2020-11-09

Welcome to Week 34

We had our fill with Haydn last week so it's time to move on. How about Tchaikovsky to get the newsletter off to a good start this week? Let us begin with the violin concerto. Here is Lisa Batiashvili with Daniel Barenboiem's West-Eastern Divan Orchestra. I've had the orchestra on before supporting soloists and they do play extremely well under their founder. Enjoy this concerto: https://www.youtube.com/watch?v=EM4gpjcWmdQ

Here is some good news from the vaccine front! Pizer and its partner BioNTech say their mRNA-based COVID-19 vaccine is more than 90% effective. They still need two months of safety data from the active arm of the trial and this is expected to be completed by later this month.

The Washington Post also reports on the Pfizer vaccine. President-elect Biden has announced a COVID-19 task force. New York City is trying block by block lockdowns to control SARS-CoV-2.

The New York Times discusses the implication of COVID-19 infections in animals. Utah is moving to implement a mask mandate after an alarming jump in cases. This should have been done a long time ago. Shanghai has seen a single case of COVID-19 and moved to isolate the area to control spread.

STAT are following along in reporting on the Pfizer vaccine press release. Are leaders at the state and local level losing the urgency to control SARS-CoV-2 outbreaks?

Kaiser Health News have an interesting story on a snakebite drug that might have applications to COVID-19. As one who trained as a lipid biochemist in grad school, I found it interesting to read about varespladib, a phospholipase inhibitor.

As always, Mondays are increasingly short of preprints. Let us hope that the other vaccine developers also report out good news in the next several months!!!!

MODELING

- Background: Since the beginning of the COVID -19 pandemic, many contact sport teams are facing major challenges to safely continue training and competition. Objective: We present the design and implementation of a structured monitoring concept for the Austrian national football league Methods: 146 professional players from five clubs of the professional Austrian football league were monitored for a period of 12 weeks. Subjective health parameters, PCR- test results and data obtained from a geo-tracking app were collected. Simulations modelling the consequences of a COVID-19 case with increasing reproduction number were computed. Results: *No COVID -19 infection occurred during the observation period in the players. Infections in the nearer surroundings lead to increased perceived risk of infection. Geo tracking was particularly hindered due to technical problems and reluctance of users. Simulation models suggested a hypothetical shut-down of all training and competition activities. Conclusions: A structured monitoring concept can help to continue contact sports safely in times of a pandemic. Cooperation of all involved is essential*. [**note: here is an approach to monitoring for COVID-19 that the Austrian football league implemented.**]
  https://www.medrxiv.org/content/10.1101/2020.11.06.20226977v1

NEWLY REGISTERED CLINICAL TRIALS

- I checked yesterday.

CLINICAL TRIAL RESULTS

- Medical journals usually do not report out on Mondays.

DRUG DEVELOPMENT

- The current pandemic of the coronavirus disease-2019 (COVID-19) has badly affected our life during the year 2020. SARS-CoV-2 is the primary causative agent of the newly emerged pandemic. Natural flavonoids, Terpenoid and Thymoquinone are tested against different viral and host-cell protein targets. These natural compounds have a good history in treating Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV). Molecular docking combined with cytotoxicity and plaque reduction assay is used to test the natural compounds against different viral (Spike, RdRp, and Mpro) and host-cell (TMPRSS II, keap 1, and ACE2) targets. The results demonstrate the binding possibility of the natural compounds (Thymol, Carvacrol, Hesperidine, and Thymoquinone) to the viral main protease (Mpro). Some of these natural compounds were approved to start clinical trail from Egypt Center for Research and Regenerative Medicine ECRRM IRB (Certificate No.IRB00012517) [**note: this is from Egypt and is an *in vitro* look at some natural products that may prove useful in treating COVID-19. There is a long history of plant derived medicines and maybe one of these will pan out.**] https://www.biorxiv.org/content/10.1101/2020.11.07.367649v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Nothing today

DIAGNOSTIC DEVELOPMENT

- Nothing today

>>

2020-11-10

Keeping with the music theme of the week here is Tchaikovsky's Fantasy Overture on Romeo and Juliet. It is played by the Frankfurt Radio Orchestra who I have featured in the past conducted by Andrés Orozco-Estrada. This is really nice: https://www.youtube.com/watch?v=3x69wuewkJM

The New York Times reports that the FDA has given an emergency use authorization for the Eli Lilly monoclonal antibody treatment. It is limited to people 12 years of age and older and applies only to people newly infected. There is a warning that it should not be used on hospitalized patients. Here are 11 things you need to know about the Pfizer COVID-19 vaccine. Returning to an office setting in a pandemic is a difficult decision. Here is a good story on the confounding results on IL-6 and cytokine storm.

The Washington Post has another in its continuing series on voices from the pandemic, this time from a physician in South Dakota who talks about the situation there. This is a cautionary story about a rural

area in mainly rural state; the virus doesn't care. The Pfizer mRNA vaccine might get an emergency use authorization in December and an accompanying good op-ed on the 'fine print.' Here is more on the Eli Lilly monoclonal antibody EUA. Planning on travel? Here is information on getting tested for COVID-19. Minks in Denmark may live after all. I'm sorry, but this behavior in Oklahoma is just stupid, it's just like not wearing a seat belt when you go out driving.

STAT talks about how the mask mandate will resemble the battle over seatbelt laws. This is too sad. The pandemic is transforming agendas in the clinical trials industry.

The Lancet have a story on psychiatric issues and COVID-19. *Survivors of COVID-19 appear to be at increased risk of psychiatric sequelae, and a psychiatric diagnosis might be an independent risk factor for COVID-19. Although preliminary, our findings have implications for clinical services, and prospective cohort studies are warranted*. Here is a commentary on this paper.

Medscape note that one of the authors of the Great Barrington Declaration is backing off the strict reliance on herd immunity. Novartis announced that its arthritis drug, canakinumab, did not help severe COVID-19 patients.

MODELING

- Surprisingly, no models today.

NEWLY REGISTERED CLINICAL TRIALS

- No, you really don't need to know anything about this right now.

