2020-06-22

Welcome to week 14 of the newsletter. I was hoping to be out of business by now but the virus has a mind of its own and I will persevere.

One of my loyal readers responded to my music selections, noting Karen Carpenter's fine voice. Most of her work was with her brother Richard, but here is a fine duet with Ella Fitzgerald from 1980: https://www.youtube.com/watch?v=HDp9b9eYoLM Carpenter had a lifelong battle with anorexia that finally took her life at the age of 32; another fine performing artist who left way too soon. The American director, Todd Haynes made a short film on Karen's life using mainly Barbie dolls to represent the characters. It is well worth watching on YouTube.

I don't have a link to this as it was in the Washington Post quick update section. Here is what they published about Scott Gottlieb's appearance on TV yesterday morning:

> Former Food and Drug Administration commissioner Scott Gottlieb said Sunday that masks should have been recommended from the start of the coronavirus pandemic. Speaking during an appearance on CBS News's "Face the Nation," he suggested that officials were initially concerned that issuing guidance that masks helped keep people safe may have encouraged them to continue going out.
>
> "I think that could have been messaged appropriately," Gottlieb said. "They didn't need to be concerned about that. We should have been recommending masks from the outset." He also said the uptick in hospitalizations that was being reported by some states was expected as the country begins reopening. But he added that it's important to understand "this isn't contained" and may not be until a vaccine or better therapeutics are available.
>
> People can "get back to some semblance of a normal life," Gottlieb said, but should continue taking precautions, including frequent hand-washing, narrowing their social circles and shopping less frequently. "We're going to see a bump in cases, and we're seeing it right now," he said. "The question is how much, and are we then going to have to reimplement some of these mitigation steps? I hope not." Gottlieb said the United States is likely to see a "slow burn" over the summer and then renewed risk in the fall, with the potential of outbreaks or even epidemics in some cities and states. He said the White House has people working to prepare for that possibility by building tools to identify and track hot spots early on. "The goal is to get good information, more real-time, so we can target the interventions so we don't have to do this national shutdown or even statewide shutdowns," he said.

As the nation gets back to work, many people will need to ride elevators up to their offices. How much of a risk does this present? This Washington Post article seeks to answer this compelling question. Lots of good information here and of course everyone recommends…….wait for it……..wear a mask!!!

I have one comment on testing statistics. It doesn't matter how much or how little testing is done, people are still going to be infected by this virus. The more important statistic is hospital bed utilization and resultant impact on critical care facilities. In some areas of Arizona, Florida, and Texas, hospitalization is going up. We may see mortality level off with better interventions. I still think that the

Case Fatality Rate for SARS-CoV-2 will be in the 0.3 to 0.5% range when we have better numbers on infections.

Here is a report of some unexplained deaths in Los Angeles that may have been COVID-19 related predating the first documented fatality. It will be important to do the pathology to confirm this.

As usual with Mondays, there are only a smattering of preprints to peruse. I'm still waiting for the big trial results to come in.

MODELING

- The clinical features and immune responses of asymptomatic individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have not been well described. We studied 37 asymptomatic individuals in the Wanzhou District who were diagnosed with RT–PCR-confirmed SARS-CoV-2 infections but without any relevant clinical symptoms in the preceding 14 d and during hospitalization. Asymptomatic individuals were admitted to the government-designated Wanzhou People's Hospital for centralized isolation in accordance with policy[1]. The median duration of viral shedding in the asymptomatic group was 19 d (interquartile range (IQR), 15–26 d). The asymptomatic group had a significantly longer duration of viral shedding than the symptomatic group (log-rank $P = 0.028$). The virus-specific IgG levels in the asymptomatic group (median S/CO, 3.4; IQR, 1.6–10.7) were significantly lower ($P = 0.005$) relative to the symptomatic group (median S/CO, 20.5; IQR, 5.8–38.2) in the acute phase. Of asymptomatic individuals, 93.3% (28/30) and 81.1% (30/37) had reduction in IgG and neutralizing antibody levels, respectively, during the early convalescent phase, as compared to 96.8% (30/31) and 62.2% (23/37) of symptomatic patients. Forty percent of asymptomatic individuals became seronegative and 12.9% of the symptomatic group became negative for IgG in the early convalescent phase. *In addition, asymptomatic individuals exhibited lower levels of 18 pro- and anti-inflammatory cytokines. These data suggest that asymptomatic individuals had a weaker immune response to SARS-CoV-2 infection. The reduction in IgG and neutralizing antibody levels in the early convalescent phase might have implications for immunity strategy and serological surveys.* [**note: it is not clear what this Chinese study means. Why do asymptomatic people seem to mount a limited immune response despite being infected? Does this have implications for vaccine development? Is this just another strange facet of this virus? I have questions but no answers.**] https://www.nature.com/articles/s41591-020-0965-6

NEWLY REGISTERED CLINICAL TRIALS

- Will Check Tomorrow

CLINICAL TRIAL RESULTS

- Surprisingly, nothing today.

DRUG DEVELOPMENT

- Clinical development of the COVID-19 vaccine candidate ChAdOx1 nCoV-19, a replication-deficient simian adenoviral vector expressing the full-length SARS-CoV-2 spike (S) protein was initiated in April 2020 following non-human primate studies using a single immunisation. Here,

we compared the immunogenicity of one or two doses of ChAdOx1 nCoV-19 in both mice and pigs. Whilst a single dose induced antigen-specific antibody and T cells responses, a booster immunisation enhanced antibody responses, particularly in pigs, with a significant increase in SARS-CoV-2 neutralising titres. [**note: this is the UK Oxford vaccine animal data. It may be that two shots will be required for maximum immunity.**]
https://www.biorxiv.org/content/10.1101/2020.06.20.159715v1

- Cytokine release syndrome (CRS) is known to be a factor in morbidity and mortality associated with acute viral infections including those caused by filoviruses and coronaviruses. IL-6 has been implicated as a cytokine negatively associated with survival after filovirus infection. However, IL-6 has also been shown to be an important mediator of innate immunity, important for the host response to an acute viral infection. Clinical studies are now being conducted by various researchers to evaluate the possible role of IL-6 blockers to improve outcomes in critically ill patients with SARS-CoV-2 infection. Most of these studies involve the use of anti-IL-6R monoclonal antibodies (mAbs). We present data showing that direct neutralization of IL-6 with an anti-IL-6 mAb in a BALB/c Ebolavirus (EBOV) challenge model produced a statistically significant improvement in outcome compared with controls when administered within the first 24 hours of challenge and repeated every 72 hours. A similar effect was seen in mice treated with the same dose of anti-IL-6R mAb when the treatment was delayed 48 hrs post-challenge. These data suggest that direct neutralization of IL-6, early during the course of infection, may provide additional clinical benefits to IL-6 receptor blockade alone during treatment of patients with virus-induced CRS. These results may have implications for selecting and managing IL-6 blockade therapy for patients with COVID-19. [**note: this is an interesting animal study of two approaches to modulating IL-6 which is present at elevated levels in cytokine storm. Many of the researchers are from Flow Pharma Inc. who have also developed an experimental SARS-CoV-2 vaccine. I don't remember seeing any preprints of this but would recommend folks visit the website and look at the information. They are targeting the nucleocapsid protein whereas a lot of other vaccines target the spike protein.**]
https://www.biorxiv.org/content/10.1101/2020.06.20.162826v1
- Fighting the COVID-19 epidemic summons deep understanding of the way SARS-CoV-2 taps into its host cell metabolic resources. We describe here the singular metabolic background that creates a bottleneck constraining coronaviruses to evolve towards likely attenuation in the long term. Cytidine triphosphate (CTP) is at the crossroad of the biosynthetic processes that allow the virus to multiply. This is because CTP is in demand for three essential steps. It is a building block of the virus genome, it is required for synthesis of the cytosine-based liponucleotide precursors of the viral envelope and, finally, it is a critical building block of the host transfer RNAs synthesis. The CCA 3'-end of all the transfer RNAs required to translate the RNA genome and further transcripts into the proteins used to build active virus copies is not coded in the human genome. It must be synthesized de novo from CTP and ATP. Furthermore, intermediary metabolism is built on compulsory steps of synthesis and salvage of cytosine-based metabolites via uridine triphosphate (UTP) that keep limiting CTP availability. As a consequence, accidental replication errors tend to replace cytosine by uracil in the genome, unless recombination events allow the sequence to return to its ancestral sequences. We document some of the consequences of this situation in the function of viral proteins. We also highlight and provide a raison d'etre to viperin, an enzyme of innate antiviral immunity, which synthesizes 3'-deoxy-3',4'-didehydro-CTP

(ddhCTP) as an extremely efficient antiviral nucleotide. [**note: well, I had to brush up on my intermediary metabolism to grasp where these researchers were heading. It is an interesting paper that focuses on cytidine triphosphate levels as a key to viral multiplication. It is not clear what the implications are for drug design and maybe if I read the paper several more times, I will figure it out. For those of you who want to brush up on nucleotide metabolism, there are some very good graphics here.**]
https://www.biorxiv.org/content/10.1101/2020.06.20.162933v1

- The Coronavirus disease 19 (COVID-19) pandemic has been ongoing since its onset in late November 2019 in Wuhan, China. To date, the SARS-CoV-2 virus has infected more than 8 million people worldwide and killed over 5% of them. Efforts are being made all over the world to control the spread of the disease and most importantly to develop a vaccine. Understanding the genetic evolution of the virus, its geographic characteristics and stability is particularly important for developing a universal vaccine covering all circulating strains of SARS-CoV-2 and for predicting its efficacy. In this perspective, we analyzed the sequences of 30,983 complete genomes from 80 countries located in six geographical zones (Africa, Asia, Europe, North & South America, and Oceania) isolated from December 24, 2019 to May 13, 2020, and compared them to the reference genome. Our in-depth analysis revealed the presence of 3,206 variant sites compared to the reference Wuhan-Hu-1 genome, with a distribution that is largely uniform over all continents. Remarkably, a low frequency of recurrent mutations was observed; only 182 mutations (5.67%) had a prevalence greater than 1%. Nevertheless, fourteen hotspot mutations (> 10%) were identified at different locations, seven at the ORF1ab gene (in regions coding for nsp2, nsp3, nsp6, nsp12, nsp13, nsp14 and nsp15), three in the nucleocapsid protein, one in the spike protein, one in orf3a, and one in orf8. Moreover, 35 non-synonymous mutations were identified in the receptor-binding domain (RBD) of the spike protein with a low prevalence (<1%) across all genomes, of which only four could potentially enhance the binding of the SARS-CoV-2 spike protein to the human receptor ACE2. These results along with the phylogenetic analysis demonstrate that the virus does not have a significant divergence at the protein level compared to the reference both among and within different geographical areas. *Unlike the influenza virus or HIV viruses, the slow rate of mutation of SARS-CoV-2 makes the potential of developing an effective global vaccine very likely* [**note: this is not an earthshaking paper and others have looked at mutation rates. However, I think it is the first paper from a Moroccan team of scientists and as such should be cited! We are all in this together.**]
https://www.biorxiv.org/content/10.1101/2020.06.20.163188v1
- Vaccines are urgently needed to combat the global coronavirus disease 2019 (COVID 19) pandemic, and testing of candidate vaccines in an appropriate non-human primate (NHP) model is a critical step in the process. Infection of African green monkeys (AGM) with a low passage human isolate of SARS-CoV-2 by aerosol or mucosal exposure resulted in mild clinical infection with a transient decrease in lung tidal volume. Imaging with human clinical-grade 18F-fluoro-2-deoxy-D-glucose positron emission tomography (18F-FDG PET) co-registered with computed tomography (CT) revealed pulmonary lesions at 4 days post-infection (dpi) that resolved over time. Infectious virus was shed from both respiratory and gastrointestinal (GI) tracts in all animals in a biphasic manner, first between 2-7 dpi followed by a recrudescence at 14-21 dpi. Viral RNA (vRNA) was found throughout both respiratory and gastrointestinal systems at necropsy with higher levels of vRNA found within the GI tract tissues. All animals seroconverted

simultaneously for IgM and IgG, which has also been documented in human COVID-19 cases. Young AGM represent an excellent species to study mild/subclinical COVID-19 disease and have shed light on unknown aspects of long-term virus shedding. They are ideally suited for preclinical evaluation of candidate vaccines and therapeutic interventions. [**note: another animal model for vaccine and drug development testing.**]
https://www.biorxiv.org/content/10.1101/2020.06.20.137687v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

2020-06-23

A couple of weeks ago I featured the Manhattan Transfer singing 'The Boy from New York City'. It is such a fave of mine that I just had to link to another recording. Here is Janis Siegel at Indiana University with two of their vocal jazz ensembles in an up tempo version: https://www.youtube.com/watch?v=7EcXR8pUImo I did my graduate work at IU and some of my best memories came from all the wonderful concerts and recitals from various groups at the IU school of music!

Derek Lowe on antibody persistence. This was in the Nature paper I linked to yesterday.

It's science Tuesday for many newspapers and the New York Times has some excellent COVID-19 pieces today. Copper is not likely to be a treatment panacea. I guess I better donate my copper bracelet. Want to know about masks? Here is an interesting interview with aerosol scientist Linsey Marr who interestingly eschews N95 masks, preferring plain old cotton ones (those are also my mask of choice as loyal readers know from my fashionista picture). If you wear a mask while exercising here are some good tips. Health columnist, Jane Brody, weighs in on vaccine development.

The Washington Post has more on the disturbing trend of harassing public health officials. Some of my loyal readers are or have been very active in this community and undoubtedly share my outrage at what has taken place!

Speaking of public health researchers, JAMA has this good piece on a possible mitigation tool for businesses; well worth reading!

The Lancet has a good commentary on the use of aerosolized medicines at home in the pandemic age. Many asthmatics and COPD sufferers rely on inhaled corticosteroids for treatment.

This STAT piece argues against vaccine human challenge trials. I am also on this side of the fence and believe there is enough knowledge at this point to look at neutralizing antibody profiles from test subjects to gauge vaccine potency.

Sanofi are accelerating their vaccine development timeline.

