2020-11-02

Welcome to Week 33 of the Pandemic Newsletter

I featured 'Will the Circle be Unbroken' some months ago. Here is a new performance that just went up the other day. Carlene Carter (daughter of June and granddaughter of Mother Maybelle) and Mary Stuart sing and play on some original instruments: https://www.youtube.com/watch?v=N-IGPWZKzJI I always find this inspiring.

The Washington Post has a story on how AI might help in solving some COVID-19 problems. If the White House won't do contact tracing, what hope is there for other areas of the US? This is a public health failure, full stop. Classical musicians are embracing the new reality of the pandemic. I miss going to live performances!

The New York Times writes that San Francisco is managing SARS-CoV-2 well but there is debate about opening up the schools. Slovakia tested half its population in one day. Here is story on the phylogenetics of the viral outbreak in the White House. This report covers the early days of the outbreak and the interaction between the WHO and China where some bad decisions were made. This Orthodox Jewish community in New York saw the virus rate drop to 2% but was there enough testing? One area of the US appears to be taking the 'herd immunity 'approach. I want to move to Australia where they appear to have good control over the virus. Restaurants in NYC get creative.

STAT summarize the political implications for tomorrow's election on the FDA, CDC, NIH and others. [**note:** regardless of the headlines, President Trump cannot fire Tony Fauci but he can reassign him.]

Kaiser Health News has an article on how science has become a hot button political issue. This is just stupid!

As is usual for Monday, not many new papers.

MODELING

- In October 2020, an outbreak of at least 50 COVID-19 cases was reported surrounding individuals employed at or visiting the White House. Here, we applied genomic epidemiology to investigate the origins of this outbreak. We enrolled two individuals with exposures linked to the White House COVID-19 outbreak into an IRB-approved research study and sequenced their SARS-CoV-2 infections. We find these viral sequences are highly genetically similar to each other, but are distinct from over 160,000 publicly available SARS-CoV-2 genomes, possessing 5 nucleotide mutations that differentiate this lineage from all other circulating lineages sequenced to date. We estimate this lineage has a common ancestor in the USA in April or May 2020, but its whereabouts for the past 5 to 6 months are not clear. Looking forwards, sequencing of additional community SARS-CoV-2 infections collected in the USA prior to October 2020 may reveal linked infections and shed light on its geographic ancestry. In sequencing of SARS-CoV-2 infections collected after October 2020, the relative rarity of this constellation of mutations may make it possible to identify infections that likely descend from the White House COVID-19 outbreak. [**note: good work from the Hutch and Univ of Washington analyzing the viral**

genome associated with the White House outbreak. A NY Times story is linked above.] https://www.medrxiv.org/content/10.1101/2020.10.31.20223925v1

NEWLY REGISTERED CLINICAL TRIALS

- You got your results yesterday!

CLINICAL TRIAL RESULTS

- It's Monday and usually no clinical study results get posted over the weekend.

DRUG DEVELOPMENT

- Recombinant neutralizing antibodies (nAbs) derived from recovered patients have proven to be effective therapeutics for COVID-19. Here, we describe the use of advanced protein engineering and modular design principles to develop tetravalent synthetic nAbs that mimic the multi-valency exhibited by IgA molecules, which are especially effective natural inhibitors of viral disease. At the same time, these nAbs display high affinity and modularity typical of IgG molecules, which are the preferred format for drugs. *We show that highly specific tetravalent nAbs can be produced at large scale and possess stability and specificity comparable to approved antibody drugs. Moreover, structural studies reveal that the best nAb targets the host receptor binding site of the virus spike protein, and thus, its tetravalent version can block virus infection with a potency that exceeds that of the bivalent IgG by an order of magnitude. Design principles defined here can be readily applied to any antibody drug, including IgGs that are showing efficacy in clinical trials. Thus, our results present a general framework to develop potent antiviral therapies against COVID-19, and the strategy can be readily deployed in response to future pathogenic threats*. [**note: here is an interesting approach to creating neutralizing antibodies.]** https://www.biorxiv.org/content/10.1101/2020.10.31.362848v1
- Effective treatment for SARS-CoV-2 is urgently needed. *We recently discovered four SARS-CoV-2 main protease (Mpro) inhibitors including boceprevir, calpain inhibitors II and XII and GC-376 with potent antiviral activity against infectious SARS-CoV-2 in cell culture. Despite the weaker enzymatic inhibition of calpain inhibitors II and XII against Mpro compared to GC-376, calpain inhibitors II and XII had more potent cellular antiviral activity. This observation promoted us to hypothesize that the cellular antiviral activity of calpain inhibitors II and XII might also involve the inhibition of cathepsin L in addition to Mpro. To test this hypothesis, we tested calpain inhibitors II and XII in the SARS-CoV-2 pseudovirus neutralization assay in Vero E6 cells and found that both compounds significantly decreased pseudoviral particle entry into cells, indicating their role in inhibiting cathepsin L.* The involvement of cathepsin L was further confirmed in the drug time-of-addition experiment. In addition, we found that these four compounds not only inhibit SARS-CoV-2, but also SARS-CoV, MERS-CoV, as well as human coronaviruses (CoVs) 229E, OC43, and NL63. The mechanism of action is through targeting the viral Mpro, which was supported by the thermal shift binding assay and enzymatic FRET assay. We further showed that these four compounds have additive antiviral effect when combined with remdesivir. Altogether, these results suggest that boceprevir, calpain inhibitors II and XII, and GC-376 are not only promising antiviral drug candidates against existing human coronaviruses, but also might work against future emerging CoVs. [**note: here are some more inhibitors of SARS-CoV-2 with broad**

spectrum activity against other coronaviruses as well.]
https://www.biorxiv.org/content/10.1101/2020.10.30.362335v1

- There is an urgent need to create novel models using human disease-relevant cells to study SARS-CoV-2 biology and to facilitate drug screening. As SARS-CoV-2 primarily infects the respiratory tract, we developed a lung organoid model using human pluripotent stem cells (hPSC-LOs). The hPSC-LOs, particularly alveolar type II-like cells, are permissive to SARS-CoV-2 infection, and showed robust induction of chemokines upon SARS-CoV-2 infection, similar to what is seen in COVID-19 patients. Nearly 25% of these patients also have gastrointestinal manifestations, which are associated with worse COVID-19 outcomes[1]. We therefore also generated complementary hPSC-derived colonic organoids (hPSC-COs) to explore the response of colonic cells to SARS-CoV-2 infection. We found that multiple colonic cell types, especially enterocytes, express ACE2 and are permissive to SARS-CoV-2 infection. Using hPSC-LOs, we performed a high throughput screen of FDA-approved drugs and identified entry inhibitors of SARS-CoV-2, including imatinib, mycophenolic acid (MPA), and quinacrine dihydrochloride (QNHC). Treatment at physiologically relevant levels of these drugs significantly inhibited SARS-CoV-2 infection of both hPSC-LOs and hPSC-COs. Together, these data demonstrate that hPSC-LOs and hPSC-COs infected by SARS-CoV-2 can serve as disease models to study SARS-CoV-2 infection and provide a valuable resource for drug screening to identify candidate COVID-19 therapeutics. [**note: this is an interesting development of two organoids that are susceptible to SARS-CoV-2 infection that can be used for drug discovery. They came up the three hits in an early study. While useful, I still want to understand the mechanism of inhibition.**]
https://www.nature.com/articles/s41586-020-2901-9

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing new

DIAGNOSTIC DEVELOPMENT

- Nothing new

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2020-11-03

If you have not done so already, GO VOTE!

One of the great rock bands of my college years was Creedence Clearwater Revival fronted by John Fogerty. Infighting led to the group's break up in 1972 and the performers went their own ways. Despite the short period of time, they recorded some iconic rock songs and also performed at Woodstock. Video recordings were not a big thing in that era but here is a studio recording of 'Bad Mood Rising': https://www.youtube.com/watch?v=w6iRNVwslM4 there are some nice recent music videos filmed to commemorate the release of a full set of all the band's recording. Here is 'Have You Ever Seen the Rain?': https://www.youtube.com/watch?v=u1V8YRJnr4Q and 'Long as I can See the Light': https://www.youtube.com/watch?v=BDfGblfOsZ4 this is great rock music and why not show their Woodstock performance: https://www.youtube.com/watch?v=75VQp8iGNF0

The Washington Posts comments on whether Arizona should relax the mask mandate that covers about 85% of the population. I say no! Here are the concerns you need to know about eating in a dining bubble. The CDC says you can still vote in person if you have tested positive for COVID-19; wear a mask, distance, and use plenty of hand sanitizer! These Utahans behaved foolishly on Halloween; let us hope that there it did not turn out to be a superspreading event! Here are some nursing homes that did not have large COVID-19 outbreaks. Dr. Birx weighs in on what is needed to tamp down the virus outbreak.

The New York Times has a story on a rapid SARS-CoV-2 antigen test that misses more infections than it spots. Here is coverage of a new CDC study showing that pregnant women may be at risk for complications from COVID-19. The Birx memo is also covered here. New cases of COVID-19 are rising in Colorado; the fight against SARS-CoV-2 requires continual vigilance! Health columnist Jane Brody discusses the twin whammy of seasonal affectation syndrome and COVID-19 fatigue.

A reader wrote to me yesterday asking about home COVID-19 testing and provided THIS LINK. It is a misnomer to call these 'at home' COVID-19 tests as they are just personal collection kits that allow one to collect either a saliva or nasal swab sample and return it to a laboratory for the actual testing. Both saliva and nasal swabs are effective in collecting samples that may contain SARS-CoV-2. A saliva sample may be more convenient and less invasive. My only suggestion is to make sure the laboratory that is doing the test is CLIA certified.

Nature have an article on age-specific mortality and immunity patterns of SARS-CoV-2. They look at death data from 45 countries and 22 seroprevalence studies.

The Lancet have an article on SARS-CoV-2 seroprevalence and transmission risk factors among high-risk close contacts. This is from Singapore. *Sharing a bedroom (multivariable odds ratio [OR] 5·38 [95% CI 1·82–15·84]; p=0·0023) and being spoken to by an index case for 30 min or longer (7·86 [3·86–16·02]; p<0·0001) were associated with SARS-CoV-2 transmission among household contacts. Among non-household contacts, exposure to more than one case (multivariable OR 3·92 [95% CI 2·07–7·40], p<0·0001), being spoken to by an index case for 30 min or longer (2·67 [1·21–5·88]; p=0·015), and sharing a vehicle with an index case (3·07 [1·55–6·08]; p=0·0013) were associated with SARS-CoV-2 transmission. Among both household and non-household contacts, indirect contact, meal sharing, and lavatory co-usage were not independently associated with SARS-CoV-2 transmission*.

Medscape have an article on how cannabis-based therapeutics might help fight COVID-19 inflammation. There are some clinical trials going on right now. Here are return to play recommendations for athletes with COVID-19 from the American College of Cardiology. J&J plans to begin testing of its COVID-19 vaccine in the 12-18 age group shortly. Novavax outlines plans for minority recruitment into its vaccine trials.

Kaiser Health News reports on Colorado State's approach to testing for COVID-19. It is a dual approach of sewage analysis and pooled saliva testing. I just looked at Purdue's COVID-19 dashboard and the latest week shows a positive test rate of 4% which is where it has been over the past several days.

MODELING

Estimating the size and infection severity of the SARS-CoV-2 epidemic is made challenging by inconsistencies in available data. The number of COVID-19 deaths is often used as a key indicator for the epidemic size, but observed deaths represent only a minority of all infections[1,2]. Additionally, the heterogeneous burden in nursing homes and variable reporting of deaths in elderly individuals can hamper direct comparisons across countries of the underlying level of transmission and mortality rates[3]. *Here we use age-specific COVID-19 death data from 45 countries and the results of 22 seroprevalence studies to investigate the consistency of infection and fatality patterns across multiple countries. We find that the age distribution of deaths in younger age groups (<65 years) is very consistent across different settings and demonstrate how this data can provide robust estimates of the share of the population that has been infected. We estimate that the infection-to-fatality ratio (IFR) is lowest among 5-9 years old, with a log-linear increase by age among individuals older than 30 years. Population age-structures and heterogeneous burdens in nursing homes explain some but not all of the heterogeneity between countries in infection-fatality ratios. Among the 45 countries included in our analysis, we estimate approximately 5% of these populations had been infected by the 1st of September 2020, with much higher transmission likely to have occurred in a number of Latin American countries*. This simple modelling framework can help countries assess the progression of the pandemic and can be applied wherever reliable age-specific death data exists. [**note: there is a great deal of heterogeneity among the countries. Large outbreaks in nursing homes and long-term care settings can skey the infection fatality rate.**] https://www.nature.com/articles/s41586-020-2918-0

Background: Close contact with children may provide cross-reactive immunity to SARs-CoV-2 due to more frequent prior coryzal infections from seasonal coronaviruses. Alternatively, close contact with children may increase risk of SARs-CoV-2 infection. We investigated whether risk of infection with SARs-CoV-2 and severe outcomes differed between adults living with and without children. Methods: Working on behalf of NHS England, we conducted a population-based cohort study using primary care data and pseudonymously-linked hospital and intensive care admissions, and death records, from patients registered in general practices representing 40% of England. Using multivariable Cox regression, we calculated fully-adjusted hazard ratios (HR) of outcomes from 1st February-3rd August 2020 comparing adults living with and without children in the household. Findings: Among 9,157,814 adults ≤65 years, living with children 0-11 years was not associated with increased risks of recorded SARS-CoV-2 infection, COVID-19 related hospital or ICU admission but was associated with reduced risk of COVID-19 death (HR 0.75, 95%CI 0.62-0.92). *Living with children aged 12-18 years was associated with a small increased risk of recorded SARS-CoV-2 infection (HR 1.08, 95%CI 1.03-1.13), but not associated with other COVID-19 outcomes. Living with children of any age was also associated with lower risk of dying from non-COVID-19 causes. Among 2,567,671 adults >65 years there was no association between living with children and outcomes related to SARS-CoV-2. We observed no consistent changes in risk following school closure. Interpretation: For adults living with children there is no evidence of an increased risk of severe COVID-19 outcomes. These findings have implications for determining the benefit-harm balance of children attending school in the COVID-19 pandemic*. [**note: here is a good cohort study of 12 million adults in England and the association of living with children during the pandemic. There appears to be no evidence of increased risk of severe COVID-19.**]
https://www.medrxiv.org/content/10.1101/2020.11.01.20222315v1

NEWLY REGISTERED CLINICAL TRIALS

- You need to be patient.