CLINICAL TRIAL RESULTS

- The severity of coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly heterogenous. Studies have reported that males and some ethnic groups are at increased risk of death from COVID-19, which implies that individual risk of death might be influenced by host genetic factors. In this project, we consider the mortality as the trait of interest and perform a genome-wide association study (GWAS) of data for 1,778 infected cases (445 deaths, 25.03%) distributed by the UK Biobank. Traditional GWAS failed to identify any genome-wide significant genetic variants from this dataset. To enhance the power of GWAS and account for possible multi-loci interactions, we adopt the concept of super-variant for the detection of genetic factors. *A discovery-validation procedure is used for verifying the potential associations. We find 8 super-variants that are consistently identified across multiple replications as susceptibility loci for COVID-19 mortality. The identified risk factors on Chromosomes 2, 6, 7, 8, 10, 16, and 17 contain genetic variants and genes related to cilia dysfunctions (DNAH7 and CLUAP1), cardiovascular diseases (DES and SPEG), thromboembolic disease (STXBP5), mitochondrial dysfunctions (TOMM7), and innate immune system (WSB1). It is noteworthy that DNAH7 has been reported recently as the most downregulated gene after infecting human bronchial epithelial cells with SARS-CoV2*. [**note: here is another analysis of genetic predisposition to COVID-19 mortality using data from the UK BioBank.**] https://www.medrxiv.org/content/10.1101/2020.11.05.20226761v1

- Objectives To assess whether gout and / or rheumatoid arthritis (RA) are risk factors for coronavirus disease 19 (COVID-19) diagnosis. To assess whether gout and / or RA are risk factors for death from COVID-19. Methods We used data from the UK Biobank. Multivariate-adjusted logistic regression was employed in the following analyses. Analysis A: to test for association between gout or RA and COVID-19 diagnosis in a population-based cohort (n=473,139). Analysis B: to test for association between gout or RA and death from COVID-19 in a case-control cohort of people who died or survived with COVID-19 (n=2,073). Analysis C: to test for association with gout or RA and death from COVID-19 in a population-based cohort (n=473,139) Results Neither RA nor gout associated with COVID-19 diagnosis in analysis A, nor did RA or gout associate with risk of death in the COVID-19-diagnosed group in analysis B. However RA associated with risk of death from COVID-19 using the population-based cohort in analysis C independent of comorbidities and other measured risk factors (OR=1.8 [95% CI 1.2 ; 2.7]). Gout was not associated with death from COVID-19 in the same population-based analysis (OR=1.2 [95% CI 0.9 ; 1.7]). Conclusions RA and gout are not risk factors for COVID-19-diagnosis. However RA, but not gout, is a risk factor for death from COVID-19 in a population-based analysis using the UK Biobank. These findings require replication in larger data sets that also allow inclusion of a wider range of factors. [**note: here is another UK BioBank study looking at patients with existing rheumatoid arthritis and gout. Neither was a risk factor for contracting COVID-19 but RA seems to be a risk factor for death from severe COVID-19.**]
  https://www.medrxiv.org/content/10.1101/2020.11.06.20227405v1
- SARS-CoV-2 causes remarkably variable disease from asymptomatic individuals to respiratory insufficiency and coagulopathy. Vitamin K deficiency was recently found to associate with clinical outcome in a cohort of COVID-19 patients. Vitamin D has been hypothesized to reduce disease susceptibility by modulating inflammation, yet little is known about its role in disease severity. Considering the critical interaction between vitamin K and vitamin D in calcium and elastic fiber metabolism, we determined vitamin D status in the same cohort of 135 hospitalized COVID-19 patients by measuring blood 25(OH)D levels. We found no difference in vitamin D status between those with good and poor outcome (defined as intubation and/or death). Instead, we found vitamin D sufficient persons (25(OH)D >50 nmol/L) had accelerated elastic fiber degradation compared to those with mild deficiency (25(OH)D 25-50 nmol/L). Based on these findings, we hypothesize that vitamin D might have both favorable anti-inflammatory and unfavorable pro-calcification effects during COVID-19 and that vitamin K might compensate for the latter. [**note: I don't know what to make of this paper. In looking at the Vitamin K reference link, I think my daily intake of this vitamin is OK.**]
  https://www.medrxiv.org/content/10.1101/2020.11.07.20227512v1
- Multisystem Inflammatory Syndrome in Children (MIS-C), a hyperinflammatory syndrome associated with SARS-CoV-2 infection, shares many clinical features with toxic shock syndrome, which is triggered by bacterial superantigens. The superantigen specificity for binding different Vbeta-chains results in Vbeta-skewing, whereby T cells with specific Vbeta-chains and diverse antigen specificity are overrepresented in the TCR repertoire. Here, we characterized the TCR repertoire of MIS-C patients and found a profound expansion of TCR Beta Variable gene (TRBV)11-2. Furthermore, TRBV11-2 skewing was remarkably correlated with MIS-C severity and serum cytokine levels. Further analysis of TRBJ gene usage and CDR3 length distribution of MIS-C expanding TRBV11-2 clones revealed extensive junctional diversity, indicating a superantigen-

mediated selection process for TRBV expansion. In silico modelling indicates that polyacidic residues in TCR Vbeta11-2 engage in strong interactions with the superantigen-like motif of SARS-CoV-2 spike glycoprotein. *Overall, our data indicate that the immune response in MIS-C is consistent with superantigenic activation*. [**note: here is a possible explanation for multi-system inflammatory syndrome in children.**]
https://www.biorxiv.org/content/10.1101/2020.11.09.372169v1

DRUG DEVELOPMENT

- User-friendly chemicals interfering at the host site with viral entry might be an approach to contain the pandemic. In addition, such an approach would work synergistic with vaccinations that miss new virus mutants. Nafamostat (NM) has been shown in vitro to interfere with cellular virus entry by inhibition of the host transmembrane protease serine 2 (TMPRSS2), an enzyme required for SARS-CoV-2 spike protein cleavage, a prerequisite for cell entry. We hypothesized that nasal application of NM in a liposomal layer (as additional mechanical barrier) could lower the nasal viral load and subsequently reduce the severity of COVID-19. *We found, indeed, that nasal viral load one day post single NM application, was lowered in a hamster SARS-CoV-2 infection model. However, severity of subsequent local tissue destruction and weight loss due to pneumonitis was not favorably altered. In conclusion, a single NM application reduced nasal viral load, but did not favorably improve the outcome of COVID-19, likely due to the short half-time of NM. Improvement of NM stability or repetitive application (which was not permitted in this animal model according to Dutch law) might circumvent these challenges*. [**note: this is a Dutch study looking at a lipid carrier for nafamostat that inhibits the main protease of the virus. They are looking to see if a nasal application can provide some protection. It appears that a single application does not work.**]
https://www.biorxiv.org/content/10.1101/2020.11.09.372375v1
- We have applied an mRNA-based technology platform, RNActive, to develop CVnCoV which contains sequence optimized mRNA coding for a stabilized form of S protein encapsulated in lipid nanoparticles (LNP). Following demonstration of protective immune responses against SARS-CoV-2 in animal models we performed a dose-escalation phase 1 study in healthy 18-60 year-old volunteers. This interim analysis shows that two doses of CVnCoV ranging from 2 µg to 12 µg per dose, administered 28 days apart were safe. No vaccine-related serious adverse events were reported. There were dose-dependent increases in frequency and severity of solicited systemic adverse events, and to a lesser extent of local reactions, but the majority were mild or moderate and transient in duration. Immune responses when measured as IgG antibodies against S protein or its receptor-binding domain (RBD) by ELISA, and SARS-CoV-2-virus neutralizing antibodies measured by micro-neutralization, displayed dose-dependent increases. Median titers measured in these assays two weeks after the second 12 µg dose were comparable to the median titers observed in convalescent sera from COVID-19 patients. Seroconversion (defined as a 4-fold increase over baseline titer) of virus neutralizing antibodies two weeks after the second vaccination occurred in all participants who received 12 µg doses. *Preliminary results in the subset of subjects who were enrolled with known SARS-CoV-2 seropositivity at baseline show that CVnCoV is also safe and well tolerated in this population, and is able to boost the pre-existing immune response even at low dose levels. Based on these results, the 12 µg dose is selected for further clinical investigation, including a phase 2b/3 study*

that will investigate the efficacy, safety, and immunogenicity of the candidate vaccine CVnCoV. [**note: here is data on the CureVac mRNA vaccine as it moves into large scale trials.**] https://www.medrxiv.org/content/10.1101/2020.11.09.20228551v1