Tuesday always sees lots of preprints!! Chinese researchers and clinicians seem not to have given up on HCQ as a treatment modality

MODELING

- Residential colleges and universities face unique challenges in providing in-person instruction during theCOVID-19 pandemic. Administrators are currently faced with decisions about whether to open during the pandemic and what modifications of their normal operations might be necessary to protect students,faculty and staff. There is little information, however, on what measures are likely to be most effective and whether existing interventions could contain the spread of an outbreak on campus. We develop a full-scale stochastic agent-based model to determine whether in-person instruction could safely continue during the pandemic and evaluate the necessity of various interventions. Simulation results indicate that large scale randomized testing, contact-tracing, and quarantining are important components of a successful strategy for containing campus outbreaks. High test specificity is critical for keeping the size of the quarantine population manageable. Moving the largest classes online is also crucial for controlling both the size of outbreaks and the number of students in quarantine. Increased residential exposure can significantly impact the size of an outbreak, but it is likely more important to control non-residential social exposure among students. Finally, necessarily high quarantine rates even in controlled outbreak simply significant absenteeism, indicating a need to plan for remote instruction of quarantined students [**note: paging Mitch Daniels of Purdue and all the other university presidents; here is must reading for you!!**] https://arxiv.org/pdf/2006.03175.pdf
- Four endemic human coronaviruses (HCoVs) are commonly associated with acute respiratory infection in humans but immune responses to these 'common cold' viruses remain incompletely understood. Moreover, there is evidence emerging from independent studies which suggests that endemic HCoVs can induce broadly cross-reactive T cell responses and may thereby affect clinical outcomes of acute infections with the phylogenetically related epidemic viruses, namely MERS-CoV and SARS-CoV-2. Here we report a comprehensive retrospective analysis of CoV-specific antibody specificities in a large number of samples from children and adults using Phage-Immunoprecipitation Sequencing (PhIP-Seq). We estimate the seroprevalence for endemic HCoVs to range from ~4% to ~27% depending on species and cohort. Most importantly, we identified a large number of novel linear B cell epitopes of HCoV proteins and demonstrate that antibody repertoires against endemic HCoVs are qualitatively different in children in comparison to the general adult population and healthy adult blood bank donors. We show that anti-HCoV IgG specificities more frequently found among children target functionally important and structurally conserved regions of the HCoV spike and nucleocapsid proteins and some antibody specificities are broadly cross-reactive with peptides of epidemic human and non-human coronavirus isolates. Our findings shed light on the humoral immune responses to natural infection with endemic HCoVs and may have important implications for understanding of the highly variable clinical outcomes of human coronavirus infections, for the development of prophylactic or therapeutic monoclonal antibodies and vaccine design. [**note: this is an interesting finding from Qatari scientists on the differential antibody profiles in adults and children.**] https://www.biorxiv.org/content/10.1101/2020.06.21.163394v1
- The SARS-CoV-2 virus has a marked affinity to the human angiotensin-converting enzyme 2 (ACE2) receptor. The ACE2 locus is highly polymorphic, and this genetic variability may affect

COVID-19 disease severity. Here, we analyzed associations between polymorphisms in the ACE2 locus and COVID-19 severity in 62 patients found to be COVID-19 positive by polymerase chain reaction. Of these patients, 23 required hospitalization due to COVID-19 infection. Of 61 ACE2 single nucleotide polymorphisms (SNPs) genotyped in this patient cohort, 10 were significantly associated with tissue expression of ACE2. Logistic regression adjusted for age and sex identified six of these ten SNPs to be significantly associated with hospitalization. These results provide preliminary evidence of a link between the ACE2 genotype and COVID-19 disease severity and suggest that the ACE2 genotype may inform COVID-19 risk stratification and need for more intense therapy. [**note: more good genetic information on the ACE2 genotype and COVID-19 disease severity. Perhaps this can be another screening tool.**]
https://www.medrxiv.org/content/10.1101/2020.06.18.20135152v1

- As the current COVID-19 pandemic continues to impact countries around the globe, refining our understanding of its transmission dynamics and the effectiveness of interventions is imperative. In particular, it is essential to obtain a firmer grasp on the effect of social distancing, potential individual-level heterogeneities in transmission such as age-specific infectivity, and impact of super-spreading. To this end, it is important to exploit multiple data streams that are becoming abundantly available during the pandemic. In this paper, we formulate an individual-level spatio-temporal mechanistic framework to statistically integrate case data with geo-location data and aggregate mobility data, enabling a more granular understanding of the transmission dynamics of COVID-19. We analyze reported cases from surveillance data, between March and early May 2020, in five (urban and rural) counties in the State of Georgia USA. We estimate natural history parameters of COVID-19 and infer unobserved quantities including infection times and transmission paths using Bayesian data-augmentation techniques. First, our results show that the overall median reproductive number was 2.88 (with 95% C.I. [1.85, 4.9]) before the state-wide shelter-in-place order issued in early April, and the effective reproductive number was reduced to below 1 about two weeks by the order. Super-spreading appears to be widespread across space and time, and it may have a particularly important role in driving the outbreak in the rural area and increasing importance towards later stages of outbreaks in both urban and rural settings. Overall, about 2% of cases may have directly infected 20% of all infections. We estimate that the infected children and younger adults (<60 years old) may be 2.38 [1.30, 3.51] times more transmissible than infected elderly (>=60), and the former may be the main driver of super-spreading. Through the synthesis of multiple data streams using our transmission modelling framework, our results enforce and improve our understanding of the natural history and transmission dynamics of COVID-19. More importantly, we reveal the roles of age-specific infectivity and characterize systematic variations and associated risk factors of super-spreading. These have important implications for the planning of relaxing social distancing and, more generally, designing optimal control measures [**note: nice analysis from Emory on super-spreaders**] https://www.medrxiv.org/content/10.1101/2020.06.20.20130476v2
- In the background of the current COVID-19 pandemic, serological tests are being used to assess past infection and immunity against SARS-CoV-2. This knowledge is paramount to determine the transmission dynamics of SARS-CoV-2 through the post pandemic period. Several individuals belonging to households with an index COVID-19 patient, reported symptoms of COVID-19 but discrepant serology results. Methods. Here we investigated the humoral and cellular immune responses against SARS-CoV-2 in seven families, including nine index patients and eight

contacts, who had evidence of serological discordances within the households. Ten unexposed healthy donors were enrolled as controls. Results. All index patients recovered from a mild COVID-19. They all developed anti-SARS-CoV-2 antibodies and a significant T cell response detectable up to 69 days after symptom onset. Six of the eight contacts reported COVID-19 symptoms within 1 to 7 days after the index patients but all were SARS-CoV-2 seronegative. Six out of eight contacts developed a SARS-CoV-2-specific T cell response against structural and/or accessory proteins that lasts up to 80 days post symptom onset suggesting a past SARS-CoV-2 infection. Conclusion. Exposure to SARS-CoV-2 can induce virus-specific T cell responses without seroconversion. T cell responses may be more sensitive indicators of SARS-Co-V-2 exposure than antibodies. Our results indicate that epidemiological data relying only on the detection of SARS-CoV-2 antibodies may lead to a substantial underestimation of prior exposure to the virus [**note: interesting stuff on immune response without seroconversion. We really need more information on this to understand why some people display clinical symptoms and positive virology testing but don't display neutralizing antibodies. Immunology can be damn tricky to understand!**] https://www.medrxiv.org/content/10.1101/2020.06.21.20132449v1

- We demonstrate that universal scaling behavior is observed in the current coronavirus (COVID-19) spread in various countries. We analyze the numbers of infected people in selected eleven countries (Japan, USA, Russia, Brazil, China, Italy, Indonesia, Spain,South Korea, UK, and Sweden). By using the double exponential function called the Gompertz function, $f_G(x)=\exp(-e^{-x})$, the number of infected people is well described as $N(t)=N_0\, f_G(\gamma(t-t_0))$, where N_0, γ and t_0 are the final total number of infected people, the damping rate of the infection probability and the peak time of $dN(t)/dt$, respectively. The scaled data of infected people in most of the analyzed countries are found to collapse onto a common scaling function $f_G(x)$ with $x=\gamma(t-t_0)$ in the range of $f_G(x) \pm 0.05$. The recently proposed indicator so-called the K value, the increasing rate of infected people in one week, is also found to show universal behavior. The mechanism for the Gompertz function to appear is discussed from the time dependence of the produced pion numbers in nucleus-nucleus collisions, which is also found to be described by the Gompertz function. [**note: yes, I know I promised to reduce the number of modeling studies but when the mysterious Gompertz function appeared in the title of a paper I just had to post this one!! I even pulled out my old statistics textbook to read up on it!**] https://www.medrxiv.org/content/10.1101/2020.06.18.20135210v1

NEWLY REGISTERED CLINICAL TRIALS

- I'll confess to binge (re)watching 'The Wire' yesterday and didn't get around to the tedious job of looking through the NLM clinical trial database. Before finding out what happens to Omar and Stringer Bell, I'll see if there are any new trials worthy of note!!

CLINICAL TRIAL RESULTS

- Background: Coronavirus disease 2019 (COVID-19) is associated with diffuse lung damage. Corticosteroids may modulate immune-mediated lung injury and reducing progression to respiratory failure and death. Methods: The Randomised Evaluation of COVID-19 therapy (RECOVERY) trial is a randomized, controlled, open-label, adaptive, platform trial comparing a range of possible treatments with usual care in patients hospitalized with COVID-19. We report the preliminary results for the comparison of dexamethasone 6 mg given once daily for up to

ten days vs. usual care alone. The primary outcome was 28-day mortality. Results: 2104 patients randomly allocated to receive dexamethasone were compared with 4321 patients concurrently allocated to usual care. Overall, 454 (21.6%) patients allocated dexamethasone and 1065 (24.6%) patients allocated usual care died within 28 days (age-adjusted rate ratio [RR] 0.83; 95% confidence interval [CI] 0.74 to 0.92; P<0.001). The proportional and absolute mortality rate reductions varied significantly depending on level of respiratory support at randomization (test for trend p<0.001): Dexamethasone reduced deaths by one-third in patients receiving invasive mechanical ventilation (29.0% vs. 40.7%, RR 0.65 [95% CI 0.51 to 0.82]; p<0.001), by one-fifth in patients receiving oxygen without invasive mechanical ventilation (21.5% vs. 25.0%, RR 0.80 [95% CI 0.70 to 0.92]; p=0.002), but did not reduce mortality in patients not receiving respiratory support at randomization (17.0% vs. 13.2%, RR 1.22 [95% CI 0.93 to 1.61]; p=0.14). Conclusions: In patients hospitalized with COVID-19, dexamethasone reduced 28-day mortality among those receiving invasive mechanical ventilation or oxygen at randomization, but not among patients not receiving respiratory support. [**note: there was some moaning and groaning when the results of the UK RECOVERY trial with dexamethasone were announced. Some scientists said they needed to see the data. Here it is!**]
https://www.medrxiv.org/content/10.1101/2020.06.22.20137273v1

- Drug repurposing may represent a rapid way to fill the urgent need for effective treatment. We evaluated the clinical utility of chloroquine and hydroxychloroquine in treating COVID-19. Forty-eight patients with moderate COVID-19 were randomized to oral treatment with chloroquine (1000 mg QD on Day 1, then 500 mg QD for 9 days; n=18), hydroxychloroquine (200 mg BID for 10 days; n=18), or control treatment (n=12). Adverse events were mild, except for one case of Grade 2 ALT elevation. Adverse events were more commonly observed in the chloroquine group (44.44%) and the hydroxychloroquine group (50.00%) than in the control group (16.67%). The chloroquine group achieved shorter time to clinical recovery (TTCR) than the control group (P=0.019). There was a trend toward reduced TTCR in the hydroxychloroquine group (P=0.049). The time to reach viral RNA negativity was significantly faster in the chloroquine group and the hydroxychloroquine group than in the control group (P=0.006 and P=0.010, respectively). The median numbers of days to reach RNA negativity in the chloroquine, hydroxychloroquine, and control groups was 2.5 (IQR: 2.0-3.8) days, 2.0 (IQR: 2.0-3.5) days, and 7.0 (IQR: 3.0-10.0) days, respectively. The chloroquine and hydroxychloroquine groups also showed trends toward improvement in the duration of hospitalization and findings on lung computerized tomography (CT). This study provides evidence that (hydroxy)chloroquine may be used effectively in treating moderate COVID-19 and supports larger trials. [**note: I guess as long at researchers are playing around with HCQ, I need to post abstracts. I have to ask the question, is a 48 patient clinical trial with two treatment arms even worthy of statistical analysis not to mention publication?**]
https://www.medrxiv.org/content/10.1101/2020.06.19.20136093v1
- Here we report our experience of using convalescent plasma at a tertiary care center in a mid-size, midwestern city that did not experience an overwhelming patient surge. Methods Hospitalized COVID-19 patients categorized as having Severe or Life-Threatening disease according to the Mayo Clinic Emergency Access Protocol were screened, consented, and treated with convalescent plasma collected from local donors recovered from COVID-19 infection. Clinical data and outcomes were collected retrospectively. Results 31 patients were treated, 16 severe patients and 15 life-threatened patients. Overall mortality was 27% (4/31) but only

patients with life-threatening disease died. 94% of transfused patients with severe disease avoided escalation to ICU care and mechanical ventilation. 67% of patients with life-threatening disease were able to be extubated. Most transfused patients had a rapid decrease in their respiratory support requirements on or about day 7 following convalescent plasma transfusion. Conclusion Our results demonstrate that convalescent plasma is associated with reducing ventilatory requirements in patients with both severe and life-threatening disease, but appears to be most beneficial when administered early in the course of disease when patients meet the criteria for severe illness. [**note: small number of patients treated with convalescent plasma. Looks like early treatment is better. It will be interesting to see if immune plasma can be used in conjunction with tocilizumab or dexamethasone to improve survival.**]
https://www.medrxiv.org/content/10.1101/2020.06.19.20135830v1

DRUG DEVELOPMENT

- Developing therapeutics against SARS-CoV-2 could be guided by the distribution of epitopes, not only on the receptor binding domain (RBD) of the Spike (S) protein, but also across the full Spike (S) protein. We isolated and characterized monoclonal antibodies (mAbs) from ten convalescent COVID-19 patients. Three mAbs showed neutralizing activities against authentic SARS-CoV-2. An mAb, named 4A8, exhibits high neutralization potency against both authentic and pseudotyped SARS-CoV-2, but does not bind the RBD. We defined the epitope of 4A8 as the N terminal domain (NTD) of the S protein by determining its cryo-EM structure in complex with the S protein to an overall resolution of 3.1 Angstrom and local resolution of 3.3 Angstrom for the 4A8-NTD interface. This points to the NTD as a promising target for therapeutic mAbs against COVID-19. [**note: it's hard to keep track of when preprints get published. I think I linked to this research earlier but here is the published version.**]
https://science.sciencemag.org/content/early/2020/06/19/science.abc6952
- COVID-19, the disease caused by SARS-CoV-2, was declared a pandemic by the World Health Organization (WHO) in March 2020. While awaiting a vaccine, several antivirals are being used to manage the disease with limited success. To expand this arsenal, we screened 4 compound libraries: a United States Food and Drug Administration (FDA) approved drug library, an angiotensin converting enzyme-2 (ACE2) targeted compound library, a flavonoid compound library as well as a natural product library. Of the 121 compounds identified with activity against SARS-CoV-2, 7 were shortlisted for validation. We show for the first time that the active form of Vitamin D, calcitriol, exhibits significant potent activity against SARS-CoV-2. This finding paves the way for consideration of host-directed therapies for ring prophylaxis of contacts of SARS-CoV-2 patients. [**note: looks like it might be time to dip into my wife's stash of Vitamin D!!!**]
https://www.biorxiv.org/content/10.1101/2020.06.21.162396v1
- The pandemic coronavirus COVID-19 affected global health from the end of 2019 to 2020 and may challenge global health in the future. There have been reports of Chloroquine (CQ) and Hydroxychloroquine (HCQ) used in clinical treatment. In our study, we used CCK-8 stain, flow cytometry and immunofluorescent stain to evaluated the toxity and autophagy of CQ and HCQ respectively on ACE2 high expressed HEK293T cells (ACE2hi cells). We further analysied the binding character of CQ and HCQ to ACE2 by molecular docking and surface plasmon resonance (SPR) assays and molecule docking, COVID-19 spike pseudotype virus was also taken to investigate the suppression viropexis effect of CQ and HCQ. Results showed that both CQ and

HCQ HCQ is slightly more toxic to ACE2hi cells than CQ, both CQ and HCQ could bind to ACE2, and they also exhibit equivalent suppression effect for the entrance of COVID-19 Spike pseudotype virus into ACE2hi cells. Our finding provide theoretical and experimental basis for the clinical treatment of CQ and HCQ for COVID-19. [**note: since everyone seems to be discontinuing HCQ trials, what relevance does this paper have? I wonder if this ever gets published.**] https://www.biorxiv.org/content/10.1101/2020.06.22.164665v1