CLINICAL TRIAL RESULTS

- Clinical and molecular characterization by Whole Exome Sequencing (WES) is reported in 35 COVID-19 patients attending the University Hospital in Siena, Italy, from April 7 to May 7, 2020. Eighty percent of patients required respiratory assistance, half of them being on mechanical ventilation. Fiftyone percent had hepatic involvement and hyposmia was ascertained in 3 patients. Searching for common genes by collapsing methods against 150 WES of controls of the Italian population failed to give straightforward statistically significant results with the exception of two genes. This result is not unexpected since we are facing the most challenging common disorder triggered by environmental factors with a strong underlying heritability (50%). The lesson learned from Autism-Spectrum-Disorders prompted us to re-analyse the cohort treating each patient as an independent case, following a Mendelian-like model. *We identified for each patient an average of 2.5 pathogenic mutations involved in virus infection susceptibility and pinpointing to one or more rare disorder(s). To our knowledge, this is the first report on WES and COVID-19. Our results suggest a combined model for COVID-19 susceptibility with a number of common susceptibility genes which represent the favorite background in which additional host private mutations may determine disease progression.* [**note: this is from the Univ of Sienna hospital (a delightful city to visit) and looks at 35 patients using whole exome sequencing. The paper will appeal to geneticists and the authors note much more work in tis area is needed to confirm these findings and look for other genetic susceptibilities or protection to SARS-CoV-2.** https://www.medrxiv.org/content/10.1101/2020.05.22.20108845v2

DRUG DEVELOPMENT

- SARS-CoV-2 has caused a global pandemic of COVID-19 that urgently needs an effective treatment. Nucleoside analog drugs including favipiravir have been repurposed for COVID-19 despite of unclear mechanism of their inhibition of the viral RNA polymerase (RdRp). Here we report the cryo-EM structures of the viral RdRp in complex with favipiravir and two other nucleoside inhibitor drugs ribavirin and penciclovir. Ribavirin and the ribosylated form of favipiravir share a similar ribose scaffold that is distinct from penciclovir. *However, the structures reveal that all three inhibitors are covalently linked to the primer strand in a monophosphate form despite the different chemical scaffolds between favipiravir and penciclovir. Surprisingly, the base moieties of these inhibitors can form mismatched pairs with the template strand. Moreover, in view of the clinical disadvantages of remdesivir mainly associated with its prodrug form, we designed several orally-available remdesivir parent nucleoside derivatives, including VV16 that showed 5-fold more potent than remdesivir in inhibition of viral replication. Together, these results demonstrate an unexpected promiscuity of the viral RNA polymerase and provide a basis for repurpose and design of nucleotide analog drugs for COVID-19*. [**note: some good drug discovery work from China. I've seen papers on favipiravir and ribavirin but this is the first I've seen on penciclovir.** https://www.biorxiv.org/content/10.1101/2020.11.01.363812v1
- SARS-CoV2 is a single strand RNA virus member of the type 2 coronavirus family, responsible for causing COVID-19 disease in humans. The objective of this study was to test the ivermectin drug in a murine model of coronavirus infection using a type 2 family RNA coronavirus similar to SARS-CoV2, the mouse hepatitis virus (MHV). BALB/cJ female mice were infected with 6,000 PFU of MHV-A59 (Group Infected; n=20) and immediately treated with one single dose of 500 ug/kg

of ivermectin (Group Infected + IVM; n=20), or were not infected and treated with PBS (Control group; n=16). Five days after infection/treatment, mice were euthanized to obtain different tissues to check general health status and infection levels. Overall results demonstrated that viral infection induces the typical MHV disease in infected animals, with livers showing severe hepatocellular necrosis surrounded by a severe lymphoplasmacytic inflammatory infiltration associated with a high hepatic viral load (52,158 AU), while ivermectin administration showed a better health status with lower viral load (23,192 AU; $p<0.05$) and few livers with histopathological damage ($p<0.05$), not showing statistical differences with control mice (P=NS). Furthermore, serum transaminase levels (aspartate aminotransferase and alanine aminotransferase) were significantly lower in treated mice compared to infected animals. In conclusion*, ivermectin seems to be effective to diminish MHV viral load and disease in mice, being a useful model for further understanding new therapies against coronavirus diseases*. [**note: this is mouse data from Uruguay. I still have not seen good clinical trial data on ivermectin.**] https://www.biorxiv.org/content/10.1101/2020.11.02.363242v1

- There is a worldwide attempt to develop prevention strategies against SARS-CoV-2 transmission. Here we examined the effectiveness of visible light-responsive photocatalyst RENECAT on the inactivation of SARS-CoV-2 under different temperatures and exposure durations. The viral activation on the photocatalyst-coated glass slides decreased from 5.93∓0.38 logTCID50/ml to 3.05∓ 0.25 logTCID50/ml after exposure to visible light irradiation for 6h at 20 degree C. On the other hand, lighting without the photocatalyst, or the photocatalyst-coat without lighting retained viral stability. Immunoblotting and electron microscopic analyses showed the reduced amounts of spike protein on the viral surface after the photocatalyst treatment. Our data suggest a possible implication of the photocatalyst on the decontamination of the SARS-CoV-2 in indoor environments, thereby preventing indirect viral spread. [**note: this is a photocatalyst that helps inactivate SARS-CoV-2 on surfaces using visible light. I'm not sure about the utility of this given surface transmission may not be a major issue. The time needed for inactivation seems too long.**] https://www.biorxiv.org/content/10.1101/2020.11.01.364364v1
- Although ozone water is one of the promising candidates for hand hygiene to prevent fomite infection, the detailed effects of ozone water on SARS-CoV-2 have not been clarified. *We evaluated the inactivating effect of ozone water against SARS-CoV-2 by its concentration and exposure time. The reduction rates of virus titer after 5 sec treatment with ozone concentrations of 1, 4, 7, and 10 mg/L were 81.4%, 93.2%, 96.6%, and 96.6%, respectively. No further decrease in virus titer was observed by the extended exposure time over 5 sec. High-concentration ozone water was considered to be effective in promptly inactivating SARS-CoV-2 virus*. [**note: in contrast to the above paper, ozone water appears to be a good disinfectant.**] https://www.biorxiv.org/content/10.1101/2020.11.01.361766v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The immune response to SARS-CoV-2 is critical in both controlling primary infection and preventing re-infection. However, there is concern that immune responses following natural infection may not be sustained and that this may predispose to recurrent infection. We analysed the magnitude and phenotype of the SARS-CoV-2 cellular immune response in 100 donors at six months following primary infection and related this to the profile of antibody level against spike, nucleoprotein and RBD over the previous six months. T-cell immune responses to SARS-CoV-2

were present by ELISPOT or ICS analysis in all donors and are characterised by predominant CD4+ T cell responses with strong IL-2 cytokine expression. Median T-cell responses were 50% higher in donors who had experienced an initial symptomatic infection indicating that the severity of primary infection establishes a set-point for cellular immunity that lasts for at least 6 months. The T-cell responses to both spike and nucleoprotein/membrane proteins were strongly correlated with the peak antibody level against each protein. *The rate of decline in antibody level varied between individuals and higher levels of nucleoprotein-specific T cells were associated with preservation of NP-specific antibody level although no such correlation was observed in relation to spike-specific responses. In conclusion, our data are reassuring that functional SARS-CoV-2-specific T-cell responses are retained at six months following infection although the magnitude of this response is related to the clinical features of primary infection*. [**note: this is good news from the UK. In a sample of 100 convalescent COVID-19 patients T-cell immunity was maintained at six months following primary infection. Is still wonder about not including the Nucleoprotein antigen in some of the vaccine preparations as per the data in this paper.**] https://www.biorxiv.org/content/10.1101/2020.11.01.362319v1

- Background: During the pandemic of coronavirus disease 2019 (COVID-19), the genetic mutations occurred in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cumulatively or sporadically. In this study, we employed a computational approach to identify and trace the emerging patterns of the SARS-CoV-2 mutations, and quantify accumulative genetic distance across different periods and proteins. Methods: Full-length human SARS-CoV-2 strains in United Kingdom were collected. We investigated the temporal variation in the evolutionary genetic distance defined by the Hamming distance since the start of COVID-19 pandemic. Findings: *Our results showed that the SARS-CoV-2 was in the process of continuous evolution, mainly involved in spike protein (S protein), the RNA-dependent RNA polymerase (RdRp) region of open reading frame 1 (ORF1) and nucleocapsid protein (N protein). By contrast, mutations in other proteins were sporadic and genetic distance to the initial sequenced strain did not show an increasing trend*. [**note: here is a temporal pattern of some of the genetic changes in SARS-CoV-2 in strains from the UK.**]
  https://www.biorxiv.org/content/10.1101/2020.11.01.363739v1

DIAGNOSTIC DEVELOPMENT

- Nothing today

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2020-11-04

Today's music selection is another Wigmore Hall recital. Soprano Mary Bevan and pianist Joseph Middleton have a program songs inspired by the 'muses' of the day, both mythological and divine: https://www.youtube.com/watch?v=Ac3auh7z0Og I really enjoyed this recital.

Given the other news of the day, it is not surprising that there is little coverage of the pandemic.

The New York Times notes that college newspapers may be the only source of outbreaks on campus. The virus is surging in the Northeast; no part of the country is immune! China has stringent rules on travelers entering the country.

The Atlantic's Sarah Zhang on how preventing infections is the key to keeping COVID-19 deaths down. Some good ideas here.

The Lancet has a letter on the use of widespread smell testing for COVID-19 and the authors of the paper offer a reply.

MODELING

- Background: Covid-19 curve can be flattened by adopting mass screening protocols with aggressive testing and isolating infected populations. The current approach largely depends on RT-PCR/rapid antigen tests that require expert personnel resulting in higher costs and reduced testing frequency. Loss of smell is reported as a major symptom of Covid-19, however, a precise olfactory testing tool to identify Covid-19 patient is still lacking. Methods: To quantitatively check for the loss of smell, we developed an odor strip, COVID-Anosmia checker, spotted with gradients of coffee and lemon grass oil. We validated its efficiency in healthy and COVID-19 positive subjects. A trial screening to identify SARS-CoV-2 infected persons was also carried out to check the sensitivity and specificity of our screening tool. Results: *It was observed that COVID positive participants were hyposmic instead of being anosmic when they were subjected to smelling higher odor concentration. Our tool identified 97% of symptomatic and 94% of asymptomatic COVID-19 positive subjects after excluding most confounding factors like concurrent chronic sinusitis. Further, it was possible to reliably predict COVID-19 infection by calculating a loss of smell score with 100% specificity. We coupled this tool with a mobile application, which takes the input response from the user, and can readily categorize the user in the appropriate risk groups. Conclusion: Loss of smell can be used as a reliable marker for screening for Covid-19. Our tool can rapidly quantitate anosmia, hyposmia, parosmia, and can be used as a first-line screening tool to trace out Covid-19 infection effectively*. [**note: this is from India and they have developed a screening tool for the loss of smell. I knew someone would jump on this!!! They may be in violation of my patent! Just joking as it is just a provisional patent.**] https://www.medrxiv.org/content/10.1101/2020.10.28.20221200v1
- Past research has established the value of social distancing as a means of deterring the spread of COVID-19 largely by examining aggregate level data. Locales in which efforts were undertaken to encourage distancing experienced reductions in their rate of transmission. However, these aggregate results tell us little about the effectiveness of social distancing at the level of the individual, which is the question addressed by the current research. Four months after participating in a study assessing their social distancing behavior, 2,120 participants indicated whether they had contracted COVID-19. Importantly, the assessment of social distancing involved not only a self-report measure of how strictly participants had followed social distancing recommendations, but also a series of virtual behavior measures of social distancing. These simulations presented participants with graphical depictions mirroring specific real-world scenarios, asking them to position themselves in relation to others in the scene. *Individual social distancing behavior, particularly as assessed by the virtual behavior measure, predicted whether they contracted COVID-19 during the intervening four months. This was true when considering*

only participants who reported having tested positively for the virus and when considering additional participants who, although untested, believed that they had contracted the virus. The findings offer a unique form of additional evidence as to why individuals should practice social distancing. What the individual does matters, not only for the health of the collective, but also for the specific individual. [**note: this is from Ohio State and shows the positive impact of social distancing. Do it for yourself, and do it for others.**]
https://www.medrxiv.org/content/10.1101/2020.10.29.20222422v1

- In a previous work [Huber et al, A minimal model for household effects in epidemics. Physical Biology, 17(6):065010], we discussed virus transmission dynamics modified by a uniform clustering of contacts in the population: close contacts within households and more distant contacts between households. In this paper, we discuss testing and tracing in such a stratified population. We propose a minimal tracing strategy consisting of random testing of the entire population plus full testing of the households of those persons found positive. We provide estimates of testing frequency for this strategy to work. [**note: this is a second paper from this group, this one looking at a minimal model for household-based testing and tracing in epidemics. There are some very useful graphs in the paper.**]
https://www.medrxiv.org/content/10.1101/2020.10.29.20222406v1
- Background. The ability to preferentially protect high-groups in COVID-19 is hotly debated. Here, the aim is to present simple metrics of such precision shielding of people at high-risk of death after infection by SARS-CoV-2; demonstrate how they can estimated; and examine whether precision shielding was successfully achieved in the first COVID-19 wave. Methods. The shielding ratio, S, is defined as the ratio of prevalence of infection among people at a high-risk group versus among people in a low-risk group. The contrasted risk groups examined here are according to age (>=70 versus <70 years), and institutionalized (nursing home) setting. For age-related precision shielding, data were used from large seroprevalence studies with separate prevalence data for elderly versus non-elderly and with at least 1000 assessed people >=70 years old. For setting-related precision shielding, data were analyzed from 10 countries where information was available on numbers of nursing home residents, proportion of nursing home residents among COVID-19 deaths, and overall population infection fatality rate. Findings. Across 17 seroprevalence studies, the shielding ratio S for elderly versus non-elderly varied between 0.4 (substantial shielding) and 1.6 (substantial inverse protection, i.e. low-risk people being protected more than high-risk people). Five studies in USA all yielded S=0.4-0.8, consistent with some shielding being achieved, while two studies in China yielded S=1.5-1.6, consistent with inverse protection. *Assuming 25% infection fatality rate among nursing home residents, S values for nursing home residents ranged from 0.07 to 3.1. The best shielding was seen in South Korea (S=0.07) and modest shielding was achieved in Israel, Slovenia, Germany, and Denmark. No shielding was achieved in Hungary and Sweden. In Belgium (S=1.9), UK (S=2.2) and Spain (S=3.1), nursing home residents were far more frequently infected that the rest of the population. Interpretation. The experience from the first wave of COVID-19 suggests that different locations and settings varied markedly in the extent to which they protected high-risk groups. Both effective precision shielding and detrimental inverse protection can happen in real-life circumstances. COVID-19 interventions should seek to achieve maximal precision shielding*. [**note: this is from Stanford's oft contrarian, Prof Ioannidis and offers a model for precision**

shielding and how to assess it.]
https://www.medrxiv.org/content/10.1101/2020.11.01.20224147v1

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been detected in domestic and wild cats. However, little is known about natural viral infections of domestic cats, although their importance for modeling disease spread, informing strategies for managing positive human-animal relationships and disease prevention. Here, we describe the SARS-CoV-2 infection in a household of two human adults and sibling cats (one male and two females) using real-time RT-PCR, an ELISA test, viral sequencing, and virus isolation. On May 5th, 2020, the cat-owners tested positive for SARS-CoV-2. Two days later, the male cat showed mild respiratory symptoms and tested positive. Four days after the male cat, the two female cats became positive, asymptomatically. Also, one human and one cat showed antibodies against SARS-CoV-2. All cats excreted detectable SARS-CoV-2 RNA for a shorter duration than humans and viral sequences analysis confirmed human-to-cat transmission. We could not determine if cat-to-cat transmission also occurred. [**note: this one is for all you cat owners though it's only a single case study.**] https://www.medrxiv.org/content/10.1101/2020.10.31.20220608v1
- How often does one perform hand disinfection while wearing a mask? In the current COVID-19 pandemic, wearing masks and hand disinfection are widely adopted hygiene practices. However, our study indicated that exposure to the vapors from alcohol-based sanitizers during hand disinfection might degrade the filtration performance of the in-use masks, and the degradation worsened with the increasing number of hand disinfection. After five times of hand disinfection, the filtration efficiencies of surgical masks decreased by >8% for 400 and 500nm particles and by 3.68±1.83% for 1μm particles. This was attributed to the dissipation of electrostatic charges on the masks when exposed to the alcohol vapor generated during hand disinfection. Simple practice of vapor-avoiding hand disinfection could mitigate the effects of alcohol vapor, which was demonstrated on two brands of surgical masks. The vapor-avoiding hand disinfection is recommended to be included in the hygiene guide to maintain the mask performance. [**note: dammed if you do damned if you don't. This study shows that alcohol based hand sanitizers can degrade the performance of certain masks.**]
https://www.medrxiv.org/content/10.1101/2020.11.01.20223982v1

NEWLY REGISTERED CLINICAL TRIALS

- Not today.