- In the ongoing SARS CoV-2 pandemic effective measures are needed, and guidance based on the methodological framework of the European committee for standardization (CEN) can help to choose effective disinfectants on an immediate basis. This study demonstrates that two commercially available formulations for surface disinfection and one formulation for hand disinfection claiming virucidal activity against enveloped viruses are effectively inactivating SARS-CoV-2. This study emphasizes that chemical disinfectants claiming virucidal activity against enveloped viruses are an effective choice to target enveloped SARS-CoV-2 as a preventive measure. [**note: here is a study of different formulations for hand and surface disinfection.]** https://www.biorxiv.org/content/10.1101/2020.11.08.373738v1
- The main protease, Mpro, of SARS-CoV-2 is required to cleave the viral polyprotein into precise functional units for virus replication and pathogenesis. Here we demonstrate a quantitative reporter for Mpro function in living cells, in which protease inhibition by genetic or chemical methods results in strong eGFP fluorescence. This robust gain-of-function system readily distinguishes between inhibitor potencies and can be scaled-up to high-throughput platforms for drug testing. [**note: here is a high throuput screening approach for the Mpro enzyme.**] https://www.biorxiv.org/content/10.1101/2020.11.09.375139v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Background. It is currently unclear whether SARS-CoV-2 re-infection will remain a rare event, only occurring in individuals who fail to mount an effective immune response, or whether it will occur more frequently when humoral immunity wanes following primary infection. Methods. A case of re-infection was observed in a Belgian nosocomial outbreak involving 3 patients and 2 health care workers. To distinguish re-infection from persistent infection and detect potential transmission clusters, whole genome sequencing was performed on nasopharyngeal swabs of all individuals including the re-infection case's first episode. IgA, IgM, and IgG and neutralizing antibody responses were quantified in serum of all individuals, and viral infectiousness was measured in the swabs of the reinfection case. Results. *Re-infection was confirmed in a young, immunocompetent health care worker as viral genomes derived from the first and second episode belonged to different SARS-CoV-2 clades. The symptomatic re-infection occurred after an interval of 185 days, despite the development of an effective humoral immune response following symptomatic primary infection. The second episode, however, was milder and characterized by a fast rise in serum IgG and neutralizing antibodies. Although contact tracing and virus culture remained inconclusive, the health care worker formed a transmission cluster with 3 patients and showed evidence of virus replication but not of neutralizing antibodies in her nasopharyngeal swabs. Conclusion. If this case is representative of most Covid-19 patients, long-lived protective immunity against SARS-CoV-2 might not be likely*. [**note: this is from Belgium and looks at a case of re-infection. This continues to be a rare phenomenon.**] https://www.medrxiv.org/content/10.1101/2020.11.05.20225052v1
- Many countries around the world have all seen a sharp rise in COVID-19 cases as the second wave since the beginning of October 2020. Decline of antibodies response to severe acute respiratory syndrome coronavirus (SARS-CoV-2) that was reported exclusively in the early month

increases the risk of reinfection for convalescent individuals. There is a current need to follow the maintenance of special antibodies against SARS-CoV-2. Here, we reported changes of antibodies against SARS-CoV-2 in convalescent patients over 8 months. Antibodies of all 20 participants targeting SARS-CoV-2 spike receptor binding-domain (RBD) had decreased from a mean OD450 value 1.78 to 0.38 over 8 months. The neutralizing antibody (NAb) titers decreased from the mean ID50 value 836 to 170. The NAb titers were significantly correlated with IgG level during 8 months ($P<0.001$). Furthermore, while RBD-specific IgG existence of 25% (5/20) convalescent plasma was undetectable, the NAb titers of 15% (3/20) convalescent plasma decreased below the threshold. *In addition, compared to wild-type SARS-CoV-2 (S-D614), lower titers of neutralizing antibodies against its G614 variant were shown at 8 months after symptom onset. This study has important implications when considering antibody protection against SARS-CoV-2 reinfection*. [**note: this eight month follow up on antibody response in convalescent patients comes from China. It is a small study on 20 individuals and shows a decrease in antibodies. We still don't now what the state of immune memory is for SARS-CoV-2 in these patients.**] https://www.medrxiv.org/content/10.1101/2020.11.06.20227439v1

- A workflow for SARS-CoV-2 epitope discovery on peptide microarrays is herein reported. The process started with a proteome-wide screening of immunoreactivity based on the use of a high-density microarray followed by a refinement and validation phase on a restricted panel of probes using microarrays with tailored peptide immobilization through a click-based strategy. Progressively larger, independent cohorts of Covid-19 positive sera were tested in the refinement processes, leading to the identification of immunodominant regions on SARS-CoV-2 Spike (S), Nucleocapsid (N) protein and Orf1ab polyprotein. A summary study testing 50 serum samples highlighted an epitope of the N protein (region 155-171) providing 92% sensitivity and 100% specificity of IgG detection in Covid-19 samples thus being a promising candidate for rapid implementation in serological tests. [**note: this is from Italy and provides more epitope mapping for the virus. There is a strong epitope on the N protein which is another reason why I want to see vaccines incorporate this.**] https://www.biorxiv.org/content/10.1101/2020.11.09.374082v1
- Rationale: The secondary thrombotic/vascular clinical syndrome of COVID-19 suggests that SARS-CoV-2 infects not only respiratory epithelium but also the endothelium activating thrombotic pathways, disrupting barrier function and allowing access of the virus to other organs of the body. However, a direct test of susceptibility to SARS-CoV-2 of authentic endothelial cell lines has not been performed. Objective: To determine infectibility of primary endothelial cell lines with live SARS-CoV-2 and pseudoviruses expressing SARS-CoV-2 spike protein. Methods and Results: Expression of ACE2 and BSG pathways genes was determined in three types of endothelial cells; blood outgrowth, lung microvascular and aortic endothelial cells. For comparison nasal epithelial cells, Vero E6 cells (primate kidney fibroblast cell line) and HEK 293T cells (human embryonic kidney cells) transfected with either ACE2 or BSG were used as controls. Endothelial and Vero E6 cells were treated with live SARS-CoV-2 virus for 1 hour and imaged at 24 and 72 hours post infection. Pseudoviruses containing SARS-CoV-2, Ebola and Vesicular Stomatis Virus glycoproteins were generated and added to endothelial cells and HEK 239Ts for 2 hours and infection measured using luminescence at 48 hours post infection. Compared to nasal epithelial cells, endothelial cells expressed low or undetectable levels of ACE2 and TMPRSS2 but comparable levels of BSG, PPIA and PPIB. *Endothelial cells showed no*

susceptibility to live SARS-CoV-2 or SARS-CoV-2 pseudovirus (but showed susceptibility to Ebola and Vesicular Stomatitis Virus). Overexpression of ACE2 but not BSG in HEK 239T cells conferred SARS-CoV-2 pseudovirus entry. Endothelial cells primed with IL-1b remained resistant to SARS-CoV-2. Conclusion: Endothelial cells are resistant to infection with SARS-CoV-2 virus, in line with relatively low levels of ACE2 and TMPRSS2, suggesting that the vascular dysfunction and thrombosis seen in severe COVID-19 is a result of factors released by adjacent infected cells (e.g. epithelial cells) and/or circulating, systemic inflammatory mediators. [**note: endothelial cells appear to be resistant to virus infection *in vitro.* The vascular effects can't result from these cells but likely adjacent epithelial cells.**]
https://www.biorxiv.org/content/10.1101/2020.11.08.372581v1