- Presentation of antigenic peptides by MHCI is central to cellular immune responses against viral pathogens. While adaptive immune responses versus SARS-CoV-2 can be of critical importance to both recovery and vaccine efficacy, how protein antigens from this pathogen are processed to generate antigenic peptides is largely unknown. Here, we analyzed the proteolytic processing of overlapping precursor peptides spanning the entire sequence of the S1 spike glycoprotein of SARS-CoV-2, by three key enzymes that generate antigenic peptides, aminopeptidases ERAP1, ERAP2 and IRAP. All enzymes generated shorter peptides with sequences suitable for binding onto HLA alleles, but with distinct specificity fingerprints. ERAP1 was the most efficient in generating peptides 8-11 residues long, the optimal length for HLA binding, while IRAP was the least efficient. The combination of ERAP1 with ERAP2 greatly limited the variability of peptide sequences produced. Less than 7% of computationally predicted epitopes were found to be produced experimentally, suggesting that aminopeptidase processing may constitute a significant filter to epitope presentation. These experimentally generated putative epitopes could be prioritized for SARS-CoV-2 immunogenicity studies and vaccine design. We furthermore propose that this in vitro trimming approach could constitute a general filtering method to enhance the prediction robustness for viral antigenic epitopes. [**note: interesting approach to identify antigenic epitopes.**] https://www.biorxiv.org/content/10.1101/2020.06.22.164681v1

DIAGNOSTIC DEVELOPMENT

- However, point-of-care (POC) testing in places such as emergency units, outpatient clinics, airport security points or the entrance of any public building is a major challenge. The need for thermal cycling and nucleic acid isolation hampers the use of standard PCR-based methods for this purpose. Methods: To avoid these obstacles, we tested PCR-independent methods for the detection of SARS-CoV-2 RNA from primary material (nasopharyngeal swabs) including loop-mediated isothermal amplification (LAMP) and specific high-sensitivity enzymatic reporter unlocking (SHERLOCK). Results: Whilst specificity of standard LAMP assays appears to be satisfactory, sensitivity does not reach the current gold-standard quantitative real-time polymerase chain reaction (qPCR) assays yet. We describe a novel multiplexed LAMP approach and validate its sensitivity on primary samples. This approach allows for fast and reliable identification of infected individuals. Primer optimization and multiplexing helps to increase sensitivity significantly. In addition, we directly compare and combine our novel LAMP assays with SHERLOCK. Conclusion: In summary, this approach reveals one-step multiplexed LAMP assays as a prime-option for the development of easy and cheap POC test kits. [**note: more good stuff on a point of care test by this joint German-American group. If you want to read a well written paper on new diagnostic approaches this is a good place to start.**] https://www.medrxiv.org/content/10.1101/2020.06.18.20130377v1

>>

2020-06-24

Though my youthful infatuation with the operas of Richard Wagner have waned, I continue to find certain parts quite moving. One of these is Wotan's farewell from Die Walküre. This excerpt is from the famous, or infamous, depending on your view; I am of the former camp, Bayreuth 1976 production. Donald McIntyre and Gwyneth Jones are the singers under the direction of Pierre Boulez: https://www.youtube.com/watch?v=4tDP-K1dQ-M as a Tuesday Twofer (obviously it is Wednesday but the selection was made on Tuesday and try to rhyme Twofer with Wednesday!), here is Gwyneth Jones in the immolation scene from the same production but the final opera of the tetralogy Götteredämmerung: https://www.youtube.com/watch?v=2JgMt8GWdyU Damn, I may need to interrupt my binging of 'The Wire' and rewatch this Ring Cycle!!!

Dr. Fauci and other public health officials testified to the House Commerce Committee yesterday on the COVID-19 pandemic. A number of issues were covered as the Washington Post and The New York Times carefully summarize. The lead editorial in the print edition of the Washington Post is on my favorite topic, yes you guessed it, masks!!

Here is a JAMA article on community containment strategies in Shenzhen, China. It's useful reading but likely cannot be successfully implemented in the US at this point in time.

Inside story on the development of a contact tracing app courtesy of STAT. Will the US or states adopt this technology? That is the critical question.

More than 35,000 new coronavirus cases were identified across the United States on Tuesday, according to a New York Times database, the highest single-day total since late April and the third-highest total of any day of the pandemic.

I have started a new category on VIRUS BIOCHEMISTRY!!! Lots of interesting stuff is happening in research and it will be useful to better track them.

MODELING

- Despite various levels of preventive measures, in 2020 many countries have suffered severely from the coronavirus 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. We show that population heterogeneity can significantly impact disease-induced immunity as the proportion infected in groups with the highest contact rates is greater than in groups with low contact rates. We estimate that if R_0 = 2.5 in an age-structured community with mixing rates fitted to social activity then the disease-induced herd immunity level can be around 43%, which is substantially less than the classical herd immunity level of 60% obtained through homogeneous immunization of the population. Our estimates should be interpreted as an illustration of how population heterogeneity affects herd immunity, rather than an exact value or even a best estimate. [**note: this is not a preprint but a publication in a mainstream journal and warrants mention here.**] https://science.sciencemag.org/content/early/2020/06/22/science.abc6810

- Background: SARS-CoV-2 may pose an occupational health risk to health care workers, but the prevalence of infections in this population is unknown. We examined the seroprevalence of SARS-CoV-2 antibodies among health care workers at a large acute care hospital in Stockholm, Sweden. We determined correlations between seroprevalence, self-reported symptoms and occupational exposure to SARS-CoV-2. Methods and findings: All employees at Danderyd Hospital (n=4375) were invited to participate in a cross-sectional study. 2149 employees from all hospital departments were enrolled in the study between April 14th and May 8th 2020. Study participants completed a questionnaire consisting of symptoms compatible with SARS-CoV-2 infection since January 2020 and occupational exposure to patients infected with SARS-CoV-2. IgG antibodies against SARS-CoV-2 were analyzed using a multiplex assay evaluated to have 99.4% sensitivity and 99.1% specificity. The over-all seroprevalence among 2149 participants was 19.1% (n=410). There was no difference in age or sex between seropositive and seronegative participants. The symptoms with the strongest correlation to seroprevalence were anosmia and ageusia, with odds ratios of 28.4 (p=2.02*10^-120) and 19.2 (p=1.67*10^-99) respectively. Seroprevalence was strongly associated with patient-related work (OR 2.9, p=4.24*10^-8), covid-19 patient contact (OR 1.43, p=0.003), and occupation as assisting nurse (OR 3.67, p=2.16*10^-9). Conclusion: These results demonstrate that anosmia and ageusia should be included in screening guidance and in the recommendations of self-isolation to reduce further spread of SARS-CoV-2. The results furthermore imply an occupational health risk for SARS-CoV-2 infection among hospital workers. Continued measures are warranted to assure healthcare worker safety and reduce transmission from health care settings to the community during the covid-19 outbreak. [**note: Sweden have taken their own road to deal with the SARS-CoV-2 pandemic. Here is a report on serological findings from healthcare workers at one hospital. Almost 20% positive finding for antibodies.**]
  https://www.medrxiv.org/content/10.1101/2020.06.22.20137646v1
- Identification of biomedical and socioeconomic predictors for the number of deaths by COVID-19 among countries will lead to the development of effective intervention. While previous multiple regression studies have identified several predictors, little is known for the effect of mask non-wearing rate on the number of COVID-19-related deaths possibly because the data is available for limited number of countries, which constricts the application of traditional multiple regression approach to screen a large number of potential predictors. In this study, we used the hypothesis-driven regression to test the effect of limited number of predictors based on the hypothesis that the mask non-wearing rate can predict the number of deaths to a large extent together with age and BMI, other relatively independent risk factors for hospitalized patients of COVID-19. The mask non-wearing rate, percentage of age ≥ 80 (male), and male BMI showed Spearman's correlations up to about 0.8, 0.7, and 0.6 with the number of deaths per million from 22 countries from mid-March to mid-June, respectively. The observed number of deaths per million were significantly correlated with the numbers predicted by the lasso regression model including four predictors, age ≥ 80 (male), male BMI, and mask non-wearing rates from mid-March and late April to early May (Pearson's coefficient = 0.918). The multiple linear regression models including the mask non-wearing rates, age, and obesity-related predictors explained up to 79% variation of the number of deaths per million. *Furthermore, 56.8% of the variation of mask non-wearing rate in mid-March, the strongest predictor of the number of deaths per million, was predicted by age ≥ 80 (male) and male BMI, suggesting the confounding*

role of these predictors. Although further verification is needed to identify causes of the national differences in COVID-19 mortality rates, these results highlight the importance of the mask, age, and BMI in predicting the COVID-19-related deaths, providing a useful strategy for future regression analyses that attempt to contribute to the mechanistic understanding of COVID-19. [**note: any paper that shows the usefulness of mask wearing will always get noted here!!! Of course this may be an application of statistics on an imperfect data set.**] https://www.medrxiv.org/content/10.1101/2020.06.22.20137745v1

- The prevalence of asymptomatic COVID-19 infections is largely unknown and may determine the course of future pandemic waves and the effectiveness of interventions. Using an epidemiological model fit to COVID-19 hospitalization counts from New York City, New York and Austin, Texas, we found that the *undocumented* attack rate in the first pandemic wave depends on the proportion of asymptomatic infections but not on the infectiousness of such individuals. Based on a recent report that 22.7% of New Yorkers are seropositive for SARS-CoV-2, we estimate that 56% (95% CI: 53-59%) of COVID-19 infections are asymptomatic. Given uncertainty in the case hospitalization rate, however, the asymptomatic proportion could be as low as 20% or as high as 80%. We find that at most 1.26% of the Austin population was infected by April 27, 2020 and conclude that immunity from undetected infections is unlikely to slow future pandemic spread in most US cities in the summer of 2020. [**note: this is a useful paper to read given the large apparent number of asymptomatic infections. An added plus that I noticed is one of the funders of the research is Titos Handmade Vodka along with NIH and NSF. Pretty cool, though I think handmade vodka to be highly overrated and expensive. I'll take small batch bourbon any day. If any of my readers want to buy me a gift, I am partial to Booker's!**] https://www.medrxiv.org/content/10.1101/2020.06.22.20137489v1
- Greece is a country with limited spread of SARS-CoV-2 and cumulative infection attack rate of 0.12% (95%CI 0.06%-0.26%). Health care workers (HCWs) are a well-recognized risk group for COVID-19. The study aimed to estimate the seroprevalence of antibodies to SARS-CoV-2 in two hospitals and assess potential risk factors. Hospital-1 was involved in the care of COVID-19 patients while hospital-2 was not. A validated, rapid, IgM/IgG antibody point-of care test was used. 1,495 individuals consented to participate (response rate 77%). The anti-SARS-CoV-2 weighted prevalence was 1.07% (95%CI 0.37-1.78) overall and 0.44% (95%CI 0.12-1.13) and 2.4% (95%CI 0.51-8.19) in hospital-1 and hospital-2, respectively. The overall, hospital-1, and hospital-2 seroprevalence was 9, 3 and 20 times higher than the estimated infection attack rate in general population, respectively. Suboptimal use of personal protective equipment was noted in both hospitals. These data have implications for the preparedness of a second wave of COVID-19 epidemic. [**note: seroprevalence among healthcare workers in Greece, a country that had a low rate of infection. I wonder if the Mediterranean diet has any positive protection.**] https://www.medrxiv.org/content/10.1101/2020.06.23.20137620v1

NEWLY REGISTERED CLINICAL TRIALS

- Surprisingly, no clinical trials for new therapeutics have been registered.

CLINICAL TRIAL RESULTS

- In addition to the overwhelming lung inflammation that prevails in COVID-19, hypercoagulation and thrombosis contribute to the lethality of subjects infected with severe acute respiratory

syndrome coronavirus 2 (SARS-CoV-2). Platelets are chiefly implicated in thrombosis. Moreover, they can interact with viruses and are an important source of inflammatory mediators. While a lower platelet count is associated with severity and mortality, little is known about platelet function during COVID-19. Objective: To evaluate the contribution of platelets to inflammation and thrombosis in COVID-19 patients. Methods and Results: We document the presence of SARS-CoV-2 RNA in platelets of COVID-19 patients. Exhaustive assessment of cytokines in plasma and in platelets revealed the modulation of platelet-associated cytokine levels in COVID-19, pointing to a direct contribution of platelets to the plasmatic cytokine load. Moreover, we demonstrate that platelets release their alpha- and dense-granule contents and phosphatidylserine-exposing extracellular vesicles. Functionally, platelets were hyperactivated in COVID-19 subjects, with aggregation occurring at suboptimal thrombin concentrations. Furthermore, platelets adhered more efficiently onto collagen-coated surfaces under flow conditions. Conclusions: These data suggest that platelets could participate in the dissemination of SARS-CoV-2 and in the overwhelming thrombo-inflammation observed in COVID-19. Thus, blockade of platelet activation pathways may improve outcomes in this disease. [**note: this Canadian/Moroccan team shows the possible involvement of platelets in COVID-19. More work is needed to confirm this.]**
https://www.medrxiv.org/content/10.1101/2020.06.23.20137596v1

- Hydroxychloroquine(HCQ) has been widely used to treat SARS-CoV-2 infection however HCQ pharmacokinetics in this condition have not been studied in non-critical care patient groups. Here we report the serum concentrations of HCQ in a small cohort of patients treated with HCQ as part of the RECOVERY trial. [**note: from the large UK trial. Interestingly, HCQ levels were lower than investigators thought would be the case based on other non-COVID-19 use data.**]
https://www.medrxiv.org/content/10.1101/2020.06.23.20137992v1

DRUG DEVELOPMENT

- Coronaviruses (CoVs) infect a wide range of animals and birds. Their tropism is primarily determined by the ability of the spike (S) protein to bind to a host cell surface receptor. The rapid outbreak of emerging novel coronavirus, SARS-CoV 2 in China inculcates the need for the development of hasty and effective intervention strategies. Medicinal plants and natural compounds have been traditionally used to treat viral infections. Here, we generated VSV based pseudotyped viruses (pvs) of SARS-, MERS-, and SARS-2 CoVs to screen entry inhibitors from natural products. In the first series of experiments, we demonstrated that pseudotyped viruses specifically bind on their receptors and enter into the cells. SARS and MERS polyclonal antibodies neutralize SARSpv and SARS-2pv, and MERSpv respectively. Incubation of soluble ACE2 inhibited entry of SARS and SARS-2 pvs but not MERSpv. In addition, expression of ACE2 and DPP4 in non-permissive BHK21 cells enabled infection by SARSpv, SARS-2pv, and MERSpv respectively. Next, we showed the antiviral properties of known enveloped virus entry inhibitors, Spirulina and Green tea extracts against CoVpvs. SARSpv, MERSpv, and SARS-2pv entry were blocked with higher efficiency when preincubated with either green tea or spirulina extracts. Green tea provided a better inhibitory effect than the spirulina extracts by binding to the S1 domain of spike and blocking the interaction of spike with its receptor. Further studies are required to understand the exact mechanism of viral inhibition. In summary, we demonstrate that pseudotyped virus is an ideal tool for screening viral entry inhibitors.