CLINICAL TRIAL RESULTS

- Abstract Background: Cognitive impairment is common following critical illness. A number of case reports and case series have suggested that cognitive deficits occur in patients with COVID-19. This study evaluated the frequency, severity, and profile of cognitive dysfunction in hospitalized patients recovering from COVID-19. Methods: We obtained and analyzed cross-sectional neuropsychological data from a cohort of N=57 patients participating in inpatient rehabilitation. Our primary outcome measure was the Brief Memory and Executive Test (BMET). We calculated the frequency of impairment based on clinician diagnosis and by the BMET subtests using age-normed classification of impairment. We explored associations with intubation and extubation as markers of illness severity and complications, as well as psychiatric diagnosis. Results: Our sample was 75% male, 61% non-white, with a mean age of 64.5 (SD =

13.9) years. Patients were evaluated at a mean of 43.2 days post-admission. 88% had documented hypoxemic respiratory failure and 77% required intubation. 81% of patients had cognitive impairment, ranging from mild to severe. Deficits were most common in working memory (55% of patients impaired), set-shifting (47%), divided attention (46%), and processing speed (40%). Executive dysfunction was not significantly associated with intubation length or the time from extubation to assessment, nor was it associated with the presence of a psychiatric diagnosis. Discussion and Conclusion: *Medically stable inpatients recovering from COVID-19 commonly have deficits in attention and executive functions. These deficits were not significantly correlated with length of intubation or time since extubation. Findings provide an early benchmark for studying the evolution of cognitive difficulties after COVID-19 and suggest that easy to disseminate interventions that remediate attention and executive dysfunctions may be important in this population*. [**note: here is a study from Cornell on cognitive deficits in hospitalized patients recovering from COVID-19.**]
https://www.medrxiv.org/content/10.1101/2020.10.28.20221887v1

- Residual SARS-CoV-2 RNA has been detected in stool samples and gastrointestinal tissues during the convalescence phase of COVID-19 infection. This raises concern for persistence of SARS-CoV-2 virus particles and faecal-oral transmissibility in recovered COVID-19 patients. *Using multiplex immunohistochemistry, we unexpectedly detected SARS-CoV-2 viral antigens in intestinal and liver tissues, in surgical samples obtained from two patients who recovered from COVID-19. We further validated the presence of virus by RT-PCR and flow cytometry to detect SARS-CoV-2-specific immunity in the tissues. These findings might have important implications in terms of disease management and public health policy regarding transmission of COVID-19 via faecal-oral and iatrogenic routes during the convalescence phase*. [**note: this is from Singapore and is only two patients. However the finding of viral antigens in gastrointestinal and hepatic tissues might be of concern if convalescent patients still harbor the virus.**]
https://www.medrxiv.org/content/10.1101/2020.10.28.20219014v1
- BACKGROUND: COVID-19 has caused havoc across the globe since, no specific treatment exists for this disease, thus, far. Hence, there is an urgent need to find an effective treatment to mitigate this scourge. Honey and Nigella sativa are two natural substances with anti-inflammatory, anti-viral, anti-microbial and immune modulating properties. They could be potentially beneficial in these patients. METHODS: We conducted an add-on, randomized, open label, placebo-controlled clinical trial using parallel group design. This was a multi-centered study with superiority framework conducted in RT-PCR confirmed COVID-19 patients showing moderate or severe disease. All patients receiving standard care were randomized into treatment and control groups. In the treatment arm, patients received HNS (honey plus Nigella sativa) in predefined doses for up to 13 days. The primary outcome measures (time taken for alleviation of symptoms, viral clearance and clinical status improvement on day 6) outcomes were assessed. RESULTS: Of 1046 patients testing positive for the SARS-CoV-2, 210 showing moderate and 103 showing severe disease were randomized into treatment and control groups as per inclusion criteria. In the moderate cases, 107 were assigned to the HNS group and 103 to the control group. Among 103 severe cases, 50 were assigned to the HNS group and 53 to the control group. In the moderate and severe cases, the HNS treatment was associated with a normalized median symptoms alleviation time reduction of 3 and 7 days (HR: 6.11; 95% CI: 4.23-8.84 and HR: 4.04; 95% CI, 2.46-6.64) respectively. The HNS treatment in both groups were

further associated with 4 days earlier reduction in median viral clearance time (Moderate HR: 5.53; 95% CI: 3.76-8.14) and Severe HR: 4.32; 95% CI: 2.62-7.13). Moreover, in the intention-to-treat analysis, the HNS groups led to a lower (better) clinical score on day 6 with resumption of normal activity among 63.6% of the moderate (OR: 0.07; 95% CI: 0.03-0.13) and 28% of severe cases (OR: 0.03; 95% CI: 0.01-0.09). Furthermore, a significant (14.87%) reduction (OR: 0.18; 95% CI: 0.02-0.92) in mortality was observed in the HNS arm. No difference in adverse effects were seen between the HNS and control arms. CONCLUSIONS: *A significant reduction in in the severity of disease, the time taken for viral clearance and mortality was observed with HNS treatment in COVID-19 patients. HNS represents a safe, effective, over the counter and affordable therapy for this pandemic essentially lowering health care burden. It can be used alone or in combination with other expensive treatments and give an additive effect. Hence, the potential of HNS against COVID-19 should be explored in future larger studies. (Funded by Smile Welfare Organization, Shaikh Zayed Medical Complex and Services Institute of Medical Sciences; NIH Clinical Trial Register number*: NCT04347382.) [**note: I was waiting for these results ever since I posted the link to the Pakistani trial. It looks like there is a treatment effect but the numbers of patients in the trial are really on the small side!**]
https://www.medrxiv.org/content/10.1101/2020.10.30.20217364v1

- Summary: No antiviral has been shown to reduce mortality in SARS-COV-2 patients to date. In the present observational and retrospective report, 3,099 patients with a definitive or highly probable diagnosis of infection due to COVID-19 were evaluated between May 1st to August 10th, 2020, at Centro Medico Bournigal (CMBO) and Centro Medico Punta Cana (CMPC), and all received compassionate treatment with Ivermectin. A total of 2,706 (87.3%) were discharged for outpatient treatment, all with mild severity of the infection. In 2,688 (99.33%) with outpatient treatment, the disease did not progress to warrant further hospitalization and there were no deaths. In 16 (0.59%) with outpatient treatment, it was necessary their subsequent hospitalization to a room without any death. In 2 (0.08%) with outpatient treatment, it was necessary their admission to the Intensive Care Unit (ICU) and 1 (0.04%) patient died. There were 411 (13.3%) patients hospitalized, being admitted at a COVID-19 room with a moderate disease 300 (9.7%) patients of which 3 (1%) died; and with a severe to critical disease were hospitalized in the ICU 111 (3.6%), 34 (30.6%) of whom died. The mortality percentage of patients admitted to the ICU of 30.6%, is similar with the percentage found in the literature of 30.9%. Total mortality was 37 (1.2%) patients, which is much lower than that reported in world statistics, which are around 3%. [**note: this is a compassionate use study of ivermectin from the Dominican Republic. It does not mean much as there was no control arm so we don't know whether it works or not.**]
https://www.medrxiv.org/content/10.1101/2020.10.29.20222505v1
- Background: Ivermectin is one among several potential drugs explored for its therapeutic and preventive role in COVID-19 infection. The study was aimed to explore the association between ivermectin prophylaxis and development of COVID-19 infection among healthcare workers. Methods: A hospital-based matched case-control study was conducted among healthcare workers of AIIMS Bhubaneswar, India, from September to October 2020. Profession, gender, age and date of diagnosis were matched for 186 case-control pairs. Cases and controls were healthcare workers who tested positive and negative, respectively, for COVID-19 by RT-PCR. Exposure was defined as the intake of ivermectin and/or hydroxychloroquine and/or vitamin-C

and/or other prophylaxis for COVID-19. Data collection and entry was done in Epicollect5, and analysis was performed using STATA version 13. Conditional logistic regression models were used to describe the associated factors for COVID-19 infection. Results: Ivermectin prophylaxis was taken by 77 controls and 38 cases. Two-dose ivermectin prophylaxis (0.27, 95% CI, 0.15-0.51) was associated with 73% reduction of COVID-19 infection among healthcare workers for the following one month, those who were involved in physical activity (3.06 95% CI, 1.18-7.93) for more than an hour/day were more likely to contract COVID-19 infection. Type of household, COVID duty, single-dose ivermectin prophylaxis, vitamin-C prophylaxis and hydroxychloroquine prophylaxis were not associated with COVID-19 infection. Conclusion: *Two-dose ivermectin prophylaxis at a dose of 300 µg/kg with a gap of 72 hours was associated 73% reduction of COVID-19 infection among healthcare workers for the following one-month. Further research is required before its large scale use*. [**note: here is a study of ivermectin as a prophylaxis treatment in healthcare providers in India. More data is needed.**]
https://www.medrxiv.org/content/10.1101/2020.10.29.20222661v1

- Background: While there was a lack of pharmacological interventions proven to be effective in early, outpatient settings for COVID-19, in a prospective, open-label observational study (pre-AndroCoV Trial) the use of nitazoxanide, ivermectin and hydroxychloroquine demonstrated similar effects, and apparent improvement of outcomes compared to untreated patients. The unexpected apparent positive results led to ethical questions on the employment of further full placebo-control studies in early stage COVID-19. The objective of the present study was to elucidate whether the conduction of a full placebo-control RCT was still ethically viable, through a comparative analysis with two control-groups. Materials and methods: Active group (AG) consisted of mild-to-moderate early stage COVID-19 patients enrolled in the Pre AndroCoV-Trial, treated with nitazoxanide ivermectin, or hydroxychloroquine in selected cases, in association with azithromycin. Vitamin D, vitamin C, zinc, glucocorticoids and anticoagulants, when clinically recommended. Control Group 1 (CG1) consisted of a retrospectively obtained group of untreated patients from the same population as those from the Pre-AndroCoV Trial, and Control Group 2 (CG2) resulted from a precise prediction of clinical outcomes, based on a thorough and structured review of articles indexed in PubMed and MEDLINE and statements by official government agencies and specific medical societies. For both CGs, patients were matched for proportion between sex, age, obesity and other comorbidities. Results: Compared to CG1 and CG2, AG showed a reduction of 31.5 to 36.5% in viral shedding ($p < 0.0001$), 70 to 85% and 70 to 73% in duration of COVID-19 clinical symptoms when including and not including anosmia and ageusia, respectively (($p < 0.0001$ for both), and 100% in respiratory complications through the parameters of the Brescia COVID-19 Respiratory Scale ($p < 0.0001$). For every 1,000 confirmed cases for COVID-19, a minimum of 140 patients were prevented from hospitalization ($p < 0.0001$), 50 from mechanical ventilation, and five deaths, when comparing to age-, sex- and comorbidity-matched non-treated patients with similar initial disease severity at the moment of diagnosis. Conclusion: *Apparent benefits of the combination between early detection and early pharmacological approaches for COVID-19 demonstrated to be consistent when when compared to different control groups of untreated patients. The potential benefits could allow a large number of patients prevented from hospitalizations, deaths and persistent symptoms after COVID-19 remission. The potential impact on COVID-19 disease course and numbers of negative outcomes and the well-established safety profile of the drugs proposed by the Pre-AndroCoV*

Trial led to ethical questions regarding the conduction of further placebo control randomized clinical trials (RCTs) for early COVID-19. Early pharmacological approaches including azithromycin in combination with any of the options between nitazoxanide, ivermectin or optionally hydroxychloroquine should be considered for those diagnosed with COVID-19 presenting less than seven days of symptoms. Of the three drugs, we opted for nitazoxanide, due to more extensive demonstration of in vitro and in vivo antiviral activity, proven efficacy against other viruses in humans, and steadier safety profile. [**note: I don't know what to make of these open label trials and whether the data are reliable enough to draw conclusions. HCQ has been tested backwards and forwards and the majority of studies show no treatment effect. Ivermectin results are all over the map and nitazoxanide trial data is not in yet. Caveat Emptor.**] https://www.medrxiv.org/content/10.1101/2020.10.31.20223883v1

DRUG DEVELOPMENT

- Bacillus Calmette Guerin (BCG) is widely used in national vaccination programs worldwide. It is accepted that BCG alleviates both pathogen and allergy induced respiratory diseases that could also include Covid-19. To investigate this possibility, we randomly assigned 60 Covid-19 patients, after admission to the hospital with pneumonia and requirement for oxygen therapy in a 1:1 ratio to receive either a single adult dose of intradermal BCG or normal saline with concomitant standard of care (SoC) medications. Primary endpoints were favorable prognosis of Covid-19 as deduced from resolution of pneumonia, viremia and secondary outcome were enumeration of ICU admissions, duration thereof and mortalities. Results Both primary and secondary endpoints were significantly improved in the BCG+SoC group. This could be seen from reduction in oxygen requirement due to Covid-19 associated pneumonia decreasing from day 3-4, improved radiological resolution from day 7-15. There were a total of 6 (10%) adverse events in the study of which 2 deaths and 4 ICU admissions were in SoC group (1 ICU admission culminated in death of the subject) and in contrast only 1 ICU admission in the BCG+SoC group. While there was an increase in Covid-19 specific IgG levels in the BCG+SoC group, there was no evidence of BCG induced cytokine storm in this group. Four patients showed localized inflammatory response at the injection site in the BCG+SoC group. Conclusions BCG+SoC administration resulted in a significantly higher percentage of patients with favorable outcomes than did SoC. A third of the patients were naive for childhood BCG vaccination. This mimicked elderly patients in countries with no universal vaccination policy for BCG. No BCG related adversity was seen in this group. *The study shows that BCG is a safe, cost-effective treatment that can be introduced as a standard of care in patients with moderate Covid-19 that can reduce requirement of oxygen supplemented beds and disease burden in low resource countries, with additional long-term benefits of reducing risk for tuberculosis*. [**note: this is a small trial of BCG vaccine and I don't think there are enough patients to draw a reliable conclusion. There are larger trials underway.**] https://www.medrxiv.org/content/10.1101/2020.10.28.20221630v1
- The ongoing of coronavirus disease 2019 (COVID-19) pandemic caused by novel SARS-CoV-2 coronavirus, resulting in economic losses and seriously threating the human health in worldwide, highlighting the urgent need of a stabilized, easily produced and effective preventive vaccine. The SARS-COV-2 spike protein receptor binding region (RBD) plays an important role in the process of viral binding receptor angiotensin-converting enzyme 2 (ACE2) and membrane fusion, making it an ideal target for vaccine development. In this study, we designed three

different RBD-conjugated nanoparticles vaccine candidates, RBD-Ferritin (24-mer), RBD-mi3 (60-mer) and RBD-I53-50 (120-mer), with the application of covalent bond linking by SpyTag-SpyCatcher system. It was demonstrated that the neutralizing capability of sera from mice immunized with three RBD-conjugated nanoparticles adjuvanted with AddaVax or Sigma Systerm Adjuvant (SAS) after each immunization was ~8- to 120-fold greater than monomeric RBD group in SARS-CoV-2 pseudovirus and authentic virus neutralization assay. Most importantly, sera from RBD-conjugated NPs groups more efficiently blocked the binding of RBD to ACE2 or neutralizing antibody in vitro, a further proof of promising immunization effect. Besides, high physical stability and flexibility in assembly consolidated the benefit for rapid scale-up production of vaccine. These results supported that our designed SARS-CoV-2 RBD-conjugated nanoparticle was competitive vaccine candidate and the carrier nanoparticles could be adopted as universal platform for future vaccine development. [**note: here is new vaccine approach from China but I've seen a similar approach to this one.**]
https://www.biorxiv.org/content/10.1101/2020.11.03.366138v1

- Respiratory viruses initially infect the naso- and oropharyngeal regions, where they amplify, cause symptoms and may also be transmitted to new hosts. Preventing initial infection or reducing viral loads upon infection might soothe symptoms, prevent dissemination into the lower airways, or transmission to the next individual. We here analyzed the potential of plant derived products to inactivate SARS-CoV-2 and influenza virus. *We found that black chokeberry (Aronia melanocarpa) juice, pomegranate (Punica granatum) juice, and green tea (Camellia sinensis) have virucidal activity against both viruses, suggesting that oral rinsing may reduce viral loads in the oral cavity thereby lowering virus transmission.* [**note: here is another paper for you natural products fans. Stock up on these items.**]
https://www.biorxiv.org/content/10.1101/2020.10.30.360545v1
- Data on potential effectiveness and prospects of treatment of new coronavirus infection of COVID-19 caused by virus SARS-CoV-2 with the help of antisense oligonucleotides acting against RNA of virus on an in vitro model are given. The ability of antisense oligonucleotides to suppress viral replication in diseases caused by coronaviruses using the example of SARS and MERS is shown. The identity of the initial regulatory section of RNA of various coronaviruses was found within 50 - 100 nucleotides from the 5'-end, which allows using antisense suppression of this RNA fragment. A new RNA fragment of the virus present in all samples of coronovirus SARS-CoV-2 has been identified, the suppression of which with the help of an antisense oligonucleotide can be effective in the treatment of COVID-19. The study of the synthesized antisense oligonucleotide 5`-AGCCGAGTGACAGCC ACACAG, complementary to the selected virus RNA sequence, was carried out. The low toxicity of the preparations of this group in the cell culture study and the ability to reduce viral load at high doses according to real time-PCR data are shown. The cytopathogenic dose exceeds 2 mg/ml. At a dosage of 1 mg/ml, viral replication is reduced by 5 - 13 times. Conclusions are made about the prospects of this direction and the feasibility of using the inhalation way of drug administration into the body. [**note: here is an antisense paper from Russia but there is a California company that already has this approach in a clinical trial.**] https://www.biorxiv.org/content/10.1101/2020.11.02.363598v1
- Infections with respiratory viruses can spread via liquid droplets and aerosols, and cause diseases such as influenza and COVID-19. Face masks and other personal protective equipment (PPE) can act as barriers that prevent the spread of respiratory droplets containing these

viruses. However, influenza A viruses and coronaviruses are stable for hours on various materials, which makes frequent and correct disposal of these PPE important. Metal ions embedded into PPE may inactivate respiratory viruses, but confounding factors such as absorption make measuring and optimizing the inactivation characteristics difficult. Here we used polyamide 6.6 (PA66) fibers that had zinc ions embedded during the polymerisation process and systematically investigated if these fibers can absorb and inactivate pandemic SARS-CoV-2 and influenza A virus H1N1. We find that these viruses are readily absorbed by PA66 fabrics and inactivated by zinc ions embedded into this fabric. The inactivation rate (pfu.gram-1.min-1) exceeds the number of active virus particles expelled by a cough and supports a wide range of viral loads. Overall, these results provide new insight into the development of "pathogen-free" PPE and better protection against RNA virus spread. [**note: when will zinc impregnated masks come on the market?]**
https://www.biorxiv.org/content/10.1101/2020.11.02.365833v1