- The COVID-19 pandemic (SARS-CoV-2) is a global infectious disease with rapid spread. Some patients have severe symptoms and clinical signs caused by an excessive inflammatory response, which increases the risk of mortality. In this study, we reanalyzed scRNA-seq data of cells from bronchoalveolar lavage fluids of patients with COVID-19 with mild and severe symptoms, focusing on antibody-producing cells. In patients with severe disease, B cells seemed to be more activated and expressed more immunoglobulin genes compared with cells from patients with mild disease, and macrophages expressed higher levels of the TNF superfamily member B-cell activating factor but not of APRIL (a proliferation-inducing ligand). In addition, macrophages from patients with severe disease had increased pro-inflammatory features and pathways associated with Fc receptor-mediated signaling, compared with patients with mild disease. CCR2-positive plasma cells accumulated in patients with severe disease, probably because of increased CCL2 expression on macrophages from patients with severe disease. *Together, these results support that different characteristics of B cells might affect the severity of COVID-19 infection*. [**note: this is from Korea and looks at immune cells in bronchoalveolar fluids from patients with mild and severe COVID-19.**]
https://www.biorxiv.org/content/10.1101/2020.11.09.374272v1
- The host response to CoV infection is complex and regulated, in part, by intracellular antiviral signaling pathways triggered in the first cells that are infected. Emerging evidence suggests that CoVs hijack these antiviral responses to reshape the production of interferons and proinflammatory cytokines. Processing bodies (PBs) are membraneless ribonucleoprotein granules that mediate decay or translational suppression of cellular mRNAs; this is particularly relevant for proinflammatory cytokine mRNA which normally reside in PBs and are repressed. Emerging evidence also suggests that PBs or their components play important direct-acting antiviral roles, providing a compelling reason for their frequent disassembly by many viruses. No information is known about how human CoVs impact PBs. Here, we provide data to show that infection with the human CoV, OC43, causes PB disassembly. Moreover, we show that several SARS-CoV-2 gene products also mediate PB loss and virus-induced PB loss correlates with elevated levels of proinflammatory cytokine mRNAs that would normally be repressed in PBs. Finally, we demonstrate that stimulating PB formation prior to OC43 infection restricts viral replication. These data suggest that SARS-CoV-2 and other CoVs disassemble PBs during infection to support viral replication and evade innate immune responses. As an unintended side effect, the disassembly of PBs enhances translation of proinflammatory cytokine mRNAs which normally reside in PBs, thereby reshaping the subsequent immune response. [**note: this is**

a new mechanism of action by the virus from a Canadian group of researchers and may help exp https://www.biorxiv.org/content/10.1101/2020.11.08.372995v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

>>

2020-11-11

Tchaikovsky wrote wonderful chamber music in addition to his larger scale pieces. Here is the first of his string quartets, #1, op 11. The performance is by the Whitman Quartet, graduate students who are being mentored by the Emerson Quartet at Stony Brook. It's good to see student performances, especially when they are as fine as this one: https://www.youtube.com/watch?v=d7dto81yZFI

The Washington Post discusses a model that projects restaurants, gyms, and coffee shops as top locations where SARS-CoV-2 can spread. The full paper was posted in Nature on-line. The EU will purchase 200 million doses of the Pfizer/BioNTech vaccine. The President of Brazil is setting a very bad example. Russia is saying its COVID-19 vaccine is 92% effective. This video interview with Sanofi CEO Paul Hudson discusses the company's two different COVID-19 vaccines.

The New York Times reports that the CDC has updated it's COVID-19 mask guidelines; mask-wearing protects you, not just those around you! The CDC science brief is HERE. Iowa's governor finally gets smart and announces a mask requirement. Here is a nice story on the husband and wife team that were behind the development of the Pfizer/BioNTech COVID-19 vaccine. Brazil stopped a trial of an experimental Chinese COVID-19 vaccine but provided no reason. Unfortunately, US hospitalizations have reached a record high number. This is why we wear masks and social distance.

STAT notes that rural hospitals lack the ultra-cold freezers needed to store the Pfizer vaccine. There tends to be less mask wearing in rural areas as well. Here is a good overview of the mRNA vaccines that are ahead in development.

The Lancet have an article on characteristics and outcomes of neonatal SARS-CoV-2 infections. *Neonatal SARS-CoV-2 infection is uncommon in babies admitted to hospital. Infection with neonatal admission following birth to a mother with perinatal SARS-CoV-2 infection was unlikely, and possible vertical transmission rare, supporting international guidance to avoid separation of mother and baby. The high proportion of babies from Black, Asian, or minority ethnic groups requires investigation.*

Nature discuss the Pfizer/BioNTech COVID-19 vaccine as does Kaiser Health News.

MODELING

- We study the relative importance of two key control measures for epidemic spreading: endogenous social self-distancing and exogenous imposed quarantine. We use the framework of adaptive networks, moment-closure, and ordinary differential equations (ODEs) to introduce several novel models based upon susceptible-infected-recovered (SIR) dynamics. First, we compare computationally expensive, adaptive network simulations with their corresponding

computationally highly efficient ODE equivalents and find excellent agreement. Second, we discover that there exists a relatively simple critical curve in parameter space for the epidemic threshold, which strongly suggests that there is a mutual compensation effect between the two mitigation strategies: as long as social distancing and quarantine measures are both sufficiently strong, large outbreaks are prevented. Third, we study the total number of infected and the maximum peak during large outbreaks using a combination of analytical estimates and numerical simulations. *Also for large outbreaks we find a similar compensation effect as for the epidemic threshold. This suggests that if there is little incentive for social distancing within a population, drastic quarantining is required, and vice versa. Both pure scenarios are unrealistic in practice. Our models show that only a combination of measures is likely to succeed to control epidemic spreading. Fourth, we analytically compute an upper bound for the total number of infected on adaptive networks, using integral estimates in combination with the moment-closure approximation on the level of an observable. This is a methodological innovation. Our method allows us to elegantly and quickly check and cross-validate various conjectures about the relevance of different network control measures. In this sense it becomes possible to adapt models rapidly to new epidemic challenges such as the recent COVID-19 pandemic*. [**note: this is an interesting paper from Austria/Germany about balancing quarantine and self-distancing measures. No single approach is acceptable.**]
https://www.medrxiv.org/content/10.1101/2020.11.07.20227595v1