Moreover, spirulina and green tea could be promising antiviral agents against emerging viruses. [**note to self: check on the supply of green tea and put it on the shopping list for tomorrow!! Wow, I didn't know about the health effects of Spirulina!! I'll have to get some of this stuff as well. Thanks to these Indian researchers for the tip.**]
https://www.biorxiv.org/content/10.1101/2020.06.20.162701v1

- much research has gone into the development of pre-clinical assays for the discovery of new or repurposing of FDA-approved therapies. Preventing viral entry into a host cell would be an effective antiviral strategy. One mechanism for SARS-CoV-2 entry occurs when the spike protein on the surface of SARS-CoV-2 binds to an ACE2 receptor followed by cleavage at two cut sites ('priming') that causes a conformational change allowing for viral and host membrane fusion. This fusion event is proceeded by release of viral RNA within the host cell. TMPRSS2 has an extracellular protease domain capable of cleaving the spike protein to initiate membrane fusion. Additionally, knock-out studies in mice have demonstrated reduced infection in the absence of TMPRSS2 with no detectable physiological impact; thus, TMPRSS2 is an attractive target for therapeutic development. A validated inhibitor of TMPRSS2 protease activity would be a valuable tool for studying the impact TMPRSS2 has in viral entry and potentially be an effective antiviral therapeutic. To enable inhibitor discovery and profiling of FDA-approved therapeutics, we describe an assay for the biochemical screening of recombinant TMPRSS2 suitable for high throughput application. We demonstrate effectiveness to quantify inhibition down to subnanomolar concentrations by assessing the inhibition of camostat, nafamostat and gabexate, clinically approved agents in Japan for pancreatitis due to their inhibition of trypsin-like proteases. Nafamostat and camostat are currently in clinical trials against COVID19. The rank order potency for the three inhibitors is: nafamostat (IC50 = 0.27 nM), camostat (IC50 = 6.2 nM) and gabexate (IC50 = 130 nM). Further profiling of these three inhibitors against a panel of proteases provides insight into selectivity and potency. [**note: I have not seen any data on the first two drugs that are in trials.**]
https://www.biorxiv.org/content/10.1101/2020.06.23.167544v1
- A high-throughput platform would greatly facilitate COVID-19 serological testing and antiviral screening. Here we report a nanoluciferase SARS-CoV-2 (SARS-CoV-2-Nluc) that is genetically stable and replicates similarly to the wild-type virus in cell culture. We demonstrate that the optimized reporter virus assay in Vero E6 cells can be used to measure neutralizing antibody activity in patient sera and produces results in concordance with a plaque reduction neutralization test (PRNT). Compared with the low-throughput PRNT (3 days), the SARS-CoV-2-Nluc assay has substantially shorter turnaround time (5 hours) with a high-throughput testing capacity. Thus, the assay can be readily deployed for large-scale vaccine evaluation and neutralizing antibody testing in humans. Additionally, we developed a high-throughput antiviral assay using SARS-CoV-2-Nluc infection of A549 cells expressing human ACE2 receptor (A549-hACE2). When tested against this reporter virus, remdesivir exhibited substantially more potent activity in A549-hACE2 cells compared to Vero E6 cells (EC50 0.115 vs 1.28 micromolar), while this difference was not observed for chloroquine (EC50 1.32 vs 3.52 micromolar), underscoring the importance of selecting appropriate cells for antiviral testing. Using the optimized SARS-CoV-2-Nluc assay, we evaluated a collection of approved and investigational antivirals and other anti-infective drugs. Nelfinavir, rupintrivir, and cobicistat were identified as the most selective inhibitors of SARS-CoV-2-Nluc (EC50 0.77 to 2.74 micromolar). In contrast, most of the clinically

approved antivirals, including tenofovir alafenamide, emtricitabine, sofosbuvir, ledipasvir, and velpatasvir were inactive at concentrations up to 10 micromolar. Collectively, this high-throughput platform represents a reliable tool for rapid neutralization testing and antiviral screening for SARS-CoV-2. [**note: this is another good assay system from Univ of Texas reasearchers that can be used for several different purposes. Gilead authors looked at using it for antiviral assays.**] https://www.biorxiv.org/content/10.1101/2020.06.22.165712v1

- The coronavirus SARS-CoV-2 is the cause of the ongoing COVID-19 pandemic. Therapeutic neutralizing antibodies constitute a key short-to-medium term approach to tackle COVID-19. However, traditional antibody production is hampered by long development times and costly production. Here, we report the rapid isolation and characterization of nanobodies from a synthetic library, known as sybodies (Sb), that target the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Several binders with low nanomolar affinities and efficient neutralization activity were identified of which Sb23 displayed high affinity and neutralized pseudovirus with an IC50 of 0.6 ug/ml. A cryo-EM structure of the spike bound to Sb23 showed that Sb23 binds competitively in the ACE2 binding site. Furthermore, the cryo-EM reconstruction revealed a novel conformation of the spike where two RBDs are in the 'up' ACE2-binding conformation. The combined approach represents an alternative, fast workflow to select binders with neutralizing activity against newly emerging viruses. [**note: this is the second paper that I've linked to on nanobodies. This is also from Karolinska researchers.**] https://www.biorxiv.org/content/10.1101/2020.06.23.165415v1
- Background. The COVID-19 pandemic goes along with increased mortality from acute respiratory disease, and measures to limit the spread of the infection go along with increased risk of vitamin D deficiency, especially among high risk groups. It has been suggested that vitamin D3 supplementation might help to reduce respiratory disease mortality. Methods. We assessed the prevalence of vitamin D insufficiency and deficiency, defined by 25(OH)D blood levels of 30-50 and <30 nmol/L, respectively, and their association with mortality from respiratory diseases during 15 years of follow-up in a cohort of 9,548 adults aged 50-75 years from Saarland, Germany. Results. Vitamin D insufficiency and deficiency were common (44% and 15%, respectively). Compared to sufficient vitamin D status, respiratory disease mortality was 2.1 (95%-CI 1.3-3.2)- and 3.0 (95%-CI 1.8-5.2)-fold increased, respectively. Although significant increases were seen in both women and men, they were much stronger among women, with 8.5 (95% CI 2.4-30.1) and 2.3 (95% CI 1.1-4.4)-fold increase of respiratory disease mortality in case of vitamin D deficiency among women and men, respectively (p-value for interaction =0.041). Overall, 41% (95% CI 20%-58%) of respiratory disease mortality was statistically attributable to vitamin D insufficiency or deficiency. Conclusion. Vitamin D insufficiency and deficiency are common and account for a large proportion of respiratory disease mortality in older adults, supporting suggestions that vitamin D3 supplementation might make a major contribution to limit the burden of the COVID-19 pandemic, particularly among women. [**note: more info on Vitamin D deficiency and COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.06.22.20137299v1 and more here: https://www.medrxiv.org/content/10.1101/2020.06.21.20136903v1

VIRUS BIOCHEMISTRY

- The Spike protein of the novel coronavirus SARS-CoV2 contains an insertion 680SPRRAR↓SV687 forming a cleavage motif RxxR for furin-like enzymes at the boundary of S1/S2 subunits. Cleavage at S1/S2 is important for efficient viral entry into target cells. The insertion is absent in other CoV-s of the same clade, including SARS-CoV1 that caused the 2003 outbreak. However, an analogous insertion was present in the Spike protein of the more distant Middle East Respiratory Syndrome coronavirus MERS-CoV. We show that a crucial third arginine at the left middle position, comprising a motif RRxR is required for furin recognition in vitro, while the general motif RxxR in common with MERS-CoV is not sufficient for cleavage. Further, we describe a surprising finding that the two serines at the edges of the insert SPRRAR↓SV can be efficiently phosphorylated by proline-directed and basophilic protein kinases. Both phosphorylations switch off furin's ability to cleave the site. Although phospho-regulation of secreted proteins is still poorly understood, further studies, supported by a recent report of ten in vivo phosphorylated sites in the Spike protein of SARS-CoV2, could potentially uncover important novel regulatory mechanisms for SARS-CoV2. [**note: first entry into this new category!**] https://www.biorxiv.org/content/10.1101/2020.06.23.166900v1
- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the COVID19 pandemic, is a highly pathogenic β-coronavirus. As other coronaviruses, SARS-CoV-2 is enveloped and remodels intracellular membranes for genome replication and assembly. Here, we report critical insights into the budding mechanism of the virus and provide structural details of virions and virus induced double-membrane vesicles by in situ cryo-electron tomography. We directly visualized double-stranded RNA within double-membrane vesicles, forming a loosely organized network with frequent RNA branching consistent with template-directed RNA synthesis intermediates. Our data indicate that membrane bending is orchestrated by the spike trimer and viral ribonucleoprotein complex recruitment into virion budding sites, suggesting the synergistic interplay of both viral components as a possible drug target for intervention. [**note: a good look at the structure and replication of SARS-CoV-2**] https://www.biorxiv.org/content/10.1101/2020.06.23.167064v1
- SARS-CoV-2 is a once-in-a-century pandemic, having emerged suddenly as a highly infectious viral pathogen. Previous phylogenetic analyses show its closest known evolutionary relative to be a virus isolated from bats (RaTG13), with a common assumption that SARS-CoV-2 evolved from a zoonotic ancestor via recent genetic changes (likely in the Spike protein receptor binding domain, or RBD) that enabled it to infect humans. We used detailed phylogenetic analysis, ancestral sequence reconstruction, and molecular dynamics simulations to examine the Spike-RBD functional evolution, finding to our surprise that it has likely possessed high affinity for human cell targets since at least 2013. [**note: interesting phylogenetic analysis of the Spike protein.**] https://www.biorxiv.org/content/10.1101/2020.06.22.165787v1
- Cell surface receptor engagement is a critical aspect of viral infection. This paper compares the dynamics of virus-receptor interactions for SARS-CoV (CoV1) and CoV2. At low (endosomal) pH, the binding free energy landscape of CoV1 and CoV2 interactions with the angiotensin-converting enzyme 2 (ACE2) receptor is almost the same. However, at neutral pH the landscape is different due to the loss of a pH-switch (His445Lys) in the receptor binding domain (RBD) of CoV2 relative to CoV1. Namely, CoV1 stabilizes a transition state above the bound state. In situations where small external strains are applied by, say, shear flow in the respiratory system, the off rate of the viral particle is enhanced. As a result, CoV1 virions are expected to detach

from cell surfaces in time scales that are much faster than the time needed for other receptors to reach out and stabilize virus attachment. On the other hand, the loss of this pH-switch, which sequence alignments show is unique to CoV2, eliminates the transition state and allows the virus to stay bound to the ACE2 receptor for time scales compatible with the recruitment of additional ACE2 receptors diffusing in the cell membrane. This has important implications for viral infection and its pathology. CoV1 does not trigger high infectivity in the nasal area because it either rapidly drifts down the respiratory tract or is exhaled. By contrast, this novel mutation in CoV2 should not only retain the infection in the nasal cavity until ACE2-rich cells are sufficiently depleted, but also require fewer particles for infection. This mechanism explains observed longer incubation times, extended period of viral shedding, and higher rate of transmission. These considerations governing viral entry suggest that number of ACE2-rich cells in human nasal mucosa, which should be significantly smaller for children (and females relative to males), should also correlate with onset of viral load that could be a determinant of higher virus susceptibility. Critical implications for the development of new vaccines to combat current and future pandemics that, like SARS-CoV2, export evolutionarily successful strains via higher transmission rates by viral retention in nasal epithelium are also discussed. [**note: intriguing research comparing virus-receptor dynamics between SARS-CoV-1 and CoV-2. The change of one amino acid alters the pH switch and binding to ACE2. Damn, this is really interesting stuff.**] https://www.biorxiv.org/content/10.1101/2020.06.16.155457v1

DIAGNOSTIC DEVELOPMENT

- The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) uses the angiotensin converting enzyme (ACE)-2 as the host receptor for target cell entry. The extent and distribution of ACE-2 has been associated with the clinical symptoms of coronavirus disease (COVID)-19. Here we show by immunofluorescence analysis that the ACE2 is abundantly expressed in oral mucosa, particularly in the surface epithelial cells suggesting that these cells could represent sites of entry for SARS-CoV-2. Further, together with the reports on ACE2 ectodomain shedding, we discuss the rationale for the hypothesis that the ACE-2 measurement in saliva could be a marker for COVID-19 infection during early phase following SARS-CoV-2 exposure. [**note: this could fit into several categories and represents another potential marker.]** https://www.biorxiv.org/content/10.1101/2020.06.22.165035v1

>>

2020-06-25

Talk about strange coincidences!!! Two weeks ago I was thinking about what songs to begin to work on for what will probably be a pandemic recital. The Schumann Op. 39 songs to Eichendorff poems are one set that I have been working on over the past several months, but what to pair them with? I though that Beethoven's 'An de Ferne Geliebte' would be a good one as it was the first 'official' song cycle written. I was discussing this with my voice teacher last week and he agreed the Beethoven cycle would be a good one to do. Yesterday's New York Times had an article on two singers who are doing one on one telephone performances! How cool and only $40 for a performance! Here is the great German

baritone Dietrich Fischer-Dieskau singing the cycle accompanied by Gerald Moore: https://www.youtube.com/watch?time_continue=14&v=KOk7EWYbyqk

STAT has set up a very nice COVID-19 Tracker.

The New York Times has a very good graphical presentation of the spread of the virus in the US. Here is an update on testing from the Times. The Washington Post discusses the lag in mortality with the current surge in infections in several US states.

It is a short day for reading! After the deluge of papers yesterday, there is hardly any preprints worth reporting on. There are three clinical trial reports in major medical journals shown below.

MODELING

- Preprints of new models and strategies continue to be published but I don't see anything that runs counter to traditional public health approaches. Most papers agree that aggressive contact tracing is the way to go but this requires resources which to date are not available in the US.

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- In this randomized clinical trial of 105 patients, the rate of the primary clinical end point (clinical deterioration) was higher in the control group than in the colchicine group, and the time to clinical deterioration was shorter in the control group than in the colchicine arm. No difference was observed in the primary biochemical end point (high-sensitivity troponin concentration), but patients in the colchicine group had a smaller increase in dimerized plasma fragment D compared with patients in the control group. [**note: this is the first report from the colchicine trials that I have seen and it is from Greece. The patient size is small and it's not clear this is statistically reliable. Most of the patients were on other clinical trial drugs as well. There is a 6000 patient trial being run out of the Montreal Heart Institute with UCSF and NYU also involved.**] https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2767593 with accompanying commentary: https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2767588
- Of 1351 patients admitted, 544 (40%) had severe COVID-19 pneumonia and were included in the study. 57 (16%) of 365 patients in the standard care group needed mechanical ventilation, compared with 33 (18%) of 179 patients treated with tocilizumab (p=0·41; 16 [18%] of 88 patients treated intravenously and 17 [19%] of 91 patients treated subcutaneously). 73 (20%) patients in the standard care group died, compared with 13 (7%; p<0·0001) patients treated with tocilizumab (six [7%] treated intravenously and seven [8%] treated subcutaneously). After adjustment for sex, age, recruiting centre, duration of symptoms, and SOFA score, tocilizumab treatment was associated with a reduced risk of invasive mechanical ventilation or death (adjusted hazard ratio 0·61, 95% CI 0·40–0·92; p=0·020). 24 (13%) of 179 patients treated with

tocilizumab were diagnosed with new infections, versus 14 (4%) of 365 patients treated with standard of care alone (p<0·0001). [**note: this is from Italy and shows the benefits of tocilizumab. Clearly we need to see more data but this is similar to past findings.**] https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30173-9/fulltext and also a Lancet commentary: https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30210-1/fulltext

- We conducted a double-blind, randomized, placebo-controlled trial of intravenous remdesivir in adults hospitalized with Covid-19 with evidence of lower respiratory tract involvement. Patients were randomly assigned to receive either remdesivir (200 mg loading dose on day 1, followed by 100 mg daily for up to 9 additional days) or placebo for up to 10 days. The primary outcome was the time to recovery, defined by either discharge from the hospital or hospitalization for infection-control purposes only. A total of 1063 patients underwent randomization. The data and safety monitoring board recommended early unblinding of the results on the basis of findings from an analysis that showed shortened time to recovery in the remdesivir group. Preliminary results from the 1059 patients (538 assigned to remdesivir and 521 to placebo) with data available after randomization indicated that those who received remdesivir had a median recovery time of 11 days (95% confidence interval [CI], 9 to 12), as compared with 15 days (95% CI, 13 to 19) in those who received placebo (rate ratio for recovery, 1.32; 95% CI, 1.12 to 1.55; P<0.001). The Kaplan-Meier estimates of mortality by 14 days were 7.1% with remdesivir and 11.9% with placebo (hazard ratio for death, 0.70; 95% CI, 0.47 to 1.04). Serious adverse events were reported for 114 of the 541 patients in the remdesivir group who underwent randomization (21.1%) and 141 of the 522 patients in the placebo group who underwent randomization (27.0%). [**note: this is the remdesivir study that was mentioned in the news a couple of weeks ago.**] https://www.nejm.org/doi/full/10.1056/NEJMoa2007764 and an accompanying editorial: https://www.nejm.org/doi/full/10.1056/NEJMe2018715

DRUG DEVELOPMENT

- No news today.

