would be protective but this pretty much says not to that.**] https://www.medrxiv.org/content/10.1101/2020.06.19.20135491v1

DRUG DEVELOPMENT

- Recently, there are several routes for COVID-19 vaccine research, yet their weaknesses lie in low efficiency, tolerability, immune adaptability and safety. We describe a new approach to COVID-19 based on engineered human mesenchymal stem cells (hu-MSC), which is like a small protein antigen response device, but will be gradually cleared and degraded by body' s immune system among recognization process. The antibody response results show that this is effective and fast. Furthermore, after several antibody response tests, we obtained an injection of a set of cocktail-like modified human mesenchymal stem cell line. This strategy opened a new avenue for vaccine design against COVID-19. [**note: here is another intriguing vaccine platform approach from China using human stem cells. They observed antibody production against the SARS-CoV-2 in mice but note that other animal models need to be studied.**] https://www.biorxiv.org/content/10.1101/2020.06.20.163030v1
- The human serine protease TMPRSS2 gene is involved in the priming of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins being one of the possible targets for COVID-19 therapy. TMPRSS2 gene is possibly co-expressed with SARS-CoV-2 cell receptor genes ACE2 and BSG, but only TMPRSS2 demonstrates tissue-specific expression in alveolar cells according to single cell RNA sequencing data. Our analysis of the structural variability of the TMPRSS2 gene based on genome-wide data of 76 human populations demonstrates that functionally significant missense mutation in exon 6/7 in TMPRSS2 gene, was found in many human populations in relatively high frequency, featuring region-specific distribution patterns. The frequency of the missense mutation encoded by the rs12329760, which previously was found to be associated with prostate cancer, is ranged between 10% and 63% being significantly higher in populations of Asian origin compared to European populations. In addition to SNPs, two copy numbers variants (CNV) were detected in the TMPRSS2 gene. Number of microRNAs have been predicted to regulate TMPRSS2 and BSG expression levels, but none of them is enriched in lung or respiratory tract cells. Several well studied drugs can downregulate the expression of TMPRSS2 in human cells, including Acetaminophen (Paracetamol) and Curcumin. Thus TMPRSS2 interaction with the SARS-CoV-2, its structural variability, gene-gene interactions, and expression regulation profiles, and pharmacogenomics properties characterize this gene as a potential target for COVID-19 therapy. [**note: more information on the TMPRSS2 gene and drug development.**] https://www.biorxiv.org/content/10.1101/2020.06.20.156224v1
- The global outbreak of this novel coronavirus has now infected >8 million people worldwide with >2 million cases in the US (June 17th, 2020). There is an urgent need for vaccines and

therapeutics to combat the spread of this coronavirus. Similarly, the development of diagnostic and research tools to determine infection and vaccine efficacy are critically needed. Molecular assays have been developed to determine viral genetic material present in patients. Serological assays have been developed to determine humoral responses to the spike protein or receptor binding domain (RBD). Detection of functional antibodies can be accomplished through neutralization of live SARS-CoV2 virus, but requires significant expertise, an infectible stable cell line, a specialized BioSafety Level 3 (BSL-3) facility. As large numbers of people return from quarantine, it is critical to have rapid diagnostics that can be widely adopted and employed to assess functional antibody levels in the returning workforce. This type of surrogate neutralization diagnostic can also be used to assess humoral immune responses induced in patients from the large number of vaccine and immunotherapy trials currently on-going. Here we describe a rapid serological diagnostic assay for determining antibody receptor blocking and demonstrate the broad utility of the assay by measuring the antibody functionality of sera from small animals and non-human primates immunized with an experimental SARS-CoV-2 vaccine and using sera from infected patients. [**note: this report from Wistar Institute and the vaccine company Inovio is important in that they develop an antibody test that can be used to qualify convalescent plasma and check for vaccine potency. They note that more work needs to be done and published by other groups on this type of approach. I think as this progresses, it will be a viable tool to do vaccine development and potentially negate the need for large community trials on potency. It does not address vaccine safety in any way and those large trials will still be needed.]**

https://www.biorxiv.org/content/10.1101/2020.06.17.158527v1

DIAGNOSTIC DEVELOPMENT

- We aimed to develop a high throughput multiplex assay to detect antibodies to SARS-CoV-2 to assess immunity to the virus in the general population. Methods Spike protein subunits S1 and RBD, and Nucleoprotein were coupled to distinct microspheres. Sera collected before the emergence of SARS-CoV-2 (N=224), and of non-SARS-CoV-2 influenza-like illness (N=184), and laboratory-confirmed cases of SARS-CoV-2 infection (N=115) with various severity of COVID-19 were tested for SARS-CoV-2-specific concentrations of IgG. Results Our assay discriminated SARS-CoV-2-induced antibodies and those induced by other viruses. The assay obtained a specificity between 95.1 and 99.0% with a sensitivity ranging from 83.6-95.7%. By merging the test results for all 3 antigens a specificity of 100% was achieved with a sensitivity of at least 90%. Hospitalized COVID-19 patients developed higher IgG concentrations and the rate of IgG production increased faster compared to non-hospitalized cases. Conclusions The bead-based serological assay for quantitation of SARS-CoV-2-specific antibodies proved to be robust and can be conducted in many laboratories. Finally, we demonstrated that testing of antibodies against different antigens increases sensitivity and specificity compared to single antigen-specific IgG determination. [**note: here is another multiplex serology test, this time from The Netherlands. Looking at multiple antigens can improve sensitivity and specificity as others have also found.**]
https://www.medrxiv.org/content/10.1101/2020.06.18.20133660v1