- Mobile contact tracing apps have been developed by many countries in response to the COVID-19 pandemic. Trials have focussed on unobserved population trials or staged scenarios aimed to simulate real life. No efficacy measure has been developed that assesses the fundamental ability of any proximity detection protocol to accurately detect, measure, and therefore assess the epidemiological risk that a mobile phone owner has been placed at. This paper provides a fair efficacy formula that can be applied to any mobile contact tracing app, using any technology, allowing it's likely epidemiological effectiveness to be assessed. This paper defines such a formula and provides results for several simulated protocols as well as one real life protocol tested according to the standard methodology set out in this paper. The results presented show that protocols that use time windows greater than 30 seconds or that bucket their distance analogue (E.g. RSSI for Bluetooth) provide poor estimates of risk, showing an efficacy rating of less than 6%. *The fair efficacy formula is shown in this paper to be able to be used to calculate the 'Efficacy of contact tracing' variable value as used in two papers on using mobile applications for contact tracing [6]. The output from the formulae in this paper, therefore, can be used to directly assess the impact of technology on the spread of a disease outbreak. This formula can be used by nations developing contact tracing applications to assess the efficacy of their applications. This will allow them to reassure their populations and increase the uptake of contact tracing mobile apps, hopefully having an effect on slowing the spread of COVID-19 and future epidemics*. [**note: I always enjoy reading papers from sole-investigators. Here is an approach to assess the effectiveness of contact tracing applications.**]
https://www.medrxiv.org/content/10.1101/2020.11.07.20227447v1

NEWLY REGISTERED CLINICAL TRIALS

- You won't get answers today.

CLINICAL TRIAL RESULTS

- Not a think today.

DRUG DEVELOPMENT

- The ongoing COVID-19 pandemic is associated with substantial morbidity and mortality. Although much has been learned in the first months of the pandemic, many features of COVID-19 pathogenesis remain to be determined. For example, anosmia is a common presentation and many patients with this finding show no or only minor respiratory signs[1]. Studies in animals experimentally infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of COVID-19, provide opportunities to study aspects of the disease not easily investigated in human patients. Although COVID-19 severity ranges from asymptomatic to lethal[2], most experimental infections provide insights into mild disease[3]. *Here, using K18-hACE2 mice that we originally developed for SARS studies[4], we show that infection with SARS-CoV-2 causes severe disease in the lung, and in some mice, the brain. Evidence of thrombosis and vasculitis was detected in mice with severe pneumonia. Furthermore, we show that infusion of convalescent plasma from a recovered patient with COVID-19 protected against lethal disease. Mice developed anosmia at early times after infection. Notably, although pre-treatment with convalescent plasma prevented notable clinical disease, it did not prevent anosmia. Thus, K18-hACE2 mice provide a useful model for studying the pathological underpinnings of both mild and lethal COVID-19 and for assessing therapeutic interventions*. [**note: here is a mouse model that can be used for drug development studies.**] https://www.nature.com/articles/s41586-020-2943-z
- Here, we report the generation of synthetic nanobodies, known as sybodies, against the receptor-binding domain (RBD) of SARS-CoV-2 spike protein. We identified a sybody pair (Sb#15 and Sb#68) that can bind simultaneously to the RBD, and block ACE2 binding, thereby neutralizing pseudotyped and live SARS-CoV-2 viruses. Cryo-EM analyses of the spike protein in complex with both sybodies revealed symmetrical and asymmetrical conformational states. In the symmetric complex each of the three RBDs were bound by both sybodies, and adopted the up conformation. The asymmetric conformation, with three Sb#15 and two Sb#68 bound, contained one down RBD, one up-out RBD and one up RBD. *Bispecific fusions of the sybodies increased the neutralization potency 100-fold, as compared to the single binders. Our work demonstrates that linking two binders that recognize spatially-discrete binding sites result in highly potent SARS-CoV-2 inhibitors for potential therapeutic applications*. [**note: this is from Switzerland and looks like a promising approach to antibody therapy. Linking the sybodies provides a much higher neutralization potency.**] https://www.biorxiv.org/content/10.1101/2020.11.10.376822v1
- Here, a SARS-CoV-2 DNA targeting the spike protein and delivered by jet injection, nCoV-S(JET), elicited neutralizing antibodies in hamsters and was protective in both wild-type and transiently immunosuppressed hamster models. This study highlights the DNA vaccine, nCoV-S(JET), we developed has a great potential to move to next stage of preclinical studies, and it also demonstrates that the transiently-immunosuppressed Syrian hamsters, which recapitulate severe and prolonged COVID-19 disease, can be used for preclinical evaluation of the protective efficacy of spike-based COVID-19 vaccine. [**note: here is animal data for a DNA vaccine developed by USAMRID.**] https://www.biorxiv.org/content/10.1101/2020.11.10.376905v1

- Nowadays, WHO recognizes traditional, complementary, and alternative medicine for treating COVID-19 symptoms. Therefore, we investigated the antiviral potential of the hydroalcoholic extract of Uncaria tomentosa stem bark from Peru against SARS-CoV-2 *in vitro*. The antiviral activity of *U. tomentosa* against SARS-CoV-2 *in vitro* was assessed in Vero E6 cells using cytopathic effect (CPE) and plaque reduction assay. After 48h of treatment, *U. tomentosa* showed an inhibition of 92.7 % of SARS-CoV-2 at 25.0 µg/mL ($p<0.0001$) by plaque reduction assay on Vero E6 cells. In addition, *U. tomentosa*, induced a reduction of 98.6 % ($p=0.02$) and 92.7 % ($p=0.03$) in the CPE caused by SARS-CoV-2 on Vero E6 cells at 25 µg/mL and 12.5 µg/mL, respectively. The EC50 calculated for *U. tomentosa* extract by plaque reduction assay was 6.6 µg/mL (4.89 – 8.85 µg/mL) for a selectivity index of 4.1. The EC50 calculated for *U. tomentosa* extract by TCID50 assay was 2.57 µg/mL (1.05 – 3.75 µg/mL) for a selectivity index of 10.54. These results showed that*U. tomentosa* known as Cat's claw has antiviral effect against SARS-CoV-2 observed as a reduction in the viral titer and CPE after 48h of treatment on Vero E6 cells. Therefore, we hypothesized that *U. tomentosa* stem bark, could be promissory to the development of new therapeutic strategies against SARS-CoV-2. [**note: here is another alternative medicine approach from Colombia. This product has a history of use as a herbal medicine.**] https://www.biorxiv.org/content/10.1101/2020.11.09.372201v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread within the human population. Although SARS-CoV-2 is a novel coronavirus, most humans had been previously exposed to other antigenically distinct common seasonal human coronaviruses (hCoVs) before the COVID-19 pandemic. Here, we quantified levels of SARS-CoV-2-reactive antibodies and hCoV-reactive antibodies in serum samples collected from 204 humans before the COVID-19 pandemic. We then quantified pre-pandemic antibody levels in serum from a separate cohort of 252 individuals who became PCR-confirmed infected with SARS-CoV-2. Finally, we longitudinally measured hCoV and SARS-CoV-2 antibodies in the serum of hospitalized COVID-19 patients. Our studies indicate that most individuals possessed hCoV-reactive antibodies before the COVID-19 pandemic. We determined that ~23% of these individuals possessed non-neutralizing antibodies that cross-reacted with SARS-CoV-2 spike and nucleocapsid proteins. These antibodies were not associated with protection against SARS-CoV-2 infections or hospitalizations, but paradoxically these hCoV cross-reactive antibodies were boosted upon SARS-CoV-2 infection. [**note: here is an interesting study from Univ of Pennsylvania showing that antibodies against circulating coronaviruses are boosted following infection with SARS-CoV-2. These antibodies cross reacted with the SARS-CoV-2 S and N protein but were non-neutralizing.**] https://www.medrxiv.org/content/10.1101/2020.11.06.20227215v1
- Animal experiments have shown that non-human primates, cats, ferrets, hamsters, rabbits and bats can be infected by SARS-CoV-2. In addition, SARS-CoV-2 RNA has been detected in felids, mink and dogs in the field. Here, we describe an in-depth investigation using whole genome sequencing of outbreaks on 16 mink farms and the humans living or working on these farms. We conclude that the virus was initially introduced from humans and has since evolved, most likely reflecting widespread circulation among mink in the beginning of the infection period several weeks prior to detection. Despite enhanced biosecurity, early warning surveillance and