VIRUS BIOCHEMISTRY

- No news today

DIAGNOSTIC DEVELOPMENT

- No news today

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

2020-06-26

I have always enjoyed Norah Jones. Her she is with Wynton Marsalis and his jazz ensemble singing the Ray Charles song, 'You Don't Know Me': https://www.youtube.com/watch?v=T2_9Rkk4f_g Here is the original audio recording by Charles: https://www.youtube.com/watch?v=jyTiyHI8g4s

Can air conditioning spread SARS-CoV-2? In my humble opinion, it depends. Room crowding is a more important variable and there is also the design of the HVAC system that is important, particularly as it relates to fresh air exchange.

Arizona presents a cautionary tale for what can go wrong in addressing the SARS-CoV-2 pandemic. Younger people appear to be driving the current outbreaks in sunbelt states.

The New York Times has a cautionary story about using dexamethasone for non-critical COVID-19 cases.

Derek Lowe editorializes (in a good way) on vaccine derangement syndrome.

Lisa Lerer of The New York Times talks with former CDC director, Tom Frieden. Good stuff.

Current CDC director, Robert Redfield, stated that the level of SARS-CoV-2 infections is likely 10 fold greater than reported (*hmmm…… I think I was saying this back in April*) at a press briefing on Thursday. He also noted that pregnant women might have greater susceptibility.

Medscape has a nice article on how to get a good night's sleep during the pandemic. It's worked for me!

Here is a news article from Nature suggesting that SARS-CoV-2 infection might trigger diabetes. Viral infection may damage insulin-producing cells in some people.

STAT has a nice story and video on how SARS-CoV-2 mounts its attack through ACE2 recptors. They also have a story on pool testing that might raise the number of possible tests that can be done on a daily basis (**note:** this is not swimming pool testing, but rather grouping samples together).

One of my loyal readers forwarded me a link to a Medscape interview on the lack of protective antibodies following SARS-CoV-2 infection. It's times like this that I wish my knowledge of immunology is better than it is. From my cursor readings of preprints and some primers on the topic, this is a complicated issue. Clearly, these individuals were ill and viral testing showed it was from the virus. There may be biochemical/genetic reasons as to why their illness did not progress to severe COVID-19. I also do not know whether the lack of antibody response means these individuals might be capable of viral shedding for a longer period of time and hence present of risk of societal spreading. This is all complicated stuff and like the onion, knowledge is improving one layer at a time (wow, maybe this is poor metaphor, but as tears come to my eyes, it is the one that popped into my brain; I can only hope it is not COVID-19 delusion!).

After a scant day of reading yesterday, a deluge of papers today! More on the use of tocilizumab and corticosteroids!

MODELING

- There is growing evidence that ethnic minorities in Europe are disproportionately affected by Covid-19. Using a name-based ethnicity classifier, we found that hospitalised Black, Asian and minority ethnic cases were younger and more likely to be admitted to intensive care (ICU). Pakistani, Bangladeshi and White - other than British or Irish, ethnic groups were most at risk. In this study, older age and male gender, but not ethnicity, were associated with death in hospitalised patients. [**note: this study confirms what is already known about racial and ethnic disparities in SARS-CoV-2 infections. However, it does break some new ground as they use name-based ethnic classification (*this worries me in the sense that this type of approach might be used for profiling in a not so good way. Onomap is the software program used in this study.)***] https://www.medrxiv.org/content/10.1101/2020.06.22.20136036v1

- COVID-19 mortality rate is higher in the elderly and in those with preexisting chronic medical conditions. The elderly also suffer from increased morbidity and mortality from seasonal influenza infection, and thus annual influenza vaccination is recommended for them. In this study, we explore a possible area-level association between influenza vaccination coverage in people aged 65 years and older and the number of deaths from COVID-19. To this end, we used COVID-19 data until June 10, 2020 together with population health data for the United States at the county level. We fit quasi-Poisson regression models using influenza vaccination coverage in the elderly population as the independent variable and the number of deaths from COVID-19 as the outcome variable. We adjusted for a wide array of potential confounding variables using both county-level generalized propensity scores for influenza vaccination rates, as well as direct adjustment. Our results suggest that influenza vaccination coverage in the elderly population is negatively associated with mortality from COVID-19. This finding is robust to using different analysis periods, different thresholds for inclusion of counties, and a variety of methodologies for confounding adjustment. In conclusion, our results suggest a potential protective effect of the influenza vaccine on COVID-19 mortality in the elderly population. The significant public health implications of this possibility point to an urgent need for studying the relationship between influenza vaccination and COVID-19 mortality at the individual level, to investigate both the epidemiology and any underlying biological mechanism. [**note: I am GLAD I got my flu shot last fall!! This is a decently done observational study showing seasonal influenza vaccination to be mildly protective against severe COVID-19. The authors are aware that there are a number of confounding issues that might impact the conclusions and not that more study of this is needed. Unrelated, CDC called on flu vaccine manufacturers to scale up production for the upcoming season.**]
  https://www.medrxiv.org/content/10.1101/2020.06.24.20129817v1
- Contact tracing can play a vital role in controlling human-to-human transmission of a highly contagious disease such as COVID-19. To investigate the benefits and costs of contact tracing, we develop an individual-based contact-network model and a susceptible-exposed-infected-confirmed (SEIC) epidemic model for the stochastic simulations of COVID-19 transmission. We estimate the unknown parameters (reproductive ratio R_0 and confirmed rate δ_2) by using observed confirmed case data. After a two month-lockdown, states in the USA have started the reopening process. We provide simulations for four different reopening situations: under "stay-at-home" order or no reopening, 25% reopening, 50% reopening, and 75% reopening. We model contact tracing in a two-layer network by modifying the basic SEIC epidemic model. The two-layer network is composed by the contact network in the first layer and the tracing network in the second layer. Since the full contact list of an infected individual patient can be hard to obtain, then we consider different fractions of contacts from 60% to 5%. The goal of this paper is to assess the effectiveness of contact tracing to control the COVID-19 spreading in the reopening process. In terms of benefits, simulation results show that increasing the fraction of traced contacts decreases the size of the epidemic. For example, tracing 20% of the contacts is enough for all four reopening scenarios to reduce the epidemic size by half. Considering the act of quarantining susceptible households as the contact tracing cost, we have observed an interesting phenomenon. When we increase the fraction of traced contacts from 5% to 20%, the number of quarantined susceptible people increases because each individual confirmed case is mentioning more contacts. However, when we increase the fraction of traced contacts from

20% to 60%, the number of quarantined susceptible people decreases because the increment of the mentioned contacts is balanced by a reduced number of confirmed cases. The main contribution of this research lies in the investigation of the effectiveness of contact tracing for the containment of COVID-19 spreading during the initial phase of the reopening process of the USA. [**note: here is a model for contact tracing in Manhattan KS, home to Kansas State University. While useful, I would not that the university's football team just shut down practice because of SARS-CoV-2 infections among the players. I wonder if we will see a college football season this fall.**] https://www.medrxiv.org/content/10.1101/2020.06.24.20139204v1

- Background: The rapid spread of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) around the world has caused a global pandemic, infecting millions of individuals worldwide, with an unprecedented impact in health care systems worldwide. Healthcare workers are one of the risk groups that need to be well characterized due to their strategic role in the management of patients, presently and in prevention of healthcare needs for future outbreaks. This study presents the results of the first SARS-CoV-2 seroprevalence study in the Northern Metropolitan Area of Barcelona, Spain. Methods: IgG SARS-CoV2 antibodies were analyzed in serum samples from 7563 healthcare workers of the Northern Metropolitan Area of Barcelona taken during the pandemia (from May 4th to May 22nd, 2020) by chemiluminescence assays. Results: A total of 779 of 7563 (10.3%) healthcare workers had detectable anti-SARS-CoV-2 IgG (specific for either S1/S2 or N antigens). No significant differences were observed between those working at primary care or at the reference hospital. Interestingly, in 29 (8.53%) of the previously confirmed positive reverse-transcriptase polymerase chain reaction (rRT-PCR) patients SARS-CoV-2 IgG (S1/S2 or recombinant N antigen) were negative. Conclusion: Seroprevalence of anti-SARS-CoV-2 IgG in the healthcare workers of the Nord Metropolitan Area of Barcelona was significantly increased in comparison with the general population in the same geographical area. These results give us an important insight for a better understanding of SARS-CoV-2 epidemiology, in a collective that is essential for the response against this pandemic. [**note: seroprevalence of COVID-19 antibodies in Barcelona healthcare workers. 10% were infected based on antibody testing.**]
  https://www.medrxiv.org/content/10.1101/2020.06.24.20135673v1 and here is a paper on Thai hospital workers that shows a more modest rate of infection:
  https://www.medrxiv.org/content/10.1101/2020.06.24.20139188v1

NEWLY REGISTERED CLINICAL TRIALS

- Study to assess the safety and efficacy of STI-5656 (Abivertinib Maleate) plus SOC versus SOC in subjects hospitalized with COVID-19 **[note: this is a third generation tyrosine kinase inhibitor and the sponsor is Sorrento therapeutics.**] NCT04440007
- Adults who suffer major COVID-19 complications appear to present a major inflammatory storm. Therefore, targeting the inflammatory response may reduce COVID-19-related complications in adults at risk or with evidence of an inflammatory storm. EB05 is a potent inhibitor of TLR4, a key component of the innate immune system which functions to detect molecules generated by pathogens, acting upstream of cytokine storm and IL-6-mediated acute lung injury. EB05 has demonstrated safety in two clinical studies (>165 patients) and was able to block LPS-induced (TLR4 agonist) IL-6 release in humans. Furthermore, TLR4 blockade rescued mice from lethal influenza-induced Acute Respiratory Distress Syndrome (ARDS), a major cause

of mortality associated with COVID-19, thus could be useful in the management of COVID-19. [**note: this is an experimental drug and the sponsor is Edesa Biotech.**] NCT04401475

- The Phase 2 portion of the study will evaluate the efficacy and safety of 2 dose levels of mavrilimumab relative to placebo (standard of care) in participants who have tested positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and have x-ray/computerized tomography (CT) evidence of bilateral pneumonia, active or recent fever, and clinical laboratory results indicative of hyper-inflammation. The Phase 3 portion is intended to confirm Phase 2 efficacy and safety findings. In both Phase 2 and Phase 3, participants will be enrolled into 2 cohorts: Cohort 1 will include non-intubated, hospitalized participants who require supplemental oxygen to maintain oxygen saturation (SpO2) ≥ 92% (ie, "non-ventilated" participants); Cohort 2 will include hospitalized participants for whom mechanical ventilation was recently initiated (ie, "ventilated" participants). Following Screening, enrolled participants in each cohort will be randomized 1:1:1 to receive one of 2 mavrilimumab dose levels, or placebo as a single intravenous (IV) infusion (Day 1). Participants will undergo primary study assessments through Day 29 and will be followed for safety through Day 90. [**note: trial is sponsored by Kiniksa Pharmaceuticals** NCT04447469
- This is a randomized, double-blind, placebo-controlled, 29-day study to assess the efficacy and safety of axatilimab plus standard of care, compared with placebo plus standard of care, in patients with respiratory signs and symptoms secondary to novel coronavirus disease (COVID-19). [**note: this is a mAb against colony stimulating factor 1 receptor. Sponsor is Syndax Pharmaceuticals.**] NCT04415073
- The proposed Allo-Prime universal viral protection mechanism involves vaccination with a bioengineered living allogeneic cellular vaccine (AlloStim) derived from healthy blood donors. The vaccine is designed to create high titers of memory immune cells that are specific to the foreign antigens in the living cell vaccine. Upon encounter with any type of virus, these memory immune cells are activated and release cytokines including an immediate release of IFN-Ɣ. This non-specific activation causes immune conditions similar to the conditions that occur in healthy younger patients that leads to rapid viral clearance and viral-specific memory immune response to clear infection and protect against recurrence. [**note: I don't know much about this other than what is in the abstract or on Immunoative Therapies, an Israeli company.**] NCT04441047

CLINICAL TRIAL RESULTS

- This multicentre cohort study involved 82 participating health-care institutions across 25 European countries, using a well established research network—the Paediatric Tuberculosis Network European Trials Group (ptbnet)—that mainly comprises paediatric infectious diseases specialists and paediatric pulmonologists. We included all individuals aged 18 years or younger with confirmed SARS-CoV-2 infection, detected at any anatomical site by RT-PCR, between April 1 and April 24, 2020, during the initial peak of the European COVID-19 pandemic. We explored factors associated with need for intensive care unit (ICU) admission and initiation of drug treatment for COVID-19 using univariable analysis, and applied multivariable logistic regression with backwards stepwise analysis to further explore those factors significantly associated with ICU admission. 582 individuals with PCR-confirmed SARS-CoV-2 infection were included, with a median age of 5·0 years (IQR 0·5–12·0) and a sex ratio of 1·15 males per female. 145 (25%) had pre-existing medical conditions. 363 (62%) individuals were admitted to hospital. 48 (8%)

individuals required ICU admission, 25 (4%) mechanical ventilation (median duration 7 days, IQR 2–11, range 1–34), 19 (3%) inotropic support, and one (<1%) extracorporeal membrane oxygenation. Significant risk factors for requiring ICU admission in multivariable analyses were being younger than 1 month (odds ratio 5·06, 95% CI 1·72–14·87; p=0·0035), male sex (2·12, 1·06–4·21; p=0·033), pre-existing medical conditions (3·27, 1·67–6·42; p=0·0015), and presence of lower respiratory tract infection signs or symptoms at presentation (10·46, 5·16–21·23; p<0·0001). The most frequently used drug with antiviral activity was hydroxychloroquine (40 [7%] patients), followed by remdesivir (17 [3%] patients), lopinavir–ritonavir (six [1%] patients), and oseltamivir (three [1%] patients). Immunomodulatory medication used included corticosteroids (22 [4%] patients), intravenous immunoglobulin (seven [1%] patients), tocilizumab (four [1%] patients), anakinra (three [1%] patients), and siltuximab (one [<1%] patient). Four children died (case-fatality rate 0·69%, 95% CI 0·20–1·82); at study end, the remaining 578 were alive and only 25 (4%) were still symptomatic or requiring respiratory support. COVID-19 is generally a mild disease in children, including infants. *However, a small proportion develop severe disease requiring ICU admission and prolonged ventilation, although fatal outcome is overall rare. The data also reflect the current uncertainties regarding specific treatment options, highlighting that additional data on antiviral and immunomodulatory drugs are urgently needed*. [**note: this is a large observational study from Europ on outcomes in children. The good news is progression to severe COVID-19 is small and mortality smaller. However, it highlights that no age group is totally risk-free.**]
https://www.thelancet.com/journals/lanchi/article/PIIS2352-4642(20)30177-2/fulltext