- The development of a vaccine against COVID-19 is a hot topic for many research laboratories all over the world. *Our aim was to design a semi-split inactivated vaccine offering a wide range of multi-epitope determinants important for the immune system including not only the spike (S) protein but also the envelope, membrane and nucleocapsid proteins*. We designed a semi-split vaccine prototype consisting of S protein-depleted viral particles and free S protein. Next, we investigated its immunogenic potential in BALB/c mice. The animals were immunized intradermally or intramuscularly with the dose adjusted with buffer or addition of aluminum hydroxide, respectively. The antibody response was evaluated by plasma analysis at 7 days after the first or second dose. The immune cell response was studied by flow cytometry analysis of splenocytes. *The data showed a very early onset of both S protein-specific antibodies and virus-neutralizing antibodies at 90% inhibition regardless of the route of vaccine administration. However, significantly higher levels of neutralizing antibodies were detected in the intradermally (geometric mean titer - GMT of 7.8 ± 1.4) than in the intramuscularly immunized mice (GMT of 6.2 ± 1.5). In accordance with this, stimulation of cellular immunity by the semi-split vaccine was suggested by elevated levels of B and T lymphocyte subpopulations in the murine spleens. These responses were more predominant in the intradermally immunized mice compared with the intramuscular route of administration. The upward trend in the levels of plasmablasts, memory B cells, Th1 and Th2 lymphocytes, including follicular helper T cells, was confirmed even in mice receiving the vaccine intradermally at a dose of 0.5 µg. We demonstrated that the semi-split vaccine is capable of eliciting both humoral and cellular immunity early after vaccination. Our prototype thus represents a promising step toward the development of an efficient anti-COVID-19 vaccine for human use*. [**note: it's likely that we have more SARS-CoV-2 vaccine prototypes than for any other infectious disease. Here is a Czech entry that uses a semi-spilt inactivated vaccine. As I have noted, including antigens other than the Spike protein may be the road to a better vaccine.**] https://www.biorxiv.org/content/10.1101/2020.11.03.366641v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Background. Real-time genomic sequencing has played a major role in tracking the global spread and local transmission of SARS-CoV-2, contributing greatly to disease mitigation strategies. After effectively eliminating the virus, New Zealand experienced a second outbreak of SARS-CoV-2 in August 2020. During this August outbreak, New Zealand utilised genomic sequencing in a

primary role to support its track and trace efforts for the first time, leading to a second successful elimination of the virus. Methods. We generated the genomes of 80% of the laboratory-confirmed samples of SARS-CoV-2 from New Zealand's August 2020 outbreak and compared these genomes to the available global genomic data. Findings. *Genomic sequencing was able to rapidly identify that the new COVID-19 cases in New Zealand belonged to a single cluster and hence resulted from a single introduction. However, successful identification of the origin of this outbreak was impeded by substantial biases and gaps in global sequencing data. Interpretation. Access to a broader and more heterogenous sample of global genomic data would strengthen efforts to locate the source of any new outbreaks*. [**note: here is a genomics study from New Zealand that points up the importance and limitations of genomic sequencing. There are large databases around and they need to be accessible to public health researchers.]** https://www.medrxiv.org/content/10.1101/2020.10.28.20221853v1

- **Background.** Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) causes Coronavirus disease-19 (COVID-19), a respiratory illness with influenza-like symptoms that can result in hospitalization or death. We investigated human genetic determinants of COVID-19 risk and severity in 455,838 UK Biobank participants, including 2,003 with COVID-19. **Methods.** We defined eight COVID-19 phenotypes (including risks of infection, hospitalization and severe disease) and tested these for association with imputed and exome sequencing variants. **Results.** We replicated prior COVID-19 genetic associations with common variants in the 3p21.31 (in *LZTFL1*) and 9q34.2 (in *ABO*) loci. The 3p21.31 locus (rs11385942) was associated with disease severity amongst COVID-19 cases (OR=2.2, $P=3x10^{-5}$), but not risk of SARS-CoV-2 infection without hospitalization (OR=0.89, P=0.25). *We identified two loci associated with risk of infection at $P<5x10^{-8}$, including a missense variant that tags the epsilon 4 haplotype in* APOE *(rs429358; OR=1.29, $P=9x10^{-9}$). The association with rs429358 was attenuated after adjusting for cardiovascular disease and Alzheimer's disease status (OR=1.15, P=0.005). Analyses of rare coding variants identified no significant associations overall, either exome-wide or with (i) 14 genes related to interferon signaling and reported to contain rare deleterious variants in severe COVID-19 patients; (ii) 36 genes located in the 3p21.31 and 9q34.2 GWAS risk loci; and (iii) 31 additional genes of immunologic relevance and/or therapeutic potential.* ***Conclusions.*** *Our analyses corroborate the association with the 3p21.31 locus and highlight that there are no rare protein-coding variant associations with effect sizes detectable at current sample sizes. Our full analysis results are publicly available, providing a substrate for meta-analysis with results from other sequenced COVID-19 cases as they become available*. Association results are available at https://rgc-covid19.regeneron.com [**note: here is another study looking at genomic determinants, this time using the UK Biobank. I want to see more papers such as this and the one from the other day. We may be honing in on genetic linkages that predispose to severe COVID-19**] https://www.medrxiv.org/content/10.1101/2020.10.28.20221804v1
- Despite the growing knowledge of T cell responses and their epitopes in COVID-19 patients, there is a lack of detailed characterizations for T cell-antigen interactions and T cell functions. *Using a peptide library predicted with HLA class I-restriction, specific CD8+ T cell responses were identified in over 75% of COVID-19 convalescent patients. Among the 15 SARS-CoV-2 epitopes identified from the S and N proteins, N361-369 (KTFPPTEPK) was the most dominant epitope. Importantly, we discovered 2 N361-369-specific T cell receptors (TCRs) with high functional avidity, and they exhibited complementary cross-reactivity to reported N361-369 mutant*

variants. In dendritic cells (DCs) and the lung organoid model, we found that the N361-369 epitope could be processed and endogenously presented to elicit the activation and cytotoxicity of CD8+ T cells ex vivo. Our study evidenced potential mechanisms of cellular immunity to SARS-CoV-2, illuminating natural ways of viral clearance with high relevancy in the vaccine development. [**note: Here is more information on T cell receptors and epitopes.]** https://www.biorxiv.org/content/10.1101/2020.11.02.364729v1

- *A polymorphism in the LZTFL1 gene located in the chemokine-receptor gene cluster (chromosome 3p) has been associated with the risk of developing COVID-19. The chemokine receptor-5 (CCR5) maps to this region, and the common 32 bp deletion variant (Δ32) has been associated with the extent of inflammatory disease and the outcome in several viral diseases*. Several studies have also suggested that the pharmacological targeting of CCR5 could reduce the impact of SARS-CoV-2 infection and the severity of COVID-19. We sought to investigate whether this polymorphism was associated with the risk of moderate-severe COVID-19. We genotyped 294 patients who required hospitalization due to COVID-19 (85 were severe cases) and 460 controls. We found a significantly lower frequency of CCR5-Δ32 among the COVID-19 patients (0.10 vs 0.18 in controls; p=0.002, OR=0.48, 95%CI=0.29-0.76). The difference was mainly due to the reduced frequency of CCR5-Δ32 carriers in the severe, significantly lower than in the non-severe patients (p=0.036). *Of note, we did not find deletion-homozygotes among the patients compared to 1% among controls. We also confirmed the association between a LZTFL1 variant and COVID-19. Our study points to CCR5 as a promising target for treatment of COVID-19, but requires validation in additional large cohorts. In confirmed by others, the genetic analysis of CCR5-variants (such as Δ32) might help to identify patients with a higher susceptibility to severe COVID-19*. [**note: this is from Spain and offers another genetic link severe COVID-19 susceptibility. I do hope someone will write a nice review summarizing all this; I'm just a one man band.**] https://www.medrxiv.org/content/10.1101/2020.11.02.20224659v1

DIAGNOSTIC DEVELOPMENT

- Background: Deep throat saliva (DTS) and pooled nasopharyngeal swab and throat swab (NPSTS) are utilized for viral detection. DTS is challenging for children. Swabbing the respiratory mucosa requires trained personnel and may trigger sneezing and coughing, which generate droplets. A reliable, simple and safe sampling method applicable to a wide age range is required for community-based surveillance. Methods: We introduced nasal strip as an easy and low-risk collection method. Asymptomatic and symptomatic SARS-CoV-2 infected patients (n = 38) were recruited. Nasal epithelial lining fluid (NELF) (n = 43) strip paired with nasal swab (n = 13) were collected by a healthcare worker to compare with NPSTS (n = 21) or DTS (n =22) collected within 24 hours as reference. All samples were subjected to viral RNA quantitation by real-time PCR targeting the nucleoprotein gene. Results: Comparable Ct values were observed between paired nasal strip and nasal swab samples. The agreement between nasal strip samples and NPSTS was 94.44% and 100% for NPSTS positive and negative samples. Higher viral RNA concentration was detected in nasal strips than DTS samples. False-negative results were recorded in six DTS specimens, of which four were from children. Storage at room temperature up to 72 (n = 3) hours did not affect diagnostic yield of nasal strips. Conclusions: Nasal strip is a reliable and non-invasive sampling method for SARS-CoV-2 detection, and viral detection remains stable for at least 72 hours. It can be used as an alternative tool for community-based surveillance. [**note:**

this is a useful approach to collecting nasal swabs from children for diagnostic testing.]
https://www.medrxiv.org/content/10.1101/2020.10.28.20220673v1

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2020-11-05

Happy Haydn day! I do not think I have featured him and what a prolific composer he was, leading the way into the classical era from the baroque. So much to choose from, but this rendition of the Symphony #52 popped up on my YouTube feed. This the Bavarian Radio Orchestra conducted by John Eliot Gardiner: https://www.youtube.com/watch?v=BUgUo_468OY I think I will go on a bit of a Haydn tear this week.

The Washington Post reports on the current outbreak. New cases and deaths are heading in the wrong direction. Denmark wants to cull all the farmed minks in the country to eliminate a possible SARS-CoV-2 host. I continue to be surprised that minks are still being raised for fur. At least one country in the world still has faith in science (two including New Zealand); maybe its time to move to Australia.

The New York Times reports that some people are getting medical bills that include a COVID-19 fee. Here is a report on the increase in hospitalizations.

STAT have an opinion piece on the need for more transparency regarding vaccine trials. Rapid COVID-19 tests will lead to a loss of valuable data says this opinion piece. I think this is right and it will further contribute to the public health failure.

The Lancet have a commentary on the challenges to the vaccine approach to herd immunity.

Medscape point to a story on social distancing possibly reducing hospitalizations for childhood respiratory illness this past spring in Massachusetts. Here is a story on the common SARS-CoV-2 mutation that has now become the dominant form (this is the one that came to the US from Europe).

Kaiser Health News has an interesting story about the search for SARS-CoV-2 in animals. It's important to find all the possible reservoirs for the virus!

The New England Journal of Medicine has the final trial report on remdesivir. Here is an editorial on the drug. There is also a study looking at 5 and 10 day dosage regimens. *In patients with severe Covid-19 not requiring mechanical ventilation, our trial did not show a significant difference between a 5-day course and a 10-day course of remdesivir. With no placebo control, however, the magnitude of benefit cannot be determined.*

MODELING

- Objective: To estimate the prevalence of anxiety symptoms and the association between moderate or severe anxiety symptoms and health and potential stressors among adults in the U.S. during the COVID-19 pandemic Methods: This analysis includes data from 5,250 adults in the Communities, Households and SARS/CoV-2 Epidemiology (CHASING) COVID Cohort Study surveyed in April 2020. Poisson models were used to estimate the association between moderate or severe anxiety symptoms and health and potential stressors among U.S. adults during the COVID-19 pandemic. Results: Greater than one-third (35%) of participants reported moderate or severe anxiety symptoms. Having lost income due to COVID-19 (adjusted

prevalence ratio [aPR] 1.27 (95% CI 1.16, 1.30), having recent COVID-like symptoms (aPR 1.17 (95% CI 1.05, 1,31), and having been previously diagnosed with depression (aPR 1.49, (95% CI 1.35, 1.64) were positively associated with anxiety symptoms. Conclusions: *Anxiety symptoms were common among adults in the U.S. during the COVID-19 pandemic. Strategies to screen and treat individuals at increased risk of anxiety, such as individuals experiencing financial hardship and individuals with prior diagnoses of depression, should be developed and implemented*. [**note: here is a study on the relationship between anxiety, health and potential stressors among adults in the US during the pandemic.**] https://www.medrxiv.org/content/10.1101/2020.10.30.20221440v1

- In response to COVID-19, the international water community rapidly developed methods to quantify the SARS-CoV-2 genetic signal in untreated wastewater. Wastewater surveillance using such methods has the potential to complement clinical testing in assessing community health. This interlaboratory assessment evaluated the reproducibility and sensitivity of 36 standard operating procedures (SOPs), divided into eight method groups based on sample concentration approach and whether solids were removed. Two raw wastewater samples were collected in August 2020, amended with a matrix spike (betacoronavirus OC43), and distributed to 32 laboratories across the U.S. Replicate samples analyzed in accordance with the project's quality assurance plan showed high reproducibility across the 36 SOPs: 80% of the recovery-corrected results fell within a band of +/- 1.15-$\log_{10}$ genome copies/L with higher reproducibility observed within a single SOP (standard deviation of 0.13-$\log_{10}$). The inclusion of a solids removal step and the selection of a concentration method did not show a clear, systematic impact on the recovery-corrected results. Other methodological variations (e.g., pasteurization, primer set selection, and use of RT-qPCR or RT-dPCR platforms) generally resulted in small differences compared to other sources of variability. *These findings suggest that a variety of methods are capable of producing reproducible results, though the same SOP or laboratory should be selected to track SARS-CoV-2 trends at a given facility. The methods showed a 7-$\log_{10}$ range of recovery efficiency and limit of detection highlighting the importance of recovery correction and the need to consider method sensitivity when selecting methods for wastewater surveillance*. [**note: here is a good overview of methods to quantify SARS-CoV-2 genetic material in wastewater**.] https://www.medrxiv.org/content/10.1101/2020.11.02.20221622v1
- Objectives Evaluate the interactions between SARS-CoV-2 positive players and other players during rugby league matches, to determine the risk of in-game SARS-CoV-2 transmission. Design Observational. Setting Super League rugby league during four matches in which SARS-CoV-2 positive players were retrospectively found to have participated (2nd August and 4th October 2020). Participants 136 male elite rugby league players: eight SARS-CoV-2 positive participants, 28 identified close contacts and 100 other players who participated in any of the four matches. Main Outcome measures Close contacts were defined by analysis of video footage for player interactions and microtechnology (GPS) data for proximity analysis. Close contacts and other players involved in the matches becoming positive for SARS-CoV-2 by RT-PCR within 14 days of the match were reported. Results The eight SARS-CoV-2 positive players were involved in up to 14 tackles with other individual players. SARS-CoV-2 positive players were within a 2 m proximity of other players for up to 316 secs, from 60 interactions. One identified contact returned a positive SARS-CoV-2 result within 14 days of the match (subsequently linked to an outbreak within their club environment, rather than in-match transmission), whereas the other

27 identified contacts returned negative SARS-CoV-2 follow up tests and no one developed COVID-19 symptoms. Ninety-five players returned negative and five players returned positive SARS-CoV-2 RT-PCR routine tests within 14 days of the match. Sources of transmission in the five cases were linked to internal club COVID-19 outbreaks and wider-community transmission. Conclusion *Despite a high number of tackle involvements and close proximity interactions between SARS-CoV-2 positive players and players on the same and opposition teams during a rugby league match, these data suggest that in-game SARS-CoV-2 transmission is limited during these types of team sport activities played outdoors*. [**note: here is a study on transmission of SARS-CoV-2 between Rugby Union players. Good information.]** https://www.medrxiv.org/content/10.1101/2020.11.03.20225284v1

- We estimate the U.S. temperature response curve for COVID-19 and show transmission is quite sensitive to temperature variation. This is despite summer outbreaks widely assumed to show otherwise. *By largely replacing the death counts states report daily, with counts based on death certificate date, we build a week-ahead statistical forecasting model that explains most of the daily variation (R-square = 0.97) and isolates the COVID-19 temperature response profile ($p < 0.001$). These counts normalized at 31C (U.S. mid-summer average) scale up nearly 160% at 5C. Positive cases are more temperature sensitive; scaling up by almost 400% between 31C and 5C. Dynamic feedback amplifies these effects. There is a short window to get COVID-19 under control before cooler weather makes the task substantially more challenging*. [**note; here is a temperature response profile for the US as it relates to COVID-19 infections. Note the final highlighted sentence; I'm afraid we missed the window for control.**] https://www.medrxiv.org/content/10.1101/2020.11.03.20225581v1

NEWLY REGISTERED CLINICAL TRIALS

- If you have to ask the question, you must not know the answer.