immediate culling of infected farms, transmission occurred between mink farms in three big transmission clusters with unknown modes of transmission. Sixty-eight percent (68%) of the tested mink farm residents, employees and/or contacts had evidence of SARS-CoV-2 infection. Where whole genomes were available, these persons were infected with strains with an animal sequence signature, providing evidence of animal to human transmission of SARS-CoV-2 within mink farms. [**note: here is data on the Dutch outbreak of SARS-CoV-2 on mink farms.**] https://science.sciencemag.org/content/early/2020/11/09/science.abe5901

DIAGNOSTIC DEVELOPMENT

- No major breakthroughs today.

>>

2020-11-12

Today's Tchaikovsky choice is Rococo Variations for cello and orchestra. The cellist is Mischa Maisky with the trusty Frankfurt Radio Symphony conducted by Paavo Järvi: https://www.youtube.com/watch?v=ygkRtPsgnf4

The Washington Post has a story that clearly shows President Trump does not understand how clinical trials are run. Pfizer did not know the preliminary results of the trial until the Data Safety Monitoring Board informed the company this past Sunday. Black and Asian people are at a higher risk for COVID-19 (The Lancet paper with these results is HERE). Turkey bans smoking in public places to curb viral spread (people should stop smoking anyway). Things are bad in Wisconsin as a health-care provider closes down testing sites so staff can care for patients.

The New York Times reports that governors are now taking a strong stance on mask wearing. Here is an article on the Naval studies that was published in The New England Journal of Medicine (see below for the links to the articles). Sometimes scientists test positive for COVID-19 but not because they are infected; it's a result of working with the virus's genetic material. Perhaps children infected with common coronaviruses develop antibodies to SARS-CoV-2. A test for specific T Cells may prove useful.

As you know I have been following Purdue University's efforts to control SARS-CoV-2. Unfortunately, the latest snapshot of their testing program shows the positive test rate approaching 7%. The majority of cases appear to be very mild or asymptomatic among students. There is a higher rate of moderate or significant illness among employees.

The Atlantic who run a good COVID-19 Testing Project cover the current wave of infections with some good graphs. When is it safe to host a dinner party for friends?

The New England Journal of Medicine has an article on the SARS-CoV-2 outbreak on the USS Theodore Roosevelt. Sticking with the military, here is one about outbreaks among Marine recruits during quarantine and an accompanying editorial.

STAT have a bleak story on the current outbreak numbers.

JAMA have an opinion piece on the need for therapeutics to treat early COVID-19. SARS-CoV-2 doesn't care how old you are and here is a perspective on how young people are becoming infected. This Emory Univ physician gives the perspective of a 'long hauler.' It's not fun. Here is a viewpoint on issues related

to monoclonal antibody approval and distribution. Yes, clinical trial results on HCQ are still being reported. Here is a multicenter randomized trial that again shows this drug does not work! What a wasted effort on so many fronts this has been as this editorial notes. *The clear, unambiguous, and compelling lesson from the hydroxychloroquine story for the medical community and the public is that science and politics do not mix. Science, by definition, requires diligence and an honest assessment of findings; politics not so much. The number of articles in the peer-reviewed literature over the last several months that have consistently and convincingly demonstrated the lack of efficacy of a highly hyped "cure" for COVID-19 represent the consequence of the irresponsible infusion of politics into the world of scientific evidence and discourse. For other potential therapies or interventions for COVID-19 (or any other diseases), this should not happen again.*

Kaiser Health News talks about the need for a communication plan as the vaccines near distribution.

Derek Lowe weighs in on the Pfizer/BioNTech vaccine data.

MODELING

- In 2019, the National Basketball Association (NBA) expanded it's mental health rules to include mandating that each team have at least one mental health professional on their full-time staff and to retain a licensed psychiatrist to assist when needed. In this work, we investigate the NBA players' discussion of mental health using historical data from players' public Twitter accounts. All current and former NBA players with Twitter accounts were identified, and each of their last 800 tweets were scraped, yielding 920,000 tweets. A list of search terms derived from the DSM5 diagnoses was then created and used to search all of the nearly one million tweets. In this work, we present the most common search terms used to identify tweets about mental health, present the change in month-by-month tweets about mental health, and identify the impact of players discussing their own mental health struggles on their box score statistics before and after their first tweet discussing their own mental health struggles. [**note: I don't use Twitter but there are some readers who are big NBA fans. This paper on the discussion of mental illness and metal health by NBA players on Twitter is for the fans!**] https://www.medrxiv.org/content/10.1101/2020.10.19.20215681v2
- While air pollution has been shown to be strongly correlated to increased SARS-CoV-2 morbidity and mortality, whether environmental pollutants such as ground level ozone affects the susceptibility of individuals to SARS-CoV-2 is not yet established. Objective: To investigate the impact of ozone inhalation on the expression levels of signatures associated with host susceptibility to SARS-CoV-2. Methods: We analyzed lung tissues collected from mice that were sub-chronically exposed to air or 0.8ppm ozone for three weeks (4h/night, 5 nights/week), and analyzed the expression of signatures associated with host susceptibility to SARS-CoV-2. Results: SARS-CoV-2 entry into the host cells requires proteolytic priming by the host-derived protease, transmembrane protease serine 2 (TMPRSS2). The TMPRSS2 protein and Tmprss2 transcripts were significantly elevated in the extrapulmonary airways, parenchyma, and alveolar macrophages from ozone-exposed mice. A significant proportion of additional known SARS-CoV-2 host susceptibility genes were upregulated in alveolar macrophages and parenchyma from ozone-exposed mice. Conclusions: Our data indicate that the unhealthy levels of ozone in the environment may predispose individuals to severe SARS-CoV-2 infection. Given the severity of this pandemic, and the challenges associated with direct testing of host-environment

interactions in clinical settings, we believe that this mice-ozone-exposure based study informs the scientific community of the potentially detrimental effects of the ambient ozone levels determining the host susceptibility to SARS-CoV-2. [**note: atmospheric ozone levels may predispose people to infection by SARS-CoV-2 from this animal study.**] https://www.biorxiv.org/content/10.1101/2020.11.10.377408v1

NEWLY REGISTERED CLINICAL TRIALS

- You need to be patient.