- COVID-19 is a deadly pulmonary disease with unique clinical features. A thorough understanding of the molecular and histological correlates of the disease is still missing, especially because post-mortem analysis of COVID-19-affected organs has been so far scant and often anecdotical. Here we report the results of the systematic analysis of 41 consecutive post-mortem samples from individuals who died of COVID-19. We found that the disease is characterized by extensive alveolar damage and thrombosis of the lung micro- and macro-vasculature. Thrombi were in different stages of organization, consistent with an ongoing, endogenous thrombotic process. In all the analyzed samples, in situ RNA hybridization showed that pneumocytes and vascular endothelial cells had massive presence of viral RNA even at the later stages of the disease. An additional feature of the disease was the presence, in the vast majority of patients, of a large number of dysmorphic pneumocytes, often forming large syncytial elements, a consequence of the fusogenic activity of the viral Spike protein, detected with specific antibodies. Despite occasional presence of virus-positive cells in the heart, no overt signs of viral infection were detected in other organs, which showed common alterations compatible with prolonged hypoxia, multifocal organ disease or previous comorbidities. In summary, COVID-19 is a unique interstitial pneumonia with extensive lung thrombosis, long-term persistence of viral replication in pneumocytes and endothelial cells, along with the presence of infected cellular syncytia in the lung. *We propose that several of the COVID-19 disease features are due to the persistence of virus-infected cells in the lungs of the infected individuals for the duration of the disease*. [**note: from Italy, lung pathology study in 41 post-mortem samples**]
https://www.medrxiv.org/content/10.1101/2020.06.22.20136358v1
- Cytokine release syndrome in COVID-19 is characterized by hyperinflammation which manifests as ARDS, multi-organ failure, and high inflammatory parameters. Tocilizumab, an IL-6 antagonist

has been used in COVID-19 acute respiratory distress syndrome (ARDS) with conflicting results from different parts of the world. We conducted a retrospective descriptive study from Feb 2020 to May 2020 on COVID-19 patients with ARDS and hyperinflammation characterized by raised CRP and/or ferritin. A total of 244 patients with COVID-19 were admitted out of which 107 had ARDS. Thirty patients had both ARDS and hyperinflammation and received tocilizumab. The mean age was 62.5 years (SD: 13.5) and the majority were male (83%). The mean CRP pre-treatment was 217.5 mg/L and post 48 to 72 hours of tocilizumab treatment was 98.5 mg/L. Twenty-one patients (70%) also received concomitant intravenous methylprednisolone. Of the 30 patients, 7 died and 20 recovered. Ten patients required intensive care unit admission and nine developed nosocomial infections. COVID-19 associated aspergillosis was diagnosed in three patients post tocilizumab treatment. Mortality was significantly higher in patients who developed a nosocomial infection and who required intermittent positive pressure ventilation (IPPV). Our study is the first to describe the treatment outcomes with tocilizumab from a low-middle income country. The availability and cost of tocilizumab in our region which makes it imperative to understand its potential for use in our setting. Our study supports the use of tocilizumab in a select patient population with COVID-19 and recommends monitoring of nosocomial infections and opportunistic infections. [**note: from Pakistan!!! Tocilizumab use is supported but this study is complicated by the co use of methylprednisolone. A subset of patients developed nosocomial infections which the authors note has to be monitored for.**] https://www.medrxiv.org/content/10.1101/2020.06.23.20134072v1

- Introduction Coronavirus disease 2019 (COVID-19) can lead to respiratory failure due to severe immune response. Treatment targeting this immune response might be beneficial but there is limited evidence on its efficacy. The aim of this study was to determine if early treatment of patients with COVID-19 pneumonia with tocilizumab and/or steroids was associated with better outcome. Methods This observational single-center study included patients with COVID-19 pneumonia who were not intubated and received either standard of care (SOC, controls) or SOC plus early (within 3 days from hospital admission) anti-inflammatory treatment. SOC consisted of hydroxychloroquine 400mg bid plus, in those admitted before March 24th, also darunavir/ritonavir. Anti-inflammatory treatment consisted of either tocilizumab (8mg/kg intravenously or 162mg subcutaneously) or methylprednisolone 1 mg/kg for 5 days or both. Failure was defined as intubation or death, and the endpoints were failure-free survival (primary endpoint) and overall survival (secondary) at day 30. Difference between the groups was estimated as Hazard Ratio by a propensity score weighted Cox regression analysis (HROW). Results Overall, 196 adults were included in the analyses. They were mainly male (67.4%), with comorbidities (78.1%) and severe COVID-19 pneumonia (83.7%). Median age was 67.9 years (range, 30-100) and median PaO2/FiO2 200 mmHg (IQR 133-289). Among them, 130 received early anti-inflammatory treatment with: tocilizumab (n=29, 22.3%), methylprednisolone (n=45, 34.6%), or both (n=56, 43.1%). The adjusted failure-free survival among tocilizumab/methylprednisolone/SOC treated patients vs. SOC was 80.8% (95%CI, 72.8-86.7) vs. 64.1% (95%CI, 51.3-74.0), HROW 0.48, 95%CI, 0.23-0.99; p=0.049. The overall survival among tocilizumab/methylprednisolone/SOC patients vs. SOC was 85.9% (95%CI, 80.7-92.6) vs. 71.9% (95%CI, 46-73), HROW 0.41, 95%CI: 0.19-0.89, p=0.025. Conclusion *Early adjunctive treatment with tocilizumab, methylprednisolone or both may improve outcomes in patients with COVID-19 pneumonia.* [**note: the tocilizumab hits keep on coming! These Italian researchers study both**

the mAb and methylprednisolone. Evidence continues to mount in favor of tocilizumab and corticosteroid treatment.]
https://www.medrxiv.org/content/10.1101/2020.06.22.20133413v1

- Diabetes is one of the most critical comorbidities linked to an increased risk of severe complications in the current coronavirus disease 2019 (COVID-19) pandemic. A better molecular understanding of COVID-19 in people with type diabetes mellitus (T2D) is mandatory, especially in countries with a high rate of T2D, such as the United Arab Emirates (UAE). Identification of the cellular and molecular mechanisms that make T2D patients prone to aggressive course of the disease can help in the discovery of novel biomarkers and therapeutic targets to improve our response to the disease pandemic. Herein, we employed a system genetics approach to explore potential genomic, transcriptomic alterations in genes specific to lung and pancreas tissues, affected by SARS-CoV-2 infection, and study their association with susceptibility to T2D in Emirati patients. Our results identified the Exocyst complex component, 6 (EXOC6/6B) gene (a component for docks insulin granules to the plasma membrane) with documented INDEL in 3 of 4 whole genome sequenced Emirati diabetic patients. Publically available transcriptomic data showed that lung infected with SARS-CoV-2 showed significantly lower expression of EXOC6/6B compared to healthy lungs. In conclusion, our data suggest that EXOC6/6B might be an important molecular link between dysfunctional pancreatic islets and ciliated lung epithelium that makes diabetic patients more susceptible to severe SARS-COV-2 complication. [**note: I think this is the first paper from the United Arab Emirates! It shows the possible genetic linkage for mortality In diabetic patients.**]
https://www.biorxiv.org/content/10.1101/2020.06.25.170936v1

DRUG DEVELOPMENT

- Currently, our knowledge of the mechanisms of COVID-19 disease pathogenesis is very limited which has hampered attempts to develop targeted antiviral strategies. Therefore, we urgently need an effective therapy for this unmet medical need. Viruses hijack and dysregulate cellular machineries in order for them to replicate and infect more cells. Thus, identifying and targeting dysregulated signaling pathways that have been taken over by viruses is one strategy for developing an effective antiviral therapy. We have developed a high-throughput drug screening system to identify potential antiviral drugs targeting SARS-CoV-2. We utilized a small molecule library of 430 protein kinase inhibitors, which are in various stages of clinical trials. Most of the tested kinase antagonists are ATP competitive inhibitors, a class of nucleoside analogs, which have been shown to have potent antiviral activity. From the primary screen, we have identified 34 compounds capable of inhibiting viral cytopathic effect in epithelial cells. Network of drug and protein relations showed that these compounds specifically targeted a limited number of cellular kinases. More importantly, we have identified mTOR-PI3K-AKT, ABL-BCR/MAPK, and DNA-Damage Response (DDR) pathways as key cellular signaling pathways critical for SARS-CoV-2 infection. Subsequently, a secondary screen confirmed compounds such as Berzosertib (VE-822), Vistusertib (AZD2014), and Nilotinib with anti SARS-CoV-2 activity. Finally, we found that Berzosertib, an ATR kinase inhibitor in the DDR pathway, demonstrated potent antiviral activity in a human epithelial cell line and human induced pluripotent stem cell (hIPSC)-derived cardiomyocytes. These inhibitors are already in clinical trials of phase 2 or 3 for cancer treatment, and can be repurposed as promising drug candidates for a host-directed therapy of

SARS-CoV-2 infection. In conclusion, we have identified small molecule inhibitors exhibiting anti SARS-CoV-2 activity by blocking key cellular kinases, which gives insight on important mechanism of host-pathogen interaction. These compounds can be further evaluated for the treatment of COVID-19 patients following additional in vivo safety and efficacy studies. [**note: this is a different screening approach from what I've seen. They look at protein kinase inhibitors. More leads on compounds that do not have a 'direct' antiviral mode of action. There are too many drugs to cover here and interested readers should download the paper.**] https://www.biorxiv.org/content/10.1101/2020.06.24.150326v1

VIRUS BIOCHEMISTRY

- I didn't see anything new today.

DIAGNOSTIC DEVELOPMENT

- Molecular testing for SARS-CoV-2 is the mainstay for accurate diagnosis of the infection, but the diagnostic performances of available assays have not been defined. We compared 12 molecular diagnostic assays, including 8 commercial kits using 155 respiratory samples (65 nasopharyngeal swabs, 45 oropharyngeal swabs, and 45 sputum) collected at 2 Japanese hospitals. Sixty-eight samples were positive for more than one assay and one genetic locus and were defined as true positive samples. All the assays showed a specificity of 100% (95% confidence interval, 95.8 to 100). The N2 assay kit of the US Centers for Disease Control and Prevention (CDC), the N2 assay of the Japanese National Institute of Infectious Disease (NIID) were the most sensitive assays with 100% sensitivity (95% confidence interval, 94.7 to 100), followed by the CDC N1 kit, E assay by Corman, and the NIID N2 assay multiplex with internal control reactions. These assays are reliable as first-line molecular assays in laboratories when combined with appropriate internal control reactions. [**note: a comparison of 12 different PCR tests but I did not see that they tested the Abbott quick test in this paper. These tests all take at least an hour to run the cycle.**]
https://www.biorxiv.org/content/10.1101/2020.06.24.170332v1
- Background Seroepidemiology is an important tool to characterize the epidemiology and immunobiology of SARS-CoV-2 but many immunoassays have not been externally validated raising questions about reliability of study findings. To ensure meaningful data, particularly in a low seroprevalence population, assays need to be rigorously characterized with high specificity. Methods We evaluated two commercial (Roche Diagnostics and Epitope Diagnostics IgM/IgG) and two non-commercial (Simoa and Ragon/MGH IgG) immunoassays against 68 confirmed positive and 232 pre-pandemic negative controls. Sensitivity was stratified by time from symptom onset. The Simoa multiplex assay applied three pre-defined algorithm models to determine sample result. Results The Roche and Ragon/MGH IgG assays each registered 1/232 false positive, the primary Simoa model registered 2/232 false positives, and the Epitope registered 2/230 and 3/230 false positives for the IgG and IgM assays respectively. Sensitivity >21 days post symptom-onset was 100% for all assays except Epitope IgM, but lower and/or with greater variability between assays for samples collected 9-14 days (67-100%) and 15-21 days (69-100%) post-symptom onset. The Simoa and Epitope IgG assays demonstrated excellent sensitivity earlier in the disease course. The Roche and Ragon/MGH IgG assays were less sensitive during early disease, particularly among immunosuppressed individuals. Conclusions

The Epitope IgG demonstrated good sensitivity and specificity. The Roche and Ragon/MGH IgG assays registered rare false positives with lower early sensitivity. The Simoa assay primary model had excellent sensitivity and few false positives. [**note: evaluation of four serology test.**] https://www.medrxiv.org/content/10.1101/2020.06.24.20139006v1

>>

2020-06-27

One of my favorite Motown songs was 'I Heard it Through the Grapevine' by Marvin Gaye. Here is a more funky live performance from the Montreux Jazz Festival: https://www.youtube.com/watch?v=Y7dGdrP3pms It was a great dance tune when I was in college as the original version shows: https://www.youtube.com/watch?v=hajBdDM2qdg

I wonder how many of my loyal readers read Sinclair Lewis's fine book, 'Arrowsmith.' I read when I was in grad school and was making my way through the Lewis canon. I think Babbitt was assigned in either a high school or college literature course but I hadn't read anything else. Here is a nice JAMA piece on the 'Arrowsmith.' I was not aware that Paul de Kruif helped Lewis out with this book. JAMA also discusses how medical journals maintain standards during a pandemic in light of the pressure to publish research findings in a timely manner.

From the National Academy of Sciences, here is a data guide for decision makers. The rapid expert consultation says that decision-makers should consider five criteria when evaluating COVID-19 data: representativeness, potential for systematic under- or over-estimation, uncertainty, time range, and geographical area.

This Starbucks barista gets amply rewarded by telling a customer to wear a mask!

One airline seems to be moving to full capacity flights.

From the New York Times, "Symptomless transmission makes the coronavirus far harder to fight. But health officials dismissed the risk for months, pushing misleading and contradictory claims in the face of mounting evidence." This is another good chapter for a 'cautionary tale' book. The Times also has a report on Coronavirus deaths in nursing homes, 'aging in place' is my mantra! The always good Gina Kolata writes on how the virus short-circuits the immune system.

How to address air filtration in schools and office buildings with a very good graphic showing an system that can clean the air.

Derek Lowe discusses vaccines and racial issues.