CLINICAL TRIAL RESULTS

- Delirium is a serious and common complication among critically ill patients with COVID-19. The objective of this study was to characterize the clinical course of delirium for COVID-19 patients in the intensive care unit, including post-discharge cognitive outcomes. A retrospective chart review was conducted for patients diagnosed with COVID-19 (n=148) admitted to an intensive care unit at Michigan Medicine between 3/1/2020 and 5/31/2020. Delirium was identified in 107/148 (72%) patients in the study cohort, with median (interquartile range) duration lasting 10 (4 - 17) days. Sedative regimens, inflammation, deviation from delirium prevention protocols, and hypoxic-ischemic injury were likely contributing factors, and the most common disposition for delirious patients was a skilled care facility (41/148, 38%). *Among patients who were delirious during hospitalization, 4/17 (24%) later tested positive for delirium at home based on caretaker assessment, 5/22 (23%) demonstrated signs of questionable cognitive impairment or cognitive impairment consistent with dementia, and 3/25 (12%) screened positive for depression within two months after discharge. Overall, patients with COVID-19 commonly experience a prolonged course of delirium in the intensive care unit, likely with multiple contributing factors. Furthermore, neuropsychological impairment may persist after discharge.* [**note: this is from Univ of Michigan medical school and follows patients suffering from delirium during hospitalization.**] https://www.medrxiv.org/content/10.1101/2020.11.03.20225466v1

- Background COVID-19 presentation ranges from asymptomatic to fatal. The variability in severity may be due in part to impaired Interferon type I response due to specific mutations in the host genome or to autoantibodies, explaining about 15% of the cases when combined. Exploring the host genome is thus warranted to further elucidate disease variability. Methods We developed a synthetic approach to genetic data representation using machine learning methods to investigate complementary genetic variability in COVID-19 infected patients that may explain disease severity, due to poly-amino acids repeat polymorphisms. Using host whole-exome sequencing data, we compared extreme phenotypic presentations (338 severe versus 300 asymptomatic cases) of the entire (men and women) Italian GEN-COVID cohort of 1178 subjects infected with SARS-CoV-2. We then applied the LASSO Logistic Regression model on Boolean gene-based representation of the poly-amino acids variability. Findings Shorter polyQ alleles (≤22) in the androgen receptor (AR) conferred protection against a more severe outcome in COVID-19 infection. In the subgroup of males with age <60 years, testosterone was higher in subjects with AR long-polyQ (≥23), possibly indicating receptor resistance (p=0.004 Mann-Whitney U test). Inappropriately low testosterone levels for the long-polyQ alleles predicted the need for intensive care in COVID-19 infected men. In agreement with the known anti-inflammatory action of testosterone, patients with long-polyQ (≥23) and age>60 years had increased levels of C Reactive Protein (p=0.018). Interpretation Our results may contribute to design reliable clinical and public health measures and provide a rationale to test testosterone treatment as adjuvant therapy in symptomatic COVID-19 men expressing AR polyQ longer than 23 repeats. [**note: here is more good human genetic work from Italy. This time they look at shorter androgen receptor polyQ alleles which my be protective against severe COVID-19.**] https://www.medrxiv.org/content/10.1101/2020.11.04.20225680v1

DRUG DEVELOPMENT

- At present, there are is no effective vaccine and only one FDA approved early-stage therapy against infection with the SARS-CoV-2 virus to prevent disease progression. The excessive inflammation and tissue damage associated with COVID-19 can lead to immediate (i.e. respiratory failure, sepsis, and ultimately, death) or long-term health problems (i.e. fatigue, dyspnea, cough, joint pain, anosmia) and the risk for these complications are higher in the elderly population, certain ethnic groups, as well as those with various co-morbid conditions. Cellular caspases play a role in the pathophysiology of a number of disorders that overlap with the list of co-morbid conditions seen in severe COVID-19. In this study, we assessed transcriptional states of caspases in immune cells from COVID-19 patients and profiled intra-cellular caspases in immune cells and red blood cells derived from a spectrum of COVID-19 patients hospitalized with acute disease or convalescent. Gene expression levels of select caspases were increased in in vitro SARS-CoV-2 infection models and single cell RNA-Seq data of peripheral blood from COVID-19 patients showed a distinct pattern of caspase expression in T cell, neutrophils, and dendritic cells. Flow cytometric evaluation of CD4 T cells showed up-regulation of caspase-1 in hospitalized COVID-19 patients compared to unexposed controls, with the exception of a subset of patients with asthma and chronic rhinosinusitis (CRS). *Convalescent COVID-19 patients with lingering symptoms (long haulers) showed persistent up-regulation of caspase-1 in CD4 T cells that was attenuated ex vivo following co-culture with a select pan-caspase inhibitor. Further, we observed elevated caspase 3 levels in red blood cells from COVID-*

19 patients compared to controls that were responsive to caspase inhibition. Taken together, our results expose an exuberant caspase response in COVID-19 that may facilitate immune-related pathological processes leading to severe outcomes. Pan-caspase inhibition could emerge as a therapeutic strategy to ameliorate, reduce, or prevent severe COVID-19 outcomes. [**note: this paper could go into several different categories. I put it here because it may lead to some new drug interventions.**] https://www.medrxiv.org/content/10.1101/2020.11.02.20223636v1

- Containment of the COVID-19 pandemic requires reducing viral transmission. SARS-CoV-2 infection is initiated by membrane fusion between the viral and host cell membranes, mediated by the viral spike protein. We have designed a dimeric lipopeptide fusion inhibitor that blocks this critical first step of infection for emerging coronaviruses and document that it completely prevents SARS-CoV-2 infection in ferrets. *Daily intranasal administration to ferrets completely prevented SARS-CoV-2 direct-contact transmission during 24-hour co-housing with infected animals, under stringent conditions that resulted in infection of 100% of untreated animals. These lipopeptides are highly stable and non-toxic and thus readily translate into a safe and effective intranasal prophylactic approach to reduce transmission of SARS-CoV-2*. [**note: this is an interesting paper on the identification of a lipopeptide that prevents direct contact transmission of SARS-CoV-2 in ferrets. It can be delivered intranasally. I wonder how easy it is to produce this at scale. They employ a segment of the Spike protein and relied on solid-phase synthesis to manufacture it.**]
https://www.biorxiv.org/content/10.1101/2020.11.04.361154v1
- Here we report on the development of a SARS-CoV-2 receptor-binding domain (RBD) protein, expressed at high levels in yeast (*Pichia pastoris*), as a suitable vaccine candidate against COVID-19. After introducing two modifications into the wild-type RBD gene to reduce yeast-derived hyperglycosylation and improve stability during protein expression, *we show that the recombinant protein, RBD219-N1C1, is equivalent to the wild-type RBD recombinant protein (RBD219-WT) in an in vitro ACE-2 binding assay. Immunogenicity studies of RBD219-N1C1 and RBD219-WT proteins formulated with Alhydrogel® were conducted in mice, and, after two doses, both the RBD219-WT and RBD219-N1C1 vaccines induced high levels of binding IgG antibodies. Using a SARS-CoV-2 pseudovirus, we further showed that sera obtained after a two-dose immunization schedule of the vaccines were sufficient to elicit strong neutralizing antibody titers in the 1:1,000 to 1:10,000 range, for both antigens tested. The vaccines induced IFN-γ, IL-6, and IL-10 secretion, among other cytokines. Overall, these data suggest that the RBD219-N1C1 recombinant protein, produced in yeast, is suitable for further evaluation as a human COVID-19 vaccine, in particular, in an Alhydrogel® containing formulation and possibly in combination with other immunostimulants*. [**note: here is another vaccine approach using a yeast vector production system. I believe there are a couple of others that have used this approach. The Baylor authors note that it has been licensed to an Indian company for development.**
https://www.biorxiv.org/content/10.1101/2020.11.04.367359v1
- At least since March 2020, the multiorgan disease COVID-19 has a firm grip on the world. Although most of the cases are mild, patients from risk populations could develop a cytokine storm, which is characterized by a systemic inflammatory response leading to acute respiratory distress syndrome and organ failure. The present paper will introduce the small molecule MP1032, describe its mode of action, and give rationale why it is a promising option for prevention/treatment of SARS-CoV-2-induced cytokine storm. MP1032 is a phase-pure

anhydrous polymorph of 5-amino-2,3-dihydro-1,4-pthalazinedione sodium salt that exhibits good stability and bioavailability. *The physiological action of MP1032 is based on a multi-target mechanism including localized, self- limiting antioxidant activities that were demonstrated in a model of lipopolysaccharide (LPS)-induced joint inflammation. Furthermore, immune-regulatory and PARP-1 modulating properties, coupled with antiviral effects against SARS-CoV-2 were shown in various cell models. Efficacy has been preclinically elucidated in LPS-induced endotoxemia, a model with excessively activated immune responses that shares many similarities to COVID-19. So far, during oral clinical development with three-months daily administrations, no serious adverse drug reactions occurred highlighting the outstanding safety profile of MP1032*. [**note: this is from a German company, MetrioPharm, who have been developing an immune modulating drug with SARS-CoV-2 antiviral activity.**]
https://www.medrxiv.org/content/10.1101/2020.11.03.20216580v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Background SARS-CoV-2 IgG antibody measurements can be used to estimate the proportion of a population exposed or infected and may be informative about the risk of future infection. Previous estimates of the duration of antibody responses vary. Methods We present 6 months of data from a longitudinal seroprevalence study of 3217 UK healthcare workers (HCWs). Serial measurements of IgG antibodies to SARS-CoV-2 nucleocapsid were obtained. Bayesian mixed linear models were used to investigate antibody waning and associations with age, gender, ethnicity, previous symptoms and PCR results. Results In this cohort of working age HCWs, antibody levels rose to a peak at 24 (95% credibility interval, CrI 19-31) days post-first positive PCR test, before beginning to fall. Considering 452 IgG seropositive HCWs over a median of 121 days (maximum 171 days) from their maximum positive IgG titre, the mean estimated antibody half-life was 85 (95%CrI, 81-90) days. The estimated mean time to loss of a positive antibody result was 137 (95%CrI 127-148) days. We observed variation between individuals; higher maximum observed IgG titres were associated with longer estimated antibody half-lives. Increasing age, Asian ethnicity and prior self-reported symptoms were independently associated with higher maximum antibody levels, and increasing age and a positive PCR test undertaken for symptoms with longer antibody half-lives. Conclusion *IgG antibody levels to SARS-CoV-2 nucleocapsid wane within months, and faster in younger adults and those without symptoms. Ongoing longitudinal studies are required to track the long-term duration of antibody levels and their association with immunity to SARS-CoV-2 reinfection*. [**note: here is another study on antibody longevity, this time in healthcare workers from the UK. Antibodies to the nucleocapsid protein were measured.**]
https://www.medrxiv.org/content/10.1101/2020.11.02.20224824v1
- Background: The genome of SARS-CoV-2 is susceptible to mutations during viral replication due to the errors generated by RNA-dependent RNA polymerases. These mutations enable the SARS-CoV-2 to evolve into new strains. Viral quasispecies emerge from de novo mutations that occur in individual patients. In combination, these sets of viral mutations provide distinct genetic fingerprints that reveal the patterns of transmission and have utility in contract tracing. Methods: Leveraging thousands of sequenced SARS-CoV-2 genomes, we performed a viral pangenome analysis to identify conserved genomic sequences. We used a rapid and highly efficient computational approach that relies on k-mers, short tracts of sequence, instead of

conventional sequence alignment. Using this method, we annotated viral mutation signatures that were associated with specific strains. Based on these highly conserved viral sequences, we developed a rapid and highly scalable targeted sequencing assay to identify mutations, detect quasispecies and identify mutation signatures from patients. These results were compared to the pangenome genetic fingerprints. Results: We built a k-mer index for thousands of SARS-CoV-2 genomes and identified conserved genomics regions and landscape of mutations across thousands of virus genomes. We delineated mutation profiles spanning common genetic fingerprints (the combination of mutations in a viral assembly) and rare ones that occur in only small fraction of patients. We developed a targeted sequencing assay by selecting primers from the conserved viral genome regions to flank frequent mutations. Using a cohort of SARS-CoV-2 clinical samples, we identified genetic fingerprints consisting of strain-specific mutations seen across populations and de novo quasispecies mutations localized to individual infections. We compared the mutation profiles of viral samples undergoing analysis with the features of the pangenome. Conclusions: *We conducted an analysis for viral mutation profiles that provide the basis of genetic fingerprints. Our study linked pangenome analysis with targeted deep sequenced SARS-CoV-2 clinical samples. We identified quasispecies mutations occurring within individual patients, mutations demarcating dominant species and the prevalence of mutation signatures, of which a significant number were relatively unique. Analysis of these genetic fingerprints may provide a way of conducting molecular contact tracing*. [**note: this study on viral mutation fingerprints comes from Stanford.**]
https://www.medrxiv.org/content/10.1101/2020.11.02.20224816v1

- In this study, we analyzed SARS-CoV-2 genomes in the Netherlands, in the context of global viral population since the beginning of the pandemic. We have identified the most variant sites on the whole genome as well as the stable, conserved ones on the S and N proteins. *We found four mutations, S:D614G, NSP12b:P314L, NSP3:F106F, to be the most frequent ones that dominate the SARS-CoV-2 population outside of China. We detected novel variants of SARS-CoV-2 almost unique to the Netherlands that form localized clusters, indicating community spread. We emphasize that while SARS-CoV-2 is evolving, and the number of mutations from the reference sequence is increasing, we observe only little diversity in the new variants as we enter the later stages of the pandemic. Our analyses suggest we have diverged away from the current SARS-CoV-2 reference enough that the reference should be re-evaluated to represent the current viral population more accurately. We assert our work provides valuable information on the genetic diversity of SARS-CoV-2 and its local dynamics in the Netherlands, especially for DNA-based diagnostic, therapeutic or vaccine development against COVID-19. We suggest sequence-based analyses should opt for a consensus representation to adequately cover the genomic variation observed*. [**note: these Dutch researchers identify a novel variant of SARS-CoV-2 in The Netherlands. This is unsurprising and I hope someone will create an atlas that documents the mutations in accordance with spread.**]
https://www.medrxiv.org/content/10.1101/2020.11.02.20224352v1
- Epidemiological studies of the COVID-19 pandemic have revealed evidence of cardiac involvement and documented that myocardial injury and myocarditis are predictors of poor outcomes. Nonetheless, little is understood regarding SARS-CoV-2 tropism within the heart and whether cardiac complications result directly from myocardial infection. Here, we develop a human engineered heart tissue model and demonstrate that SARS-CoV-2 selectively infects

cardiomyocytes. Viral infection is dependent on expression of angiotensin-I converting enzyme 2 (ACE2) and endosomal cysteine proteases, suggesting an endosomal mechanism of cell entry. After infection with SARS-CoV-2, engineered tissues display typical features of myocarditis, including cardiomyocyte cell death, impaired cardiac contractility, and innate immune cell activation. Consistent with these findings, autopsy tissue obtained from individuals with COVID-19 myocarditis demonstrated cardiomyocyte infection, cell death, and macrophage-predominate immune cell infiltrate. These findings establish human cardiomyocyte tropism for SARS-CoV-2 and provide an experimental platform for interrogating and mitigating cardiac complications of COVID-19. [**note: here is a model for myocardial injury by SARS-CoV-2.**] https://www.biorxiv.org/content/10.1101/2020.11.04.364315v1

- The COVID-19 pandemic has revealed a range of disease phenotypes in infected patients with asymptomatic, mild or severe clinical outcomes, but the mechanisms that determine such variable outcomes remain unresolved. In this study, we identified immunodominant CD8 T-cell epitopes in the RBD and the non-RBD domain of the spike antigen using a novel TCR-binding algorithm. A selected pool of 11 predicted epitopes induced robust T-cell activation in unexposed donors demonstrating pre-existing CD4 and CD8 T-cell immunity to SARS-CoV-2 antigen. The T-cell reactivity to the predicted epitopes was higher than the Spike-S1 and S2 peptide pools containing 157 and 158 peptides both in unexposed donors and in convalescent patients suggesting that strong T-cell epitopes are likely to be missed when larger peptide pools are used in assays. *A key finding of our study is that pre-existing T-cell immunity to SARS-CoV-2 is contributed by TCRs that recognize common viral antigens such as Influenza and CMV, even though the viral epitopes lack sequence identity to the SARS-CoV-2 epitopes. This finding is in contrast to multiple published studies in which pre-existing T-cell immunity is suggested to arise from shared epitopes between SARS-CoV-2 and other common cold-causing coronaviruses. Whether the presence of pre-existing T-cell immunity provides protection against COVID-19 or contributes to severe disease phenotype remains to be determined in a larger cohort. However, our findings raise the expectation that a significant majority of the global population is likely to have SARS-CoV-2 reactive T-cells because of prior exposure to flu and CMV viruses, in addition to common cold-causing coronaviruses*. [**note: this paper comes from the company MedGenome. This is an interesting finding but runs counter to some other papers on this topic. Not being an immunologist, I cannot comment on the possible impact.**] https://www.biorxiv.org/content/10.1101/2020.11.03.367375v1
- Infection is associated with development of variable levels of antibodies with neutralizing activity that can protect against infection in animal models. Antibody levels decrease with time, but the nature and quality of the memory B cells that would be called upon to produce antibodies upon re-infection has not been examined. Here we report on the humoral memory response in a cohort of 87 individuals assessed at 1.3 and 6.2 months after infection. We find that IgM, and IgG anti-SARS-CoV-2 spike protein receptor binding domain (RBD) antibody titers decrease significantly with IgA being less affected. Concurrently, neutralizing activity in plasma decreases by five-fold in pseudotype virus assays. In contrast, the number of RBD-specific memory B cells is unchanged. Memory B cells display clonal turnover after 6.2 months, and the antibodies they express have greater somatic hypermutation, increased potency and resistance to RBD mutations, indicative of continued evolution of the humoral response. Analysis of intestinal biopsies obtained from asymptomatic individuals 3 months after COVID-19 onset,

using immunofluorescence, electron tomography or polymerase chain reaction, revealed persistence of SARS-CoV-2 in the small bowel of 7 out of 14 volunteers. *We conclude that the memory B cell response to SARS-CoV-2 evolves between 1.3 and 6.2 months after infection in a manner that is consistent with antigen persistence*. [**note: this is from a large Rockefeller Univ group and shows evolution of antibody immunity to the virus.**] https://www.biorxiv.org/content/10.1101/2020.11.03.367391v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

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2020-11-06

We are continuing with a Haydn journey. Here is the 'Sunrise String Quartet' in B flat major. The artists are the Dover String Quartet and this is from the Banff International String Quartet Competition. It is a wonderful performance: https://www.youtube.com/watch?v=vcrKzQ1_2R4

The Washington Post covers how COVID-19 can warp some of your senses. Cases of COVID-19 continue to mount in the US.