CLINICAL TRIAL RESULTS

- Some trials were mentioned above.

DRUG DEVELOPMENT

- The predominant approach for antibody generation remains animal immunization, which can yield exceptionally selective and potent antibody clones owing to the powerful evolutionary process of somatic hypermutation. However, animal immunization is inherently slow, has poor compatibility with certain antigens (e.g., integral membrane proteins), and suffers from self-tolerance and immunodominance, which limit the functional spectrum of antibodies that can be obtained. Here, we describe Autonomous Hypermutation yEast surfAce Display (AHEAD), a synthetic recombinant antibody generation technology that imitates somatic hypermutation inside engineered yeast. In AHEAD, antibody fragments are encoded on an error-prone orthogonal DNA replication system, resulting in Saccharomyces cerevisiae populations that continuously mutate surface-displayed antibody repertoires. Simple cycles of yeast culturing and enrichment for antigen binding drive the evolution of high-affinity antibody clones in a readily parallelizable process that takes as little as 2 weeks. We applied AHEAD to generate nanobodies against the SARS-CoV-2 S glycoprotein, a GPCR, and other targets. The SARS-CoV-2 nanobodies, concurrently evolved from an open-source naive nanobody library in 8 independent experiments, reached subnanomolar affinities through the sequential fixation of multiple mutations over 3-8 AHEAD cycles that saw ~580-fold and ~925-fold improvements in binding affinities and pseudovirus neutralization potencies, respectively. These experiments highlight the defining speed, parallelizability, and effectiveness of AHEAD and provide a template for streamlined antibody generation at large with salient utility in rapid response to current and future viral outbreaks. [**note: here is new method for rapid generation of potent antibodies using yeast.**] https://www.biorxiv.org/content/10.1101/2020.11.11.378778v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The Nsp1 protein of SARS-CoV-2 regulates the translation of host and viral mRNAs in cells. Nsp1 inhibits host translation initiation by binding to the entry channel of the 40S ribosome subunit. The structural study of SARS-CoV-2 Nsp1-ribosomal complexes reported post-termination 80S complex containing Nsp1 and the eRF1 and ABCE1 proteins. Considering the presence of Nsp1 in the post-termination 80S ribosomal complex simultaneously with eRF1, we hypothesized that Nsp1 may be involved in translation termination. We show the direct influence of Nsp1 on translation termination. Using a cell-free translation system and reconstituted in vitro translation system, we reveal that Nsp1 stimulates translation termination in the stop codon

recognition stage. We identify that activity of Nsp1 in translation termination is localized in its N-terminal domain. The data obtained will enable an investigation of new classes of potential therapeutic agents from coronavirus infection competing with Nsp1 for binding with the termination complex. [**note: this is from Russia and points to the Nsp1 protein as having an impact on cellular protein synthesis by inhibiting it. The virus has lots of moving parts.**] https://www.biorxiv.org/content/10.1101/2020.11.11.377739v1

- ORF3a in both SARS-CoV-1 and SARS-CoV-2 are ion channels (viroporins) and involved in virion assembly and membrane budding. Using sensitive profile-based homology detection methods, we unify the SARS-CoV ORF3a family with several families of viral proteins, including ORF5 from MERS-CoVs, proteins from beta-CoVs (ORF3c), alpha-CoVs (ORF3b), most importantly, the Matrix (M) proteins from CoVs, and more distant homologs from other nidoviruses. By sequence analysis and structural modeling, we show that these viral families utilize specific conserved polar residues to constitute an ion-conducting pore in the membrane. We reconstruct the evolutionary history of these families, objectively establish the common origin of the M proteins of CoVs and Toroviruses. We show that the divergent ORF3a/ORF3b/ORF5 families represent a duplication stemming from the M protein in alpha- and beta-CoVs. By phyletic profiling of major structural components of primary nidoviruses, we present a model for their role in virion assembly of CoVs, ToroVs and Arteriviruses. *The unification of diverse M/ORF3 ion channel families in a wide range of nidoviruses, especially the typical M protein in CoVs, reveal a conserved, previously under-appreciated role of ion channels in virion assembly, membrane fusion and budding. We show that the M and ORF3 are under differential evolutionary pressures; in contrast to the slow evolution of M as core structural component, the CoV-ORF3 clade is under selection for diversification, which indicates it is likely at the interface with host molecules and/or immune attack*. [**note: this paper looks at two proteins that appear to be distributed in several different viral familys.]** https://www.biorxiv.org/content/10.1101/2020.11.10.377366v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.

>>

2020-11-13

Today's focus is on some of Tchaikovsky's work for ballet. Here is Nina Kaptsova in the 'Dance of the Sugar Plum Fairy' from The Nutcracker: https://www.youtube.com/watch?v=Wz_f9B4pPtg and the 'Waltz of the Flowers' as choreographed by George Balanchine for the NY City Ballet: https://www.youtube.com/watch?v=LKcZL8q1eBw and finally, two great icons: Rudolf Nureyev and Margot Fonteyn in the pas de deux from 'Swan Lake': https://www.youtube.com/watch?v=qG7JvpPGdEU

It did not escape my attention that today is of great concern for those suffering from triskaidekaphobia. This is of no concern to me. 😀

The Washington Post notes that the BioNTech vaccine may be available in the UK as early as next month. I think this op-ed gets it right about how to open schools safely. Here is the back story on the new CDC recommendation on mask-wearing. The Midwest is facing a major crunch on hospital bed

utilization. This Pennsylvania school district is holding classes. According to the CDC, guests at a Maine superspreader wedding returned to work when they should not have.

The New York Times discusses how Pfizer will distribute their vaccine which requires ultra-cold temperatures. Tony Fauci urges Americans to double down on what they should already be doing. The CDC reports that children's visits to ERs for mental health issues has risen sharply. Israel are buying enough of the Pfizer/BioNTech vaccine to inoculate 4 million people. Europe has the right idea; keep schools open and control access to bars and restaurants.

The New Yorker has an article on 'the pandemic's winter surge.' This is a good explanation of the virus impact on the human immune system.

Here are CDC recommended masks.

The Lancet have the first report that I have seen on a randomized controlled trial of beta-Interferon. It is a small number but those on the active arm appeared to do better than the placebo.
Patients who received SNG001 had greater odds of improvement and recovered more rapidly from SARS-CoV-2 infection than patients who received placebo, providing a strong rationale for further trials.

Kaiser Health News has an article on autoantibodies to Interferon that is linked below in the immunology section.

Derek Lowe weighs in on recent studies of T-Cells.

We are finally seeing some clinical trial results trickle in.