MODELING

- The fundamental dose-response relation is still missing for better evaluating and controlling the transmission risk of COVID-19. A recent study by Chu et al. has indicated that the anticipated probability of viral infection is about 12.8% within 1 m and about 2.6% at further distance through a systematic review and meta-analysis. This important information provides us a unique

opportunity to assess the dose-response relation of the viruses, if reasonable exposure dose could be estimated. Here we developed a simple framework to integrate the a priori dose-response relation for SARS-CoV based on mice experiments, and the recent data on infection risk and viral shedding, to shed light on the dose-response relation for human. The developed dose-response relation is an exponential function with a constant k in the range of 6.19E4 to 7.28E5 virus copies. The result mean that the infection risk caused by one virus copy in viral shedding is about 1.5E-6 to 1.6E-5. The developed dose-response relation provides a tool to quantify the magnitude of the infection risk. [**note: I've been asked on several occasions about the infectious dose of SARS-CoV-2. I've not seen many papers on this but here is one that constructs a model based on mice experiments.**]
https://www.medrxiv.org/content/10.1101/2020.06.26.20140624v1

- Importance: Reported cases of SARS-CoV-2 infection likely underestimate the prevalence of infection in affected communities. Large-scale seroprevalence studies provide better estimates of the proportion of the population previously infected. Objective: To estimate prevalence of SARS-CoV-2 antibodies in convenience samples from several geographic sites in the United States. Design: Serologic testing of convenience samples using residual sera obtained for routine clinical testing by two commercial laboratory companies. Setting: Connecticut (CT), south Florida (FL), Missouri (MO), New York City metro region (NYC), Utah (UT), and Washington State's (WA) Puget Sound region. Participants: Persons of all ages with serum collected during intervals from March 23 through May 3, 2020. Exposure: SARS-CoV-2 virus infection. Main outcomes and measures: We estimated the presence of antibodies to SARS-CoV-2 spike protein using an ELISA assay. We standardized estimates to the site populations by age and sex. Estimates were adjusted for test performance characteristics (96.0% sensitivity and 99.3% specificity). We estimated the number of infections in each site by extrapolating seroprevalence to site populations. We compared estimated infections to number of reported COVID-19 cases as of last specimen collection date. Results: We tested sera from 11,933 persons. Adjusted estimates of the proportion of persons seroreactive to the SARS-CoV-2 spike protein ranged from 1.13% (95% confidence interval [CI] 0.70-1.94) in WA to 6.93% (95% CI 5.02-8.92) in NYC (collected March 23-April 1). For sites with later collection dates, estimates ranged from 1.85% (95% CI 1.00-3.23, collected April 6-10) for FL to 4.94% (95% CI 3.61-6.52) for CT (April 26-May 3). The estimated number of infections ranged from 6 to 24 times the number of reported cases in each site. Conclusions and relevance: Our seroprevalence estimates suggest that for five of six U.S. sites, from late March to early May 2020, >10 times more SARS-CoV-2 infections occurred than the number of reported cases. Seroprevalence and under-ascertainment varied by site and specimen collection period. Most specimens from each site had no evidence of antibody to SARS-CoV-2. Tracking population seroprevalence serially, in a variety of specific geographic sites, will inform models of transmission dynamics and guide future community-wide public health measures. [**note: this is the CDC serology survey that was mentioned in yesterday's newsletter.**] https://www.medrxiv.org/content/10.1101/2020.06.25.20140384v1

NEWLY REGISTERED CLINICAL TRIALS

- Will check tomorrow.

CLINICAL TRIAL RESULTS

- Surprisingly nothing today.

DRUG DEVELOPMENT

- I don't have a link to the paper as it is being published in Cell today, but the Krogan research group at UCSF has expanded on their virus/cell proteome interaction study that was published a short while ago in Nature. They looked at a number of kinase inhibitors and found that some were better inhibitors than remdesivir. Krogan said tests of kinase inhibitors showed some, including Gilteritnib and Ralimetinib, required lower concentrations than remdesivir in order to kill off 50% of the virus. There are some kinase inhibitors in trials right now. This is a different drug discovery approach than looking at compounds that directly inhibit viral enzymes. [**note: this came across my newsfeed. this group is really doing some nice research! I'll post a link as soon as I find it.**]
- We previously showed that oral administration of cystine and theanine (CT) to mice confers resistance to influenza virus infection. In human studies, CT prevented colds in healthy subjects and enhanced antibody production after influenza vaccination in elderly individuals with a poor nutritional status. The mechanism of action of CT is thought to be glutathione (GSH)-mediated regulation of intracellular redox, which might affect innate immune systems such as macrophages to exert physiological effects. The effect of CT on influenza is independent of viral type, and this treatment has a broad range of antiviral activities. To explore the mechanisms of CT in viral infection, we performed transcriptome profiling of spleen tissues isolated from influenza A virus (IAV)-infected mice. We identified unique gene signatures in response to CT in the IAV-infected mice. Genes upregulated by CT included redox-regulated genes such as GCLC/GCLM (subunits of glutamate cysteine ligase, a rate-limiting enzyme of GSH biosynthesis), TXN1, TXN2, TXNRD2, and SOD1, suggesting that the intracellular redox environment is substantially altered by CT. However, genes downregulated in response to CT included chemokine/chemokine receptor genes (CCL5, CCL19, CXCL9, CXCL12, CXCR3, CXCR4, and ACKR3), some of which are related to cytokine storm. A comparison with public COVID-19-related gene set data showed that the upregulated gene signature was highly similar to the downregulated gene sets of SARS-CoV/SARS-CoV-2-infected cells and the upregulated gene set of attenuated SARS-CoV-infected cells. In conclusion, the unique gene signatures observed in response to orally administered CT in IAV-infected mouse spleen tissues suggested that CT may attenuate viral infection, replication and associated symptoms such as cytokine storm. [**note: others have written about the role glutathione might play. This certainly is a low cost therapy 'if it works.'**] https://www.biorxiv.org/content/10.1101/2020.06.25.149427v1
- Pathogens (bacteria, fungus and virus) are becoming a potential threat to the health of human beings and environment worldwide. They widely exist in the environment, with characteristics of variety, spreading quickly and easily causing adverse reactions. In this work, an Ag-based material is used to be incorporated and functionalized in polycotton fabrics using pad-dry-cure method. This composite proved to be effective for inhibiting the SARS-CoV-2 virus, decreasing the number of replicates in 99.99% after an incubation period of 2 minutes. In addition, it caused 99.99% inhibition of the pathogens S. aureus, E. coli and C. albicans, preventing cross-infections and does not cause allergies or photoirritation processes, demonstrating the safety of its use. [**note: this is pretty cool technology. They can affix the nano-particles to poly-cotton**

fabric to give it antimicrobial properties.]
https://www.biorxiv.org/content/10.1101/2020.06.26.152520v1

- SARS-CoV-2 Nsp15 is a uridylate-specific endoribonuclease with C-terminal catalytic domain belonging to the EndoU family. It degrades the polyuridine extensions in (-) sense strand of viral RNA and some non-translated RNA on (+) sense strand. This activity seems to be responsible for the interference with the innate immune response and evasion of host pattern recognition. Nsp15 is highly conserved in coronaviruses suggesting that its activity is important for virus replication. Here we report first structures with bound nucleotides and show that SARS-CoV-2 Nsp15 specifically recognizes U in a pattern previously predicted for EndoU. In the presence of manganese ions, the enzyme cleaves unpaired RNAs. Inhibitors of Nsp15 have been reported but not actively pursued into therapeutics. The current COVID-19 pandemic brought to attention the repurposing of existing drugs and the rapid identification of new antiviral compounds. Tipiracil is an FDA approved drug that is used with trifluridine in the treatment of colorectal cancer. Here, we combine crystallography, biochemical and whole cell assays, and show that this compound inhibits SARS-CoV-2 Nsp15 and interacts with the uridine binding pocket of the enzyme's active site, providing basis for the uracil scaffold-based drug development. [**note: I've not seen this enzyme mentioned before. Maybe it is another useful target to pursue.**] https://www.biorxiv.org/content/10.1101/2020.06.26.173872v1
- Murine models of SARS-CoV-2 infection are critical for elucidating the biological pathways underlying COVID-19 disease. Because human ACE2 is the receptor for SARS-CoV-2, mice expressing the human ACE2 gene have shown promise as a potential model for COVID-19. Five mice from the transgenic mouse strain K18-hACE2 were intranasally inoculated with SARS-CoV-2 Hong Kong/VM20001061/2020. Mice were followed twice daily for five days and scored for weight loss and clinical symptoms. Infected mice did not exhibit any signs of infection until day four, when weight loss, but no other obvious clinical symptoms were observed. By day five all infected mice had lost around 10% of their original body weight, but exhibited variable clinical symptoms. All infected mice showed high viral titers in the lungs as well as altered lung histology associated with immune cell infiltration and alveolar septal thickening. Overall, these results show that symptomatic SARS-CoV-2 infection can be established in the K18-hACE2 transgenic background and this mouse strain should be a useful for COVID-19 disease. [**note: this might be a useful transgenic mouse model for studying SARS-CoV-2 infections.]**
https://www.biorxiv.org/content/10.1101/2020.06.26.171033v1

VIRUS BIOCHEMISTRY

- The spike (S) protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is required for cell entry and is the major focus for vaccine development. We combine cryo electron tomography, subtomogram averaging and molecular dynamics simulations to structurally analyze S in situ. Compared to recombinant S, the viral S is more heavily glycosylated and occurs predominantly in a closed pre-fusion conformation. We show that the stalk domain of S contains three hinges that give the globular domain unexpected orientational freedom. We propose that the hinges allow S to scan the host cell surface, shielded from antibodies by an extensive glycan coat. The structure of native S contributes to our understanding of SARS-CoV-2 infection and the development of safe vaccines. The large scale tomography data set of SARS-CoV-2 used for this study is therefore sufficient to resolve structural features to below 5

Ångstrom, and is publicly available at EMPIAR-10453. [**note: more on the structure of the Spike protein.**] https://www.biorxiv.org/content/10.1101/2020.06.26.173476v1

DIAGNOSTIC DEVELOPMENT

- Here we present a rapid and versatile method for capturing and concentrating SARS-CoV-2 from transport medium and saliva using affinity-capture magnetic hydrogel particles. We demonstrate that the method concentrates virus prior to RNA extraction, thus significantly improving detection of the virus using a real-time RT-PCR assay across a range of viral titers, from 100 to 1,000,000 viral copies/mL; in particular, detection of virus in low viral load samples is enhanced when using the method coupled with the IDT 2019-nCoV CDC EUA Kit. This method is compatible with commercially available nucleic acid extraction kits, as well with a simple heat and detergent method. Using transport medium diagnostic remnant samples that previously had been tested for SARS-CoV-2 using either the Abbott RealTime SARS-CoV-2 EUA Test (n=14) or the Cepheid Xpert Xpress SARS-CoV-2 EUA Test (n=35), we demonstrate that our method not only correctly identifies all positive samples (n = 17) but also significantly improves detection of the virus in low viral load samples. The average improvement in cycle threshold (Ct) value as measured with the IDT 2019-nCoV CDC EUA Kit was 3.1; n = 10. Finally, to demonstrate that the method could potentially be used to enable pooled testing, we spiked infectious virus or a confirmed positive diagnostic remnant sample into 5 mL and 10 mL of negative transport medium and observed significant improvement in the detection of the virus from those larger sample volumes. [**note: this is an interesting approach to concentrating test samples**] https://www.biorxiv.org/content/10.1101/2020.06.25.172510v1
- SARS-CoV-2 causes substantial morbidity and mortality in elderly and immunocompromised individuals, particularly in retirement homes, where transmission from asymptomatic staff and visitors may introduce the infection. Here we present a cheap and fast approach to detect SARS-CoV-2 in single or pooled gargle lavages ('mouthwashes'). With this approach, we test all staff at a nursing home daily over a period of three weeks in order to reduce the risk that the infection penetrates the facility. This or similar approaches could be implemented to protect hospitals, nursing homes and other institutions in this and future viral epidemics. [**note: this is a different take on getting oral samples to test for SARS-CoV-2. More work needs to be don't to validate how this 'gargle' approach compares with other sample collection techniques.**] https://www.medrxiv.org/content/10.1101/2020.06.24.20139501v1
- The currently used methods for diagnostics are time consuming and also hindered by the limited availability of reagents and reaction costs, thus presenting a bottle neck for prevention of COVID-19 spread. Here, we present a new ultra-fast test method which is ten times faster than conventional diagnostic tests using real time quantitative PCR (RT-qPCR). In addition, this ultra-fast method is easy to handle as well as cost effective. We translated published SARS-CoV-2 testing protocols from the Centers of Disease Control and Prevention (Atlanta, Georgia, USA) and the Charité Berlin (Germany) to the NEXTGENPCR (NGPCR) machine and combined it with a fluorescence-based endpoint measurement. Fluorescence was measured with a commercial blue light scanner. We confirmed the NEXTGENPCR results with commercially available positive controls. In addition, we isolated RNA from SARS-CoV-2 infected patients and achieved similar results to clinical RT-qPCR assays. Here, we could show correlation between the results obtained

by NEXTGENPCR and conventional RT-qPCR. [**note: another fast, in this case 'ultra-fast' approach to PCR testing.]** https://www.medrxiv.org/content/10.1101/2020.06.25.20137398v1

>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

2020-06-28

For reflection Sunday, let us turn to Felix Mendelssohn's 5th symphony also known as the Reformation Symphony. Here is my current favorite orchestra, the Frankfurt Radio Symphony under the direction of Jérémie Rhorer: https://www.youtube.com/watch?v=otcrnrQAwD8

I will have to set up a CNN news feed! Here is a very interesting article on David Fajgenbaum of UPenn school of medicine and the leader of their Center of Cytokine Storm Treatment and Laboratory. He suffers from Castleman disease and a repurposed drug saved his life. He has put his energies into looking at repurposed drugs for COVID-19 and has established a database that is extremely useful.

One of the better podcasts around is Tim Harford's 'Cautionary Tales' An economist by training, Harford always finds twists to both current and past events. This week's podcast 'The Spreadsheet of Life and Death' is all about quantifying the value of life in making medical decisions. Harford lives in the UK so much of the focus is on the NHS and cost effectiveness of various interventions. It's well worth listening to.

A good family friend and today a new reader of the newsletter asked me about whether the MMR vaccine, now standard for all other than anti-vaxxers, might be protective. I had not seen anything in my readings about this. The reader sent me a provocative preprint 'MMR Vaccine Appears to confer Strong Protection from COVID-19', a linkage study looking at populations, vaccine rates, and mortality from the virus. This study is no different from the linkage studies on the BCG vaccine of which there have been many. At least with the BCG vaccine, researchers are taking it seriously and there are some ongoing clinical trials, mostly in health care workers. I have seen no mention of the MMR vaccine other than the one paper sent to me. Boosting the immune system is also leading to some suggestions that the Sabin oral polio vaccine be repurposed for this and more on this approach from Russia. [**note: I just posted a link to a registered Egyptian trial on measles vaccine! While thinking about this issue, I wonder if the new shingles vaccine that some of us have taken is protective. It sure generated a huge immune response for me!!**]

A physician in Houston reflects on the surge they could have seen coming.

Here is a brief note on my favorite topic, masks! Don't go for the mask that has the one way valve on it. These are made for construction work not protection against viral infections. The tried and true cotton mask just works!

Three reasons why the median age of reported COVID infections are dropping.