The New York Times notes that COVID-19 cases at US colleges has hit the quarter million mark. Here is an article on the COVID-19 prevention nasal spray that was covered yesterday in the Drug Development section. I do think this is an interesting approach and the question of manufacturing scale up needs to be addressed as well as how one would do a clinical trial.

Science have a story on the Novavax vaccine which is one of the approaches I like.

Derek Lowe talks about the Danish mink farms and the low quality of some research that is coming out. I try to pass by articles that appear to be of dubious quality but it does suck up time.

STAT discuss the Danish decision to cull the mink population because of SARS-CoV-2 transmission fears.

The Lancet has a commentary on the use of vaccines under an Emergency Use Authorization using the lessons learned from Ebola. Do we know enough about the sex differences in COVID-19 case fatality? Here is a good report on the effect of HCQ as a pre-exposure treatment for COVID-19. It is based on an observational look at rheumatology based on 30,000 patients on the drug for lupus or rheumatoid arthritis. *We found no evidence of a difference in COVID-19 mortality among people who received hydroxychloroquine for treatment of rheumatological disease before the COVID-19 outbreak in England. Therefore, completion of randomised trials investigating pre-exposure prophylactic use of hydroxychloroquine for prevention of severe outcomes from COVID-19 are warranted.* There is also an editorial commentary on this paper.

Medscape notes that coughs can send virus particles further than six feet. This is not surprising, and you should always cover your mouth when you cough!!! Public health practice #4.

For you mouthwash fans, The Journal of Dental Research has an article on the antiviral activity of mouth rinses against SARS-CoV-2.

Kaiser Health News discuss how the VA and the Pentagon are helping to recruit volunteers for COVID-19 vaccine trials. Escalating COVID-19 cases are forcing Montana to change its mask strategy.

MODELING

- Policymakers make decisions about COVID-19 management in the face of considerable uncertainty. *We convened multiple modeling teams to evaluate reopening strategies for a mid-sized county in the United States, in a novel process designed to fully express scientific uncertainty while reducing linguistic uncertainty and cognitive biases. For the scenarios considered, the consensus from 17 distinct models was that a second outbreak will occur within 6 months of reopening, unless schools and non-essential workplaces remain closed. Up to half the population could be infected with full workplace reopening; non-essential business closures reduced median cumulative infections by 82%. Intermediate reopening interventions identified no win-win situations; there was a trade-off between public health outcomes and duration of workplace closures. Aggregate results captured twice the uncertainty of individual models, providing a more complete expression of risk for decision-making purposes*. [**note: here is another paper with lots of authors. The paper provides multiple models for outbreak decision support for reopening strategies at the county level.**]
https://www.medrxiv.org/content/10.1101/2020.11.03.20225409v1
- Background: Evidence-based infection control strategies are needed for healthcare workers (HCWs) following high-risk exposure to SARS-CoV-2. This study evaluated the negative predictive value (NPV) of a home-based 7-day infection control strategy. Methods: HCWs advised by their Infection Control or Occupational Health officer to self-isolate due to a high-risk SARS-CoV-2 exposure were enrolled between May-September 2020. The strategy consisted of symptom-triggered nasopharyngeal SARS-CoV-2 RNA testing from day 0-6 post exposure, followed by standardized home-based nasopharyngeal swab and saliva testing on day 7. The NPV of this strategy was calculated for i) clinical COVID-19 diagnosis from day 8-14 post exposure, and for ii) asymptomatic SARS-CoV-2 detected by standardized nasopharyngeal swab and saliva specimens collected at days 9-10 and 14 post exposure. Interim results are reported in the context of a second wave threatening this essential workforce. Results: Among 30 HCWs enrolled to date (age 31±9 years, 24 [80.0%] female), 3 were diagnosed with COVID-19 by day 14 post exposure (secondary attack rate 10.0%), with all cases detected by the 7-day infection control strategy: NPV for subsequent clinical COVID-19 or asymptomatic SARS-CoV-2 detection by day 14 was 100.0% (95%CI: 93.1-100.0%). Interpretation: *Among HCWs with high-risk exposure to SARS-CoV-2, a home-based 7-day infection control strategy may have a high NPV for subsequent COVID-19 and asymptomatic SARS-CoV-2 detection. While ongoing data collection and data sharing are needed to improve the precision of the estimated NPV, we report interim results to inform infection control strategies in light of a second wave threatening this essential workforce*. [**note: from Canada here is an evaluation of a home-based control strategy for healthcare worker at high-risk exposure to SARS-CoV-2.**]
https://www.medrxiv.org/content/10.1101/2020.11.05.20224618v1
- Wastewater monitoring for SARS-CoV-2 has been suggested as an epidemiological indicator of community infection dynamics and disease prevalence. We report wastewater viral RNA levels of SARS-CoV-2 in a major metropolis serving over 3.6 million people geographically spread over

39 distinct sampling sites. *Viral RNA levels were followed weekly for 22 weeks, both before, during, and after a major surge in cases, and simultaneously by two independent laboratories. We found SARS-CoV-2 RNA wastewater levels were a strong predictive indicator of trends in the nasal positivity rate two-weeks in advance. Furthermore, wastewater viral RNA loads demonstrated robust tracking of positivity rate for populations served by individual treatment plants, findings which were used in real-time to make public health interventions, including deployment of testing and education strike teams*. [**note: this is the last of the 'poop scoop' papers that I will post.😃 Sewage monitoring works as this Houston TX study shows. I suspect many more communities world-wide will adopt this approach.**] https://www.medrxiv.org/content/10.1101/2020.11.04.20226191v1

- Nursing homes and other long term care facilities have been disproportionately impacted by the COVID-19 pandemic. Strategies are urgently needed to reduce transmission in these vulnerable populations. We aimed to evaluate the reduction in transmission in nursing homes achieved through contact-targeted interventions and testing and to evaluate the effectiveness of two types of screening tests conducted with varying frequency: 1) rapid antigen testing and 2) PCR testing. Methods: We developed an agent-based Susceptible-Exposed-Infectious(Asymptomatic/Symptomatic)-Recovered (SEIR) model to examine SARS-CoV-2 transmission in nursing homes. Residents and staff are modelled individually; residents are split into two cohorts based on COVID-19 diagnosis. In the resident cohorting intervention, recovered residents are moved back from the COVID (infected) cohort to the non-COVID (susceptible/uninfected) cohort. In the immunity-based staffing intervention, recovered staff, who we assume have protective immunity, are assigned to work in the non-COVID cohort, while susceptible staff work in the COVID cohort and are assumed to have high levels of protection from personal protective equipment. These interventions aim to reduce the fraction of people's contacts that are presumed susceptible (and therefore potentially infected) and replace them with recovered (immune) contacts. Results: The frequency and type of testing has a larger impact on the size of outbreaks than the cohorting and staffing interventions. *The most effective testing strategies modeled are daily antigen testing of everyone and daily antigen testing of staff with weekly PCR testing for residents. Under all screening testing strategies, the immunity-based staffing intervention reduces the final size of the outbreak. The resident cohorting intervention reduces the final outbreak size under some, but not all, testing scenarios. Conclusions: Increasing the frequency of screening testing of all residents and staff, or even staff alone, in nursing homes has the potential to greatly reduce outbreaks in this vulnerable setting. Immunity-based staffing can further reduce spread at little or no additional cost and becomes particularly important when daily testing is not feasible*. [**note: here is proposal to improve protection of nursing home residents.**] https://www.medrxiv.org/content/10.1101/2020.11.04.20224758v1

NEWLY REGISTERED CLINICAL TRIALS

- Seriously, you really want to know?

CLINICAL TRIAL RESULTS

- Background. With increasing rates of SARS-CoV-2 infections and the intention to avoid a lock-down, the risks for the working population are of great interest. No large studies have been conducted which allow risk assessment for this population. Methods. DKMS is a non-profit

donor center for stem cell donation and reaches out to registered volunteers between 18 and 61 years of age. To identify risk factors for severe COVID-19 courses in this population we performed a cross-sectional study. Self-reported data on oro- or nasopharyngeal swabs, risk factors, symptoms and treatment were collected with a health questionnaire and linked to existing genetic data. We fitted multivariable logistic regression models for the risk of contracting SARS-CoV-2, risk of severe respiratory infection and risk of hospitalization. Findings. Of 4,440,895 contacted volunteers 924,660 (20.8%) participated in the study. Among 157,544 participants tested, 7,948 reported SARS-CoV-2 detection. Of those, 947 participants (11.9%) reported an asymptomatic course, 5,014 (63.1%) mild/moderate respiratory infections, and 1,987 (25%) severe respiratory tract infections. In total, 286 participants (3.6%) were hospitalized for respiratory tract infections. *The risk of hospitalization in comparison to a 20-year old person of normal weight was 2.1-fold higher (95%-CI, 1.2-3.69, p=0.01) for a person of same age with a BMI between 35-40 kg/m2, it was 5.33-fold higher (95%-CI, 2.92-9.70, p<0.001) for a 55-year old person with normal weight and 11.2-fold higher (95%-CI, 10.1-14.6, p<0.001) for a 55-year old person with a BMI between 35-40 kg/m2. Blood group A was associated with a 1.15-fold higher risk for contracting SARS-CoV-2 (95%-CI 1.08-1.22, p<0.001) than blood group O but did not impact COVID-19 severity. Interpretation. In this relatively healthy population, the risk for hospitalizations due to SARS-CoV-2 infections was moderate. Age and BMI were major risk factors. These data may help to tailor risk-stratified preventive measures*. [**note: maintain a health body weight particularly if you are older. This German study notes that age and BMI are the major risk factors for respiratory infections and outweigh ABO blood groups.**] https://www.medrxiv.org/content/10.1101/2020.11.05.20226100v1

DRUG DEVELOPMENT

- Cost-effective, efficacious therapeutics are urgently needed against the COVID-19 pandemic. Here, we used camelid immunization and proteomics to identify a large repertoire of highly potent neutralizing nanobodies (Nbs) to the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD). We discovered Nbs with picomolar to femtomolar affinities that inhibit viral infection at sub-ng/ml concentration and determined a structure of one of the most potent in complex with RBD. Structural proteomics and integrative modeling revealed multiple distinct and non-overlapping epitopes and indicated an array of potential neutralization mechanisms. *We constructed multivalent Nb constructs that achieved ultrahigh neutralization potency (IC50s as low as 0.058 ng/ml) and may prevent mutational escape. These thermostable Nbs can be rapidly produced in bulk from microbes and resist lyophilization, and aerosolization*. [**note: I can't remember if this paper came out as a pre-print but these Univ of Pittsburgh researchers have generated some highly potent nanobodies to SARS-CoV-2**] https://science.sciencemag.org/content/early/2020/11/04/science.abe4747
- We developed a de novo protein design strategy to swiftly engineer decoys for neutralizing pathogens that exploit extracellular host proteins to infect the cell. Our pipeline allowed the design, validation, and optimization of de novo hACE2 decoys to neutralize SARS-CoV-2. *The best decoy, CTC-445.2, binds with low nanomolar affinity and high specificity to the RBD of the spike protein. Cryo-EM shows that the design is accurate and can simultaneously bind to all three RBDs of a single spike protein. Because the decoy replicates the spike protein target interface in hACE2, it is intrinsically resilient to viral mutational escape. A bivalent decoy, CTC-445.2d, shows*

~10-fold improvement in binding. CTC-445.2d potently neutralizes SARS-CoV-2 infection of cells in vitro and a single intranasal prophylactic dose of decoy protected Syrian hamsters from a subsequent lethal SARS-CoV-2 challenge. [**note: here is another paper on the design of an ACE2 decoy that can bind the virus with high affinity.**]
https://science.sciencemag.org/content/early/2020/11/04/science.abe0075

- The pathogen SARS-CoV-2 replicates in the lower respiratory tract and causes fatal pneumonia. Although tremendous efforts have been put into investigating the pathogeny of SARS-CoV-2, the underlying mechanism of how SARS-CoV-2 interacts with its host is largely unexplored. Here, by comparing the genomic sequences of SARS-CoV-2 and human, we identified five fully conserved elements in SARS-CoV-2 genome, which were termed as "human identical sequences (HIS)". HIS are also recognized in both SARS-CoV and MERS-CoV genome. Meanwhile, HIS-SARS-CoV-2 are highly conserved in the primate. Mechanically, HIS-SARS-CoV-2 RNA directly binds to the targeted loci in human genome and further interacts with host enhancers to activate the expression of adjacent and distant genes, including cytokines gene and angiotensin converting enzyme II (ACE2), a well-known cell entry receptor of SARS-CoV-2, and hyaluronan synthase 2 (HAS2), which further increases hyaluronan formation. *Noteworthily, hyaluronan level in plasma of COVID-19 patients is tightly correlated with severity and high risk for acute respiratory distress syndrome (ARDS) and may act as a predictor for the progression of COVID-19. HIS antagomirs, which downregulate hyaluronan level effectively, and 4-Methylumbelliferone (MU), an inhibitor of hyaluronan synthesis, are potential drugs to relieve the ARDS related ground-glass pattern in lung for COVID-19 treatment. Our results revealed that unprecedented HIS elements of SARS-CoV-2 contribute to the cytokine storm and ARDS in COVID-19 patients. Thus, blocking HIS-involved activating processes or hyaluronan synthesis directly by 4-MU may be effective strategies to alleviate COVID-19 progression*. [**note: this is from China and looks at some of the genomic sequences in the virus and in humans. They find another marker that may be predictive of severe COVID-19 and a possible therapy.**]
https://www.biorxiv.org/content/10.1101/2020.11.04.361576v1
- A safe, effective, and scalable vaccine is needed to halt the ongoing SARS-CoV-2 pandemic. We describe the structure-based design of self-assembling protein nanoparticle immunogens that elicit potent and protective antibody responses against SARS-CoV-2 in mice. The nanoparticle vaccines display 60 SARS-CoV-2 spike receptor-binding domains (RBDs) in a highly immunogenic array and induce neutralizing antibody titers ten-fold higher than the prefusion-stabilized spike despite a five-fold lower dose. Antibodies elicited by the RBD-nanoparticles target multiple distinct epitopes, suggesting they may not be easily susceptible to escape mutations, and exhibit a lower binding: neutralizing ratio than convalescent human sera, which may minimize the risk of vaccine-associated enhanced respiratory disease. The high yield and stability of the assembled nanoparticles suggest that manufacture of the nanoparticle vaccines will be highly scalable. These results highlight the utility of robust antigen display platforms and have launched cGMP manufacturing efforts to advance the SARS-CoV-2-RBD nanoparticle vaccine into the clinic. [**note: this is from Univ of Washington and another example of a nanoparticle protype vaccine based on the Spike protein.**] https://www.cell.com/cell/pdf/S0092-8674(20)31450-1.pdf
- To address the need for a safe, efficacious vaccine against SARS-CoV-2 infection with the critical properties of enabling both blocking viral entry into cells and clearing virus from cells already