MODELING

- Available information on Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) transmission by small particle aerosols continues to evolve rapidly. To assess the potential role of heating, ventilation, and air conditioning (HVAC) systems in airborne viral transmission, this

study sought to determine the viral presence, if any, on air handling units in a healthcare setting where Coronavirus Disease 2019 (COVID-19) patients were being treated. The presence of SARS-CoV-2 RNA was detected in approximately 25% of samples taken from nine different locations in multiple air handlers. While samples were not evaluated for viral infectivity, the presence of viral RNA in air handlers raises the possibility that viral particles can enter and travel within the air handling system of a hospital, from room return air through high efficiency MERV-15 filters and into supply air ducts. Although no known transmission events were determined to be associated with these specimens, the findings suggest the potential for HVAC systems to facilitate transmission by environmental contamination via shared air volumes with locations remote from areas where infected persons reside. More work is needed to further evaluate the risk of SARS-CoV-2 transmission via HVAC systems and to verify effectiveness of building operations mitigation strategies for the protection of building occupants. These results are important within and outside of healthcare settings and may present a matter of some urgency for building operators of facilities that are not equipped with high-efficiency filtration. [**note: I am not surprised at all by this finding. The more critical, and missing, piece of information is whether these samples are infectious. It would have been useful for the investigators to have done some *in vitro* studies on this point. Several days ago I posted a link regarding HVAC systems in office buildings. I'm not a building engineer and I'll leave any further discussion of this point to experts.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20141085v1

- Background: Fierce debate about the health and financial tradeoffs presented by different COVID-19 pandemic mitigation strategies highlights the need for rigorous quantitative evaluation of policy options. Objective: To quantify the economic value of the costs and benefits of a policy of continued limited reopening with social distancing relative to alternative COVID-19 response strategies in the United States. Design: We estimate the number and value of quality-adjusted life-years (QALY) gained from mortality averted, with a value of $125,000 per QALY, and compare these benefits to the associated costs in terms of plausible effects on US GDP under a policy of continued limited reopening with social distancing relative to a policy of full reopening toward herd immunity. Using the same QALY value assumptions, we further evaluate cost-effectiveness of a return to Shelter-in-Place relative to a policy of limited reopening. Setting: United States Measurements: QALY and cost as percent of GDP of limited reopening with continued social distancing relative to a strategy of full reopening aimed at achieving herd immunity; a limited reopening budget measured in the number of months before this strategy fails to demonstrate cost-effectiveness relative to a full reopening; a shelter-in-place threshold measured in the number of lives saved at which a month of sheltering in place demonstrates cost effectiveness relative to the limited reopening strategy. Results: QALY benefits from mortality averted by continued social distancing and limited reopening relative to a policy of full reopening exceed projected GDP costs if an effective vaccine or therapeutic can be developed within 11.1 months from late May 2020. White House vaccine projections fall within this date, supporting a partial reopening strategy. One month of shelter-in-place restrictions provides QALY benefits from averted mortality that exceed the associated GDP costs relative to limited reopening if the restrictions prevent at least 154,586 additional COVID-19 deaths over the course of the pandemic. Current models of disease progression suggest that limited reopening will not cause this many additional deaths, again supporting a limited reopening strategy. Limitation: Limited horizon of COVID-19 mortality projections; infection fatality ratio stable

across strategies, ignoring both the potential for ICU overload to increase mortality and the deployment of partially effective therapeutics to decrease mortality; effect on GDP modeled as constant within a given phase of the pandemic; accounts for age and sex distribution of QALYs, but not effect of comorbidities; only considers impact from QALY lost due to mortality and from changes in GDP, excluding numerous other considerations, such as non-fatal COVID-19 morbidity, reduced quality of life caused by prolonged social distancing, or educational regression associated with prolonged school closures and restrictions. Conclusions: A limited reopening to achieve partial mitigation of COVID-19 is cost effective relative to a full reopening if an effective therapeutic or vaccine can be deployed within 11.1 months of late May 2020. One additional month of shelter-in-place restrictions should only be imposed if it saves at least 154,586 lives per month before the development of an effective therapeutic or vaccine relative to limited reopening. [**note: several academic economists are among my loyal readers. This is for them! It's also similar to some of what Tim Harford discusses in his podcast referred to in today's introduction.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20141044v1

NEWLY REGISTERED CLINICAL TRIALS

- A Phase 2 study to evaluate the safety and preliminary efficacy of ATYR1923, compared to placebo, in hospitalized patients with SARS-CoV-2 (COVID-19) severe pneumonia not requiring mechanical ventilation [**note: this trial is sponsored by aTyr Pharma and ATYR1923 is a selective modulator of NRP2 that downregulates the innate and adaptive immune response in inflammatory disease states.]** NCT04412668
- The following two mechanisms that explain the ability of measles vaccine to cause partial protection against COVID-19. The first is that measles vaccine may increase the ability of the immune system to fight off pathogens other than measles due to the generated bystander immunity that would enhance the overall immunity against the new coronavirus. The second is that SARS-CoV-2 is proven to have structure similarities with measles, which may cause cross-reactivity and immunity between measles vaccines and COVID-19, leading to partial protection against COVID-19 in vaccinated subjects [**note: SURPRISE! Not only did I get a question on MMR vaccines but these Egyptian researchers want to see if the measles vaccine is protective!!! I guess my one question is whether those of us who actually had measles as kids (pre-vaccine era) have any protection. One might expect the antibody response to the disease to have been quite robust. How does one correct for this confounder?**] NCT04445610
- This is a randomized, double-blind, placebo-controlled, phase I clinical study to evaluate the tolerability, safety, pharmacokinetic profile and immunogenicity of JS016 (anti-SARS-CoV-2 monoclonal antibody) injection in Chinese healthy subjects after intravenous infusion of single dose.Eligible patients will be injection JS016 (anti-SARS-CoV-2 monoclonal antibody) [**note: China enters the mAb race!**] NCT04441918
- The objective of our study is to evaluate safety, tolerability, and immunogenicity of COVID-19 preventive DNA vaccine in healthy volunteers [**note: another vaccine this time from the South Korean company Genexine.]** NCT04445389
- The aim of this study is to investigate the levels of insomnia 3 months after (T2) the strict physical distancing government initiated physical distancing protocols related to the COVID-19 pandemic (T1). The study also aims to investigate how predictors measured after and before the COVID-19 pandemic are associated with sleep problems at T2. [**note: more countries should follow the lead of these Norwegian researchers! My COVID-19 nightmares have largely**

disappeared, but sleep disorders will likely persist. I will have to read up on the Likert scale that is used for this research.] NCT04443361

- This study aims to evaluate the safety and reactogenicity profile after 1 and 2 dose administrations of CVnCoV at different dose levels. [**note: this is the mRNA vaccine from CureVac, the German company that was in the news several months ago.]** NCT04449276

CLINICAL TRIAL RESULTS

- Background Pandemic COVID-19 caused by the coronavirus SARS-CoV-2 has a high incidence of patients with severe acute respiratory syndrome (SARS). Many of these patients require admission to an intensive care unit (ICU) for invasive artificial ventilation and are at significant risk of developing a secondary, ventilator-associated pneumonia (VAP). Objectives To study the incidence of VAP, as well as differences in secondary infections, and bacterial lung microbiome composition of ventilated COVID-19 and non-COVID-19 patients. Methods In this prospective observational study, we compared the incidence of VAP and secondary infections using a combination of a TaqMan multi-pathogen array and microbial culture. In addition, we determined the lung microbime composition using 16S RNA analyisis. The study involved eighteen COVID-19 and seven non-COVID-19 patients receiving invasive ventilation in three ICUs located in a single University teaching hospital between April 13th 2020 and May 7th 2020. Results We observed a higher percentage of confirmed VAP in COVID-19 patients. However, there was no statistical difference in the detected organisms or pulmonary microbiome when compared to non-COVID-19 patients. Conclusion COVID-19 makes people more susceptible to developing VAP, partly but not entirely due to the increased duration of ventilation. The pulmonary dysbiosis caused by COVID-19, and the array of secondary infections observed are similar to that seen in critically ill patients ventilated for other reasons. [**note: ventilator associated pneumonia is a serious consequence from the use of this tool in the management of serious respiratory disease. However, the consequences in COVID-19 patients do not appear to be heightened compared to non-COVID-19 infection management.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20139873v1
- Convalescent plasma is currently one of the leading treatments for COVID-19, but there is a paucity of data identifying therapeutic efficacy. A comprehensive analysis of the antibody responses in potential plasma donors and an understanding of the clinical and demographic factors that drive variant antibody responses is needed. Among 126 potential convalescent plasma donors, the humoral immune response was evaluated by a SARS-CoV-2 virus neutralization assay using Vero-E6-TMPRSS2 cells, commercial IgG and IgA ELISA to Spike (S) protein S1 domain (Euroimmun), IgA, IgG and IgM indirect ELISAs to the full-length S or S-receptor binding domain (S-RBD), and an IgG avidity assay. Multiple linear regression and predictive models were utilized to assess the correlations between antibody responses with demographic and clinical characteristics. IgG titers were greater than either IgM or IgA for S1, full length S, and S-RBD in the overall population. Of the 126 plasma samples, 101 (80%) had detectable neutralizing titers. Using neutralization titer as the reference, the sensitivity of the IgG ELISAs ranged between 95-98%, but specificity was only 20-32%. Male sex, older age, and hospitalization with COVID-19 were all consistently associated with increased antibody responses across the serological assays. Neutralizing antibody titers were reduced over time in contrast to overall antibody responses. There was substantial heterogeneity in the antibody

response among potential convalescent plasma donors, but sex, age and hospitalization emerged as factors that can be used to identify individuals with a high likelihood of having strong antiviral antibody levels. [**note: interesting finding that males tend to have more robust antibody response but this may result from more severe disease symptoms.]** https://www.medrxiv.org/content/10.1101/2020.06.26.20139063v1

- Purpose Vitamin D has been proposed as a potential causal factor in COVID-19 risk. We aimed to establish whether blood 25-hydroxyvitamin D (25(OH)D) concentration was associated with COVID-19 mortality, and inpatient confirmed COVID-19 infection, in UK Biobank participants. Methods UK Biobank recruited 502,624 participants aged 37-73 years between 2006 and 2010. Baseline exposure data, including 25(OH)D concentration, were linked to COVID-19 mortality. Univariable and multivariable Cox proportional hazards regression analyses were performed for the association between 25(OH)D and COVID-19 death, and poisson regression analyses for the association between 25(OH)D and severe COVID-19 infection. Results Complete data were available for 341,484 UK Biobank participants, of which 656 had inpatient confirmed COVID-19 infection and 203 died of COVID-19 infection. Vitamin D was associated with severe COVID-19 infection and mortality univariably (mortality HR=0.99; 95% CI 0.98-0.998; p=0.016), but not after adjustment for confounders (mortality HR=0.998; 95% CI=0.99-1.01; p=0.696). Conclusions *Our findings do not support a potential link between vitamin D concentrations and risk of severe COVID-19 infection and mortality. Recommendations for vitamin D supplementation to lessen COVID-19 risks may provide false reassurance*. [**note: don't you wish we had a US Biobank so that these types of studies could be more easily done? I guess I should not be raiding my wife's supply of Vitamin D pills as this observational study suggests that there is no effect. There continue to be some ongoing clinical trials that will, we hope, provide a firm answer to this.**] https://www.medrxiv.org/content/10.1101/2020.06.26.20140921v1

DRUG DEVELOPMENT

- There is a great need for the development of vaccines for preventing SARS-CoV-2 infection and mitigating the COVID-19 pandemic. Here, we developed two modified vaccinia Ankara (MVA) based vaccines which express either a membrane anchored full-length spike protein (MVA/S) stabilized in a prefusion state or the S1 region of the spike (MVA/S1) which forms trimers and is secreted. Both immunogens contained the receptor-binding domain (RBD) which is a known target of antibody-mediated neutralization. Following immunizations with MVA/S or MVA/S1, both spike protein recombinants induced strong IgG antibodies to purified full-length SARS-CoV-2 spike protein. The MVA/S induced a robust antibody response to purified RBD, S1 and S2 whereas MVA/S1 induced an antibody response to the S1 region outside of the RBD region. Both vaccines induced an antibody response in the lung and that was associated with induction of bronchus-associated lymphoid tissue. MVA/S but not MVA/S1 vaccinated mice generated robust neutralizing antibody responses against SARS-CoV-2 that strongly correlated with RBD antibody binding titers. Mechanistically, S1 binding to ACE-2 was strong but reduced following prolonged pre-incubation at room temperature suggesting confirmation changes in RBD with time. These results demonstrate MVA/S is a potential vaccine candidate against SARS-CoV-2 infection. [**note: sound the trumpets and bang the drums, here is another SARS-CoV-2 vaccine candidate! This vector system has been used to develop several vaccines that have been clinically tested and there is a thorough discussion in the paper. Move vaccine candidates increase the odds of**

success but is it too late for this one?]
https://www.biorxiv.org/content/10.1101/2020.06.27.175166v1

VIRUS BIOCHEMISTRY

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virions are surrounded by a lipid bilayer from which spike (S) protein trimers protrude. Heavily glycosylated S trimers bind the ACE2 receptor and mediate entry of virions into target cells. S exhibits extensive conformational flexibility: it modulates the exposure of its receptor binding site and later undergoes complete structural rearrangement to drive fusion of viral and cellular membranes. The structures and conformations of soluble, overexpressed, purified S proteins have been studied in detail using cryo-electron microscopy. The structure and distribution of S on the virion surface, however, has not been characterised. Here we applied cryo-electron microscopy and tomography to image intact SARS-CoV-2 virions, determining the high-resolution structure, conformational flexibility and distributions of S trimers in situ on the virion surface. These results provide a basis for understanding the conformations of S present on the virion, and for studying their interactions with neutralizing antibodies. [**note: more on the structure and confirmation on the Spike protein.**] https://www.biorxiv.org/content/10.1101/2020.06.27.174979v1
- The COVID-19 pandemic has spread to almost every country in the world since it started in China in late 2019. Controlling the pandemic requires a multifaceted approach including whole genome sequencing to support public health interventions at local and national levels. One of the most widely used methods for sequencing is the ARTIC protocol, a tiling PCR approach followed by Oxford Nanopore sequencing (ONT) of up to 24 samples at a time. There is a need for a higher throughput method to reduce cost per genome. Here we present CoronaHiT, a method capable of multiplexing up to 95 small genomes on a single Nanopore flowcell, which uses transposase mediated addition of adapters and PCR based addition of symmetric barcodes. We demonstrate the method using 48 and 94 SARS-CoV-2 genomes per flowcell, amplified using the ARTIC protocol, and compare performance with Illumina and ARTIC ONT sequencing. Results demonstrate that all sequencing methods produce inaccurate genomes when the RNA extract from SARS-CoV-2 positive clinical sample has a cycle threshold (Ct) >= 32. Results from set same set of 23 samples with a broad range of Cts show that the consensus genomes have >90% coverage (GISAID criteria) for 78.2% of samples for CoronaHiT-48, 73.9% for CoronaHiT-94, 78.2% for Illumina and 73.9% for ARTIC ONT, and all have the same clustering on a maximum likelihood tree. In conclusion, we demonstrate that CoronaHiT can multiplex up to 94 SARS-CoV-2 genomes per nanopore flowcell without compromising the quality of the resulting genomes while reducing library preparation complexity and significantly reducing cost. This protocol will aid the rapid expansion of SARS-CoV-2 genome sequencing globally, to help control the pandemic. [**note: thanks to a loyal reader as I missed this one when it came out. This is a useful approach to doing large genomic sequencing of viral samples. It should help fill in gaps in the phylogenetic path of SARS-CoV-2**]
https://www.biorxiv.org/content/10.1101/2020.06.24.162156v1

DIAGNOSTIC DEVELOPMENT

- Mass cytometry (CyTOF) represents one of the most powerful tools in immune phenotyping, allowing high throughput quantification of over 40 single parameters at single-cell resolution.

However, wide deployment of CyTOF-based immune phenotyping studies are limited by complex experimental workflows and the need for specialized CyTOF equipment and technical expertise. Furthermore, differences in cell isolation and enrichment protocols, antibody reagent preparation, sample staining and data acquisition protocols can all introduce technical variation that can potentially confound integrative analyses of large data-sets of samples processed across multiple labs. Here, we present a streamlined whole blood CyTOF workflow which addresses many of these sources of experimental variation and facilitates wider adoption of CyTOF immune monitoring across sites with limited technical expertise or sample-processing resources or equipment. Our workflow utilizes commercially available reagents including the Fluidigm MaxPar Direct Immune Profiling Assay (MDIPA), a dry tube 30-marker immunophenotyping panel, and SmartTube Proteomic Stabilizer, which allows for simple and reliable fixation and cryopreservation of whole blood samples. We validate a workflow that allows for streamlined staining of whole blood samples with minimal processing requirements or expertise at the site of sample collection, followed by shipment to a central CyTOF core facility for batched downstream processing and data acquisition. We further demonstrate the application of this workflow to characterize immune responses in a cohort of hospitalized COVID-19 patients, highlighting key disease-associated changes in immune cell frequency and phenotype. [**note: more good work from Mt. Sinai on doing antibody profiling.**]
https://www.medrxiv.org/content/10.1101/2020.06.26.20141341v1