infected, we have developed a bivalent, human adenovirus serotype 5 (hAd5) SARS-CoV-2 S-Fusion + N-ETSD vaccine that is currently in clinical testing. This vaccine uses the next-generation hAd5 [E1-, E2b-, E3-] platform previously used successfully in cancer patients with pre-existing adenovirus immunity, engineered to express both SARS-CoV-2 spike (S) protein modified to improve the generation of neutralizing antibodies to block entry of the virus, and nucleocapsid (N) protein with an Enhanced T cell Stimulation Domain (ETSD) to activate CD4+ and CD8+ T cells to clear the virus and block replication by killing infected cells. The targeting of N to endosomes and lysosomes to enhance CD4+ and CD8+ T-cell responses distinguishes our vaccine. In our previously reported pre-clinical studies we showed that in mice, the hAd5 S-Fusion + N-ETSD vaccine elicits both humoral and T-cell responses that are robust and T helper cell 1 (Th1) dominant. Here we report that the hAd5 S-Fusion + N-ETSD vaccine is recognized by anti-sera and T cells from previously SARS-CoV-2 infected patients, and that the presence of N is vital for T-cell recall. The findings presented herein: i. demonstrate specific recognition of hAd5 S-Fusion + N-ETSD infected cells by plasma antibodies from previously SARS-CoV-2 infected patients, but not antibodies from virus-naive subjects; ii. show enhanced binding of plasma SARS-CoV-2 antibodies from previously infected patients to monocyte-derived dendritic cells (MoDCs) expressing the hAd5 S-Fusion + N-ETSD vaccine as compared to hAd5 S-Fusion alone; iii. reveal N-ETSD localizes to vesicles associated with MHC class II antigen presentation, including endosomes, lysosomes, and autophagosomes in MoDCs; iv. demonstrate endosome/lysosome-targeted N-ETSD elicits higher interferon-gamma T-cell responses than cytoplasm-localized N; and v. N-ETSD alone or in the hAd5 S-Fusion + N-ETSD construct induces both CD4+ and CD8+ T cell memory recall. *This recognition of hAd5 S-Fusion + N-ETSD vaccine antigens by T cells from previously SARS-CoV-2 infected patients, together with the ability of this vaccine candidate to elicit de novo immune responses in naive mice suggests that it re-capitulates the natural immune response to SARS-CoV-2 to activate both B and T cells towards viral neutralization and recognition of infected cells, critical for prevention of COVID-19 disease. Intriguingly, our hAd5 S-Fusion + N-ETSD T-cell biased vaccine has the potential to not only provide protection for uninfected individuals, but also to be utilized as a therapeutic for already infected patients to induce rapid clearance of the virus by activating T cells to kill the virus-infected cells, thereby reducing viral replication and lateral transmission*. [**note: this vaccine candidate is being developed by ImmunityBio and NantKwest. It includes both the Spike and Nucleocapsid proteins in an adenovirus vector system. I've noted in the past that inclusion of the N protein may prove beneficial. This vaccine is in clinical trials.**]
https://www.medrxiv.org/content/10.1101/2020.11.04.20225417v1

- Background: The stabilized prefusion form of the SARS-CoV-2 spike protein is produced by transient expression in Nicotiana benthamiana. The trimeric spike glycoproteins are displayed at the surface of self-assembling Virus-Like-Particles that mimic the shape and the size of the virus. The candidate vaccine (CoVLP) administered alone or with AS03 or CpG1018 adjuvants was evaluated in a Phase 1 trial in healthy adults. (ClinicalTrials.gov number NCT04450004) Methods: The study was a randomized, partially-blinded, prime-boost 21 days apart, dose-escalation Phase 1 study intended to assess the safety, tolerability, and immunogenicity of CoVLP at three dose levels (3.75 microgram, 7.5 microgram, and 15 microgram) unadjuvanted or adjuvanted with either CpG 1018 or AS03 in 180 SARS-CoV-2 seronegative healthy adults 18 to 55 years of age. Enrollment was staggered for dose-escalation. At each dose level, the vaccine was initially

administered to a small number of subjects. Vaccination of the remaining subjects at the same dose level and the next higher vaccine dose level was administered with approval of an Independent Data Monitoring Committee (IDMC). The same procedure was followed for the second vaccine administration. Monitoring of safety signals was performed throughout the study with pre-determined pausing/stopping rules if there was clear evidence of harmful effects such as severe adverse events (AEs) related to the treatment. The primary endpoints were the safety and tolerability of the vaccine after each dose and the immunogenicity as assessed by neutralizing antibody responses assessed using a vesicular stomatitis virus (VSV) pseudovirion assay and interferon-gamma and interleukin-4 (IL-4) ELISpot assays at Days 0, 21 and 42. Secondary endpoints were anti-spike antibody responses by ELISA and neutralizing antibodies measured by live virus plaque reduction neutralization test (PRNT) assay at Days 0, 21 and 42 and immunogenicity with additional safety and immunogenicity endpoints planned for 6-months following the last vaccination. The anti-spike and neutralizing antibody responses were compared with 23 convalescent serum samples from symptomatic Covid-19 patients. We performed a primary analysis at day 42. Results: A total of 180 subjects (102 females: 78 males: average 34.3 years) were recruited to the study and interim safety and immunogenicity data up to day 42 after the first dose are reported here. There was no obvious CoVLP dose effect in safety outcomes for any of the formulations tested and all formulations were generally well-tolerated. Most solicited local and systemic AEs were mild-moderate and transient. Reactogenicity was increased in all adjuvanted formulations and was generally highest in the CoVLP+AS03 groups. Local and systemic adverse events were reported with similar frequency after the first and second doses in subjects who received either CoVLP alone or CoVLP+CpG1018 but increased in both frequency and severity after the second dose in the CoVLP+AS03 groups. CoVLP alone only elicited a weak total anti-spike IgG response at the highest dose level and little-to-no neutralization antibody response, even after the second dose. Cellular responses in the CoVLP alone groups (IFN-gamma and IL-4) were detectable after the second dose but were still only marginally above background levels. The addition of either adjuvant substantially increased both antibody and cellular responses at most CoVLP dose levels and changes were most pronounced after the second dose. However, a substantial neutralizing antibody response after the first dose was only seen in all CoVLP+AS03 groups. After the second dose, both total anti-spike IgG and neutralizing antibody titers in the CoVLP+AS03 groups were higher than those in the CoVLP+CpG1018 groups. *The antibody titers achieved were either similar to (CoVLP+CpG1018) or at least 10-times higher (CoVLP+AS03) than those seen in convalescent plasma. Administration of CoVLP with either adjuvant also significantly increased the cellular responses. After 2 doses, both IFN-gamma and IL-4 responses were significantly increased in the CoVLP+CpG1018 groups. In the CoVLP+AS03 groups, significant increases in the cellular responses were observed after the first dose while IFN-gamma and IL-4 increased further in both magnitude and number of subjects responding after the second dose. Again, the cellular responses in the CoVLP+AS03 groups were higher than those seen in the CoVLP+CpG1018 groups. Conclusion: These data demonstrate that CoVLP administered with either CpG1018 or AS03 has a safety profile similar to other candidate vaccines for SARS-CoV-2. When administered with either AS03 or CpG1018, several of the CoVLP dose levels elicited strong humoral and T cell responses after the second dose. When administered with AS03, even the 3.75 microgram CoVLP dose elicited neutralizing antibody titers that were ~10-times higher than those observed in*

individuals recovering from Covid-19 as well as consistent and balanced IFN-gamma and IL-4 responses. Although many CoVLP formulations were immunogenic, in the absence of established correlates of protection and given the advantages of dose-sparing in the context of the on-going pandemic, these findings suggest that CoVLP (3.75 microgram)+AS03 has a good benefit/risk ratio and support the transition of this formulation to studies in expanded populations and to efficacy evaluations [**note: this is Phase 1 study of the plant-based COVID-19 vaccine produced by Medicago, a Canadian biotech company.**]
https://www.medrxiv.org/content/10.1101/2020.11.04.20226282v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Clinical manifestations of COVID-19 caused by the new coronavirus SARS-CoV-2 are associated with age. Adults develop respiratory symptoms, which can progress to acute respiratory distress syndrome (ARDS) in the most severe form, while children are largely spared from respiratory illness but can develop a life-threatening multisystem inflammatory syndrome (MIS-C). Here, we show distinct antibody responses in children and adults after SARS-CoV-2 infection. Adult COVID-19 cohorts had anti-spike (S) IgG, IgM and IgA antibodies, as well as anti-nucleocapsid (N) IgG antibody, while children with and without MIS-C had reduced breadth of anti-SARS-CoV-2-specific antibodies, predominantly generating IgG antibodies specific for the S protein but not the N protein. Moreover, children with and without MIS-C had reduced neutralizing activity as compared to both adult COVID-19 cohorts, indicating a reduced protective serological response. These results suggest a distinct infection course and immune response in children independent of whether they develop MIS-C, with implications for developing age-targeted strategies for testing and protecting the population. [**note: this is from Columbia and shows that children have a reduced antibody response compared to adults. It's unclear what this means.**]
https://www.nature.com/articles/s41590-020-00826-9
- SARS-CoV-2 can mutate to evade immunity, with consequences for the efficacy of emerging vaccines and antibody therapeutics. Herein we demonstrate that the immunodominant SARS-CoV-2 spike (S) receptor binding motif (RBM) is the most divergent region of S, and provide epidemiological, clinical, and molecular characterization of a prevalent RBM variant, N439K. We demonstrate that N439K S protein has enhanced binding affinity to the hACE2 receptor, and that N439K virus has similar clinical outcomes and in vitro replication fitness as compared to wild-type. *We observed that the N439K mutation resulted in immune escape from a panel of neutralizing monoclonal antibodies, including one in clinical trials, as well as from polyclonal sera from a sizeable fraction of persons recovered from infection. Immune evasion mutations that maintain virulence and fitness such as N439K can emerge within SARS-CoV-2 S, highlighting the need for ongoing molecular surveillance to guide development and usage of vaccines and therapeutics*. [**note: this may or may not be good news. Here is a mutation in the Spike protein that may escape the immune system. This is from Scotland and also Vir Biotechnology, a California company.**]
https://www.biorxiv.org/content/10.1101/2020.11.04.355842v1
- Angiotensin-converting enzyme 2 (ACE2) is the primary host cell receptor that interacts with the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Although pneumonia is the main symptom in severe cases of SARS-CoV-2 infection, the expression levels of ACE2 in the lung is low, suggesting the presence of another receptor for the spike protein. In order to identify the

additional receptors for the spike protein, we screened a receptor for the SARS-CoV-2 spike protein from the lung cDNA library. We cloned L-SIGN as a specific receptor for the N-terminal domain (NTD) of the SARS-CoV-2 spike protein. The RBD of the spike protein did not bind to L-SIGN. In addition, not only L-SIGN but also DC-SIGN, a closely related C-type lectin receptor to L-SIGN, bound to the NTD of the SARS-CoV-2 spike protein. Importantly, cells expressing L-SIGN and DC-SIGN were both infected by SARS-CoV-2. Furthermore, L-SIGN and DC-SIGN induced membrane fusion by associating with the SARS-CoV-2 spike protein. Serum antibodies from infected patients and a patient-derived monoclonal antibody against NTD inhibited SARS-CoV-2 infection of L-SIGN or DC-SIGN expressing cells. *Our results highlight the important role of NTD in SARS-CoV-2 dissemination through L-SIGN and DC-SIGN and the significance of having anti-NTD neutralizing antibodies in antibody-based therapeutics*. [**note: this is from Japan and looks at the importance of the N-terminal domain of the Spike glycoprotein and how it influences infection.**] https://www.biorxiv.org/content/10.1101/2020.11.05.369264v1

DIAGNOSTIC DEVELOPMENT

- The long-lasting global COVID-19 pandemic demands timely genomic investigation of SARS-CoV-2 viruses. Here we report a simple and efficient workflow for whole genome sequencing utilizing one-step RT-PCR amplification on a microfluidic platform, followed by MiSeq amplicon sequencing. The method uses Fluidigm IFC and instruments to amplify 48 samples with 39 pairs of primers in a single step. Application of this method on RNA samples from both viral isolate and clinical specimens demonstrate robustness and efficiency of this method in obtaining the full genome sequence of SARS-CoV-2. [**note: this is from Walter Reed and Univ of Missouri and offers a rapid high throughput whole genome sequencing approach.**] https://www.biorxiv.org/content/10.1101/2020.11.04.369165v1

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2020-11-07

Yes, we are still here with good old Franz Joseph Haydn. He wrote some exquisite vocal music. One of my favorites is the cantata, 'Arianna a Naxos' that is written for mezzo-soprano. Here is a live performance from this past September with Anna Reinhold accompanied by Florent Albrecht: https://www.youtube.com/watch?v=e7Lv39y-ug8 The text and translation are HERE. This is a glorious performance and interpretation.

I'm sure you all are reading about the large numbers of cases of COVID-19 in the US and Europe. I'm not going to post a lot of links to those stories.

The New York Times reports on a Boston study that shows a large percent of grocery workers who had asymptomatic COVID-19. The UK RECOVERY trials team is now studying aspirin. If it works, it certainly will be a low cost treatment. Here is a story on the finding that children produce weaker antibodies which I posted the abstract of yesterday. It may be they clear the virus quicker than adults.

The Washington Post discusses how choral groups are trying to find their voice. I miss singing!!! Here is a useful op-ed to ponder from the director of public health in Bismarck, ND.

Nature have a useful article on rapid COVID-19 tests.

The Annals of Internal Medicine have an opinion piece on the use of emergency approvals for COVID-19 treatments and the impact on patient care.

There were not many articles posted on the servers I monitor.

MODELING

- Nothing today.

NEWLY REGISTERED CLINICAL TRIALS

- Be patient, one more day!!!!

CLINICAL TRIAL RESULTS

- Nothing today

DRUG DEVELOPMENT

- In the present report, we describe two small molecules with broad-spectrum antiviral activity. These drugs block formation of the nodosome. The studies were prompted by the observation that infection of human fetal brain cells with Zika virus (ZIKV) induces expression of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), a host factor that was found to promote ZIKV replication and spread. A drug that targets NOD2 was shown to have potent broad-spectrum antiviral activity against other flaviviruses, alphaviruses and SARS-CoV-2, the causative agent of COVID-19. Another drug that inhibits the receptor-interacting serine/threonine-protein kinase 2 (RIPK2) which functions downstream of NOD2, also decreased replication of these pathogenic RNA viruses. The broad-spectrum action of nodosome targeting drugs is mediated, at least in part, by enhancement of the interferon response. Together, these results suggest that further preclinical investigation of nodosome inhibitors as potential broad-spectrum antivirals is warranted. [**note: this is from Univ of Alberta and looks at two small molecules with broad antiviral activity. I don't know what the structures of these two compounds are as they appear to have been developed by GSK at one point in time. I'll leave it to those of you who are interested to read the paper and pursue it.**]
  https://www.biorxiv.org/content/10.1101/2020.11.05.370767v1
- Leading SARS-CoV-2 vaccine candidates immunize with the viral spike protein delivered on viral vectors, encoded by injected mRNAs, or as purified protein. *Here we describe a different approach to SARS-CoV-2 vaccine development that uses exosomes to deliver mRNAs that encode antigens from multiple SARS-CoV-2 structural proteins. Approach: Exosomes were purified and loaded with mRNAs designed to express (i) an artificial fusion protein, LSNME, that contains portions of the viral spike, nucleocapsid, membrane, and envelope proteins, and (ii) a functional form of spike.* The resulting combinatorial vaccine, LSNME/SW1, was injected into thirteen weeks-old, male C57BL/6J mice, followed by interrogation of humoral and cellular immune responses to the SARS-CoV-2 nucleocapsid and spike proteins, as well as hematological and histological analysis to interrogate animals for possible adverse effects. Results: Immunized mice developed CD4+, and CD8+ T-cell reactivities that respond to both the SARS-CoV-2 nucelocapsid protein and the SARS-CoV-2 spike protein. These responses were apparent nearly two months

after the conclusion of vaccination, as expected for a durable response to vaccination. In addition, the spike-reactive CD4+ T-cells response was associated with elevated expression of interferon gamma, indicative of a Th1 response, and a lesser induction of interleukin 4, a Th2-associated cytokine. Vaccinated mice showed no sign of altered growth, injection-site hypersensitivity, change in white blood cell profiles, or alterations in organ morphology. Consistent with these results, we also detected moderate but sustained anti-nucleocapsid and anti-spike antibodies in the plasma of vaccinated animals. Conclusion: Taken together, these results validate the use of exosomes for delivering functional mRNAs into target cells in vitro and in vivo, and more specifically, establish that the LSNME/SW1 vaccine induced broad immunity to multiple SARS-CoV-2 proteins. [**note: this is from Johns Hopkins that has a different way to prepare mRNA vaccines using exosomes to deliver the mRNA. I like this approach as they deliver several different antigens in this study rather than just the Spike protein. I wish more researchers would be doing this.**]
https://www.biorxiv.org/content/10.1101/2020.11.06.371419v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Zoonotic introduction of novel coronaviruses may encounter preexisting immunity in humans. Using diverse assays for antibodies recognizing SARS-CoV-2 proteins, we detect preexisting humoral immunity. SARS-CoV-2 spike glycoprotein (S)-reactive antibodies were detectable by a flow cytometry-based method in SARS-CoV-2-uninfected individuals and were particularly prevalent in children and adolescents. They were predominantly of the IgG class and targeted the S2 subunit. By contrast, SARS-CoV-2 infection induced higher titers of SARS-CoV-2 S-reactive IgG antibodies, targeting both the S1 and S2 subunits, and concomitant IgM and IgA antibodies, lasting throughout the observation period. Notably, SARS-CoV-2-uninfected donor sera exhibited specific neutralizing activity against SARS-CoV-2 and SARS-CoV-2 S pseudotypes. Distinguishing preexisting and de novo immunity will be critical for our understanding of susceptibility to and the natural course of SARS-CoV-2 infection. [**note: this is from a large UK team and looks at humoral immunity in humans. There may be some preexisting immunity because of exposure to the other common coronaviruses.**]
https://science.sciencemag.org/content/early/2020/11/05/science.abe1107
- Background: The longevity of the immune response against SARS-CoV-2 is currently debated. We thus profiled the serum anti-SARS-CoV-2 antibody levels and virus specific memory B- and T-cell responses over time in convalescent COVID-19 patients. Methods: A cohort of COVID-19 patients from the Lombardy region in Italy who experienced mild to critical disease and Swedish volunteers with mild symptoms, were tested for the presence of elevated anti-spike and anti-receptor binding domain antibody levels over a period of eight months. In addition, specific memory B- and T-cell responses were tested in selected patient samples. Results: Anti-SARS-CoV-2 antibodies were present in 85% samples collected within 4 weeks after onset of symptoms in COVID-19 patients. *Levels of specific IgM or IgA antibodies declined after 1 month while levels of specific IgG antibodies remained stable up to 6 months after diagnosis. Anti-SARS-CoV-2 IgG antibodies were still present, though at a significantly lower level, in 80% samples collected at 6-8 months after symptom onset. SARS-CoV-2-specific memory B- and T-cell responses were developed in vast majority of the patients tested, regardless of disease severity, and remained detectable up to 6-8 months after infection. Conclusions: Although the serum*

levels of anti-SARS-CoV-2 IgG antibodies started to decline, virus-specific T and/or memory B cell responses increased with time and maintained during the study period (6-8 months after infection). [**note: here is a another time study of antibody decay in COVID-19 patients. As I've noted in the past, loss of antibodies does not mean loss of immunity.**]
https://www.biorxiv.org/content/10.1101/2020.11.06.371617v1

DIAGNOSTIC DEVELOPMENT

- Nothing new

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2020-11-08

For reflection Sunday, we need to all listen to Haydn's 'The Creation' and gain inspiration from this work. This masterful performance is in commemoration of the 200th anniversary of Haydn's death and take place at the Esterhazy Palace in Austria. Soloists include Annette Dasch, Chrisoph Strehl and Thomas Quasthoff. Quastoff, in addition to being an incredibly fine singer was born with defects from thalidomide exposure before the teratogenic effects of the drug were discovered. The orchestra is conducted by Adam Fischer. https://www.youtube.com/watch?v=xIpSNMQZH9M Enjoy and reflect on what was and what can be.

The Washington Post notes that Shakespeare's 'King Lear' was written during a plague and want to know what literature will come from the COVID-19 pandemic. If you like dystopian fiction, I can strongly recommend the 'Oryx and Crake' trilogy by Margaret Atwood. As Italy is finding out, the battle against SARS-CoV-2 requires constant vigilance. There is another outbreak of the virus in the White House.

The New York Times has a good story on whether IL-6 is an important factor in the observed cytokine storm accompanying severe COVID-19. There is so much confounding data on the efficacy of tocilizumab, the mAb against the interleukin. Biden is expected to announce a COVID-19 task force tomorrow. There is no good news on the case and mortality levels in the US.

JAMA have a news article on the testing quagmire in the US and note that some are refusing to be tested. Here is an article on the track and trace effort in San Francisco during the early stage of the pandemic.

Yes, you fans of new clinical research will be overjoyed that I scanned the NIH Clinical Trials Database and reported out on new trials today!!!!!👍

MODELING

- Background: Changes in autonomic nervous system function, characterized by heart rate variability (HRV), have been associated with and observed prior to the clinical identification of infection. We performed an evaluation of this metric collected by wearable devices, to identify and predict Coronavirus disease 2019 (COVID-19) and its related symptoms. Methods: Health care workers in the Mount Sinai Health System were prospectively followed in an ongoing observational study using the custom Warrior Watch Study App which was downloaded to their

smartphones. Participants wore an Apple Watch for the duration of the study measuring HRV throughout the follow up period. Surveys assessing infection and symptom related questions were obtained daily. Findings*: Using a mixed-effect COSINOR model the mean amplitude of the circadian pattern of the standard deviation of the interbeat interval of normal sinus beats (SDNN), a HRV metric, differed between subjects with and without COVID-19 (p=0.006). The mean amplitude of this circadian pattern differed between individuals during the 7 days before and the 7 days after a COVID-19 diagnosis compared to this metric during uninfected time periods (p=0.01). Significant changes in the mean MESOR and amplitude of the circadian pattern of the SDNN was observed between the first day of reporting a COVID-19 related symptom compared to all other symptom free days (p=0.01). Interpretation: Longitudinally collected HRV metrics from a commonly worn commercial wearable device (Apple Watch) can identify the diagnosis of COVID-19 and COVID-19 related symptoms. Prior to the diagnosis of COVID-19 by nasal PCR, significant changes in HRV were observed demonstrating its predictive ability to identify COVID-19 infection*. [**note: this is another paper from Mt Sinai and looks at the use of a wearable device (Apple watch) among health staff there. Perhaps there is some utility to using this approach as a predictive measure for infection.**]
https://www.medrxiv.org/content/10.1101/2020.11.06.20226803v1

- Projections of the stage of the Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) pandemic and local, regional and national public health policies designed to limit the spread of the epidemic as well as reopen cities and states, are best informed by reproducible, high throughput, and statically credible antibody (Ab) assays. To date, a myriad of Ab tests, both available and authorized for emergency use by the FDA, has led to confusion rather than insight per se. The present study reports the results of a rapid, point-in-time 1,000-person cohort study using serial blood donors in the New York City metropolitan area (NYC) using multiple serological tests, including enzyme-linked immunosorbent assays (ELISAs) and high throughput serological assays (HTSAs). These were then tested and associated with assays for neutralizing Ab (NAb). Of the 1,000 NYC blood donor samples in late June and early July 2020, 12.1% and 10.9% were seropositive using the Ortho Total Ig and the Abbott IgG HTSA assays, respectively. These serological assays correlated with neutralization activity specific to SARS-CoV-2. The data reported herein suggest that seroconversion in this population occurred in approximately 1 in 8 blood donors from the beginning of the pandemic in NYC (considered March 1, 2020). These findings deviate with an earlier seroprevalence study in NYC showing 13.7% positivity. *Collectively however, these data demonstrate that a low number of individuals have serologic evidence of infection during this first wave and suggest that the notion of herd immunity at rates of ~60% or higher are not near. Furthermore, the data presented herein show that the nature of the Ab-based immunity is not invariably associated with the development of NAb. While the blood donor population may not mimic precisely the NYC population as a whole, rapid assessment of seroprevalence in this cohort and serial reassessment could aid public health decision making*. [**note: this is another serological study of New York City.**]
https://www.medrxiv.org/content/10.1101/2020.11.06.20220087v1
- The control of shipborne disease outbreaks represents a vexing but entirely predictable challenge at the start of any pandemic. Passenger ships, with large numbers of people confined in close quarters, can serve as incubators of disease, seeding the pandemic across the globe as infected passengers return home. Short-term steps taken by local authorities can exacerbate

this problem, creating humanitarian crises and worsening the scale of the outbreak. In this work, we have undertaken a model-based examination of the USS Theodore Roosevelt outbreak to understand the dynamics of COVID-19 spread aboard the aircraft carrier. We have used a series of counterfactual "what-if" analyses to better understand the options available to public health authorities in such situations. The models suggest that rapid mass evacuation and widespread surveillance testing can be effective in these settings. Our results lead to a set of generalizable recommendations for disease control that are broadly applicable to the current COVID-19 crisis as well as to future pandemics. [**note: this model is based on the outbreak on the USS Theodore Roosevelt that took place early in the pandemic.**]
https://www.medrxiv.org/content/10.1101/2020.11.05.20226712v1

NEWLY REGISTERED CLINICAL TRIALS

- This is the first study of COVI-VAC in humans. The purpose of the study is to evaluate the safety and immune response of COVI-VAC (a live attenuated vaccine to prevent COVID-19) in healthy adults aged 18 to 30 years. Approximately 48 participants will be enrolled into 1 of 3 dose groups (low, medium, high). Within each of these dose groups, participants will be assigned randomly to receive either 2 doses of COVI-VAC 28 days apart, 2 doses of placebo (saline), or 1 dose of COVI-VAC and 1 dose of placebo. COVI-VAC or placebo is administered by drops into each nostril. Neither the participants nor the researchers will know whether COVI-VAC or placebo has been received. [**note: this is a single dose live attenuated nasally delivered COVID-19 vaccine from Codagenix. I like this approach.]** NCT04619628
- In this study, a vaccine developed by IIBR for SARS-CoV-2 virus will be assessed for its safety and potential efficacy in volunteers. The study is comprised of two phases, a dose-escalation phase (phase I) during which subjects (18-55 years old) will be randomly allocated to receive a single administration of IIBR-100 100 at low, mid or high dose or saline or two administrations of IIBR-100 at low dose, or saline, 28 days apart. [**note: this is an Israeli vaccine developed by their Institute of Biological Research. I don't see much more details on this and perhaps it uses vesicular stomatitis virus as the vector. If so, this makes it similar to one of the Merck approaches.**] NCT04608305
- Cyclophilins are cellular (host) peptidyl-prolyl cis/trans isomerases (molecular chaperones) involved in protein folding, maturation, and trafficking. Cyclophilins have been shown to play a key role in the lifecycle of many coronaviruses, including human coronaviruses 229E (HCoV-229E) and NL-63 (HCoV-NL63), feline infectious peritonitis coronavirus (FPIV), SARS-CoV and Middle-East-Respiratory-Syndrome coronavirus (MERS-CoV). Cyclosporin A (CsA), a potent cyclophilin inhibitor, blocks the replication of various coronaviruses in vitro, including HCoV-229E, HCoV-NL63, FPIV, mouse hepatitis virus (MHV), avian infectious bronchitis virus, and SARS-CoV. Alisporivir is a non-immunosuppressive analogue of CsA with potent cyclophilin inhibition properties. In vitro, alisporivir inhibits the replication of HCoV-229E, HCoV-NL63, MHV, SARS-CoV and MERS-CoV at low micromolar concentrations without cytotoxic effect. Although alisporivir has not demonstrated activity against coronaviruses in in vivo models to date, recent experiments showed that alisporivir bears concentration-dependent properties against CoV-2 in vitro. [**note: this is a French trial of alisporivir.**] NCT04608214

CLINICAL TRIAL RESULTS

- Nothing today.

DRUG DEVELOPMENT

- The SARS-CoV-2 pandemic is continuing to disrupt personal lives, global healthcare systems and economies. Hence, there is an urgent need for a vaccine that prevents viral infection, transmission and disease. Here, we present a two-component protein-based nanoparticle vaccine that displays multiple copies of the SARS-CoV-2 spike protein. Immunization studies show that this vaccine induces potent neutralizing antibody responses in mice, rabbits and cynomolgus macaques. The vaccine-induced immunity protected macaques against a high dose challenge, resulting in strongly reduced viral infection and replication in upper and lower airways. These nanoparticles are a promising vaccine candidate to curtail the SARS-CoV-2 pandemic. [**note: this is another vaccine candidate based on nanoparticles. I can't be totally sure as it is not clear in the paper, but it looks like this comes from Icosavax.**]
https://www.biorxiv.org/content/10.1101/2020.11.07.365726v1
- The COVID-19 pandemic has prompted the search for animal models that recapitulate the pathophysiology observed in humans infected with SARS-CoV-2 and allow rapid and high throughput testing of drugs and vaccines. Exposure of larvae to SARS-CoV-2 Spike (S) receptor binding domain (RBD) recombinant protein was sufficient to elevate larval heart rate and treatment with captopril, an ACE inhibitor, reverted this effect. Intranasal administration of SARS-CoV-2 S RBD in adult zebrafish recombinant protein caused severe olfactory and mild renal histopathology. Zebrafish intranasally treated with SARS-CoV-2 S RBD became hyposmic within minutes and completely anosmic by 1 day to a broad-spectrum of odorants including bile acids and food. Single cell RNA-Seq of the adult zebrafish olfactory organ indicated widespread loss of expression of olfactory receptors as well as inflammatory responses in sustentacular, endothelial, and myeloid cell clusters. Exposure of wildtype zebrafish larvae to SARS-CoV-2 in water did not support active viral replication but caused a sustained inhibition of ace2 expression, triggered type 1 cytokine responses and inhibited type 2 cytokine responses. Combined, our results establish adult and larval zebrafish as useful models to investigate pathophysiological effects of SARS-CoV-2 and perform pre-clinical drug testing and validation in an inexpensive, high throughput vertebrate model. [**note: more data on the use of the Zebrafish as a model for SARS-CoV-2 pathologies.**]
https://www.biorxiv.org/content/10.1101/2020.11.06.368191v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Although neutralizing antibodies against the SARS-CoV-2 spike (S) protein are a goal of most COVID-19 vaccines and being developed as therapeutics, escape mutations could compromise such countermeasures. To define the immune-mediated mutational landscape in S protein, we used a VSV-eGFP-SARS-CoV-2-S chimeric virus and 19 neutralizing monoclonal antibodies (mAbs) against the receptor binding domain (RBD) to generate 48 escape mutants. These variants were mapped onto the RBD structure and evaluated for cross-resistance by convalescent human plasma. Although each mAb had unique resistance profiles, many shared residues within an epitope, as several variants were resistant to multiple mAbs. Remarkably, we identified mutants that escaped neutralization by convalescent human sera, suggesting that some humans induce a narrow repertoire of neutralizing antibodies. By comparing the antibody-mediated mutational

landscape in S protein with sequence variation in circulating SARS-CoV-2 strains, we identified single amino acid substitutions that could attenuate neutralizing immune responses in some humans. [**note: this is a useful paper that looks at possible escape variants of SARS-CoV-2 spike mutations that can attenuate antibody response. This is always going to be an issue with viruses as we well know from the yearly influenza mutations that lead to new outbreaks. Whether SARS-CoV-2 experiences this naturally is still unknown.**] https://www.biorxiv.org/content/10.1101/2020.11.06.372037v1

- SARS-CoV-2 accessory proteins ORF3a, ORF6, ORF7a, ORF7b, ORF8, and ORF10 with putative functions to manipulate host immune mechanisms such as interferons, immune signaling receptor NLRP3 (NOD-, LRR-, and pyrin domain-containing 3) inflammasome, inflammatory cytokines such as interleukin β (IL-1β) are critical in COVID-19 pathology. Outspread variations of each of the six accessory proteins of all complete proteomes (available as of October 26, 2020, in the National Center for Biotechnology Information depository) of SARS-CoV-2, were observed across six continents. Across all continents, the decreasing order of percentage of unique variations in the accessory proteins was found to be ORF3a>ORF8>ORF7a>ORF6>ORF10>ORF7b. The highest and lowest unique variations of ORF3a were observed in South America and Oceania, respectively. This finding suggests that the wide variations of accessory proteins seem to govern the pathogenicity of SARS-CoV-2, and consequently, certain propositions and recommendations can be made in the public interest. [**note: this is part of the ongoing effort to look at other proteins encoded by the SARS-CoV-2 virus and their possible role in the pathology of the infection.**] https://www.biorxiv.org/content/10.1101/2020.11.06.372227v1

DIAGNOSTIC DEVELOPMENT

- Nothing today

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