MODELING

- Objectives: Norway and Sweden are similar countries regarding ethnicity, socioeconomics and health care. To combat Covid-19, Norway implemented extensive measures such as school closures and lock-downs, while Sweden has been criticised for relaxed measures against Covid-19. We compared the effect of the different national strategies on all-cause and Covid-19 associated mortality. Design: Retrospective cohort. Setting: The countries Norway and Sweden. Participants: All inhabitants. Main outcome measures: We calculated weekly mortality rates (MR) with 95% confidence intervals (CI) per 100,000 individuals as well as mortality rate ratios (MRR) comparing the epidemic year (29th July, 2019 to 26th July, 2020) to the four preceding years (July 2015 to July 2019). We also compared Covid-19 associated deaths and mortality rates for the weeks of the epidemic in Norway and Sweden (16th March to 26th July, 2020). Results: In Norway, mortality rates were stable during the first three 12-month periods of 2015/16; 2016/17 and 2017/18 (MR 14.8 to 15.1 per 100,000), and slightly lower in the two most recent periods including during epidemic period (2018/19 and 2019/20; 14.5 per 100,000). In Sweden, all-cause mortality was stable during the first three 12-month periods of 2015/16; 2016/17 and 2017/18 (MR 17.2 to 17.5 per 100,000), but lower in the year 2018/19 immediately preceding the epidemic (16.2 per 100,000). Covid-19 associated mortality rates were 0.2 per 100,000 (95%CI 0.1 to 0.4) in Norway and 2.9 (95%CI 1.9 to 3.9) in Sweden. The increase in mortality was confined to individuals in 70 years or older. Conclusions: *All-cause mortality remained unaltered in Norway. In Sweden, the observed increase in all- cause mortality during Covid-19 was partly due to a lower than expected mortality preceding the epidemic and the observed excess*

mortality, was followed by a lower than expected mortality after the first Covid-19 wave. This may suggest mortality displacement. [**note: for those interested in how the Swedish approach to managing COVID-19 compares to a neighbor this one is for you.**
https://www.medrxiv.org/content/10.1101/2020.11.11.20229708v1

- Given the continued burden of severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) disease (COVID-19) across the U.S., there is a high unmet need for data to inform decision-making regarding social distancing and universal masking. We examined the association of community-level social distancing measures and individual masking with risk of predicted COVID-19 in a large prospective U.S. cohort study of 198,077 participants. Individuals living in communities with the greatest social distancing had a 31% lower risk of predicted COVID-19 compared with those living in communities with poor social distancing. Self-reported masking was associated with a 63% reduced risk of predicted COVID-19 even among individuals living in a community with poor social distancing. These findings provide support for the efficacy of mask-wearing even in settings of poor social distancing in reducing COVID-19 transmission. In the current environment of relaxed social distancing mandates and practices, universal masking may be particularly important in mitigating risk of infection. [**note: I don't think I need to post any more abstracts about mask-wearing and/or social distancing. It's clear that these two things will help control the virus until the arrival of a vaccine. Why people do not understand this continues to baffle me. I wonder if any of my readers have any better ideas, if so please email me. I may pick the best answers for a future newsletter without attribution of course!!**]
https://www.medrxiv.org/content/10.1101/2020.11.11.20229500v1
- There is striking racial disparity in the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection rates in the United States. We hypothesize that the disparity is significantly smaller in areas with a higher ratio of green spaces at the county level because green spaces provide health benefits to people of different races through multiple pathways. We suggest that they are an equalizing salutogenic factor, modifying infection exposure. This study used the 135 most urbanized counties across the United States as sample sites. County level data on the SARS-CoV-2 infection rates of blacks and whites in each county were collected. *The ratio of green spaces by land-cover type at the county level was calculated from satellite imagery. An ecological hierarchical regression analysis measured cross-sectional associations between racial disparity in infection rates and green space, after controlling for confounding factors. We found a significantly higher infection rate among black individuals compared to white individuals. More importantly, a higher ratio of green spaces at the county level is significantly associated with a lower racial disparity in the SARS-CoV-2 infection rate. Further, we identified four green space factors that have significant negative associations with the racial disparity in SARS-CoV-2 infection rates, including open space in developed areas, forest, shrub-scrub, and herbaceous-grassland*. [**note: this is quite intriguing. More green space may mean a lower racial disparity in SARDS associated with COVID-19.}**
https://www.medrxiv.org/content/10.1101/2020.11.11.20228130v1

NEWLY REGISTERED CLINICAL TRIALS

- Seriously, why are you even looking at this section? It's only Friday.

CLINICAL TRIAL RESULTS

- Therapeutics for Inpatients with COVID-19 (TICO), is a global multi-arm, multi-stage (MAMS) platform master protocol, which facilitates the rapid evaluation of the safety and efficacy of candidate anti-viral therapeutic agents for adults hospitalized with COVID-19. The protocol design allows multiple therapeutic agents to be evaluated in an efficient and scientifically rigorous manner, with efficiencies delivered by the MAMS design, and began by studying neutralizing monoclonal antibodies. TICO employs an agile and robust approach to futility and safety evaluation at 300 patients enrolled (Stage 1), with subsequent expansion to full sample size and an expanded target population (Stage 2) if the agent shows an acceptable safety profile and evidence of efficacy. Two ordinal outcomes applied early (Day 5) determine the efficacy signals of the investigational agents(s) and progression to Stage 2. These ordinal outcomes assess both respiratory and other organ failure events, recognizing the broad range of COVID-19 morbidity. In Stage 2, overall efficacy is assessed using the primary outcome of time to sustained recovery, assessed over 90 days. This approach to early futility assessment using an early intermediate outcome and a primary endpoint out to 90 days allows the study team to make rapid decisions on safety and potential efficacy of novel agents while ultimately focusing on patient-centered, longer-term outcomes. The implementation of TICO across a global network allows for continued enrollment despite variations in geographic epidemiology. Study Status: *The TICO master protocol moved from conception to first patient enrolled in approximately 9 weeks, a testament to the expedited regulatory and ethics review, coupled with flexible and responsive study operations. The first agent to be tested using this protocol, LY-CoV-555, enrolled N=326 participants before undergoing Stage 1 futility and safety assessment. Two additional agents will enter the study in November 2020, with other agents planned. Conclusion: The TICO MAMS platform trial has been implemented efficiently across a global network of sites and several trial networks. It will generate results rapidly for multiple novel neutralizing monoclonal antibodies and other therapeutics agents*. [**note: this is a master protocol for clinical trial studies.**] https://www.medrxiv.org/content/10.1101/2020.11.08.20227876v1
- A randomized controlled trial of calcifediol (25-hydroxyvitamin D3) as a treatment for hospitalized COVID-19 patients in Córdoba, Spain, found that the treatment was associated with reduced ICU admissions with very large effect size and high statistical significance, but the study has had limited impact because it had only 76 patients and imperfect blinding, and did not measure vitamin D levels pre- and post-treatment or adjust for several comorbidities. Here we reanalyze the results of the study using rigorous and well established statistical techniques, and find that the randomization, large effect size, and high statistical significance address many of these concerns. *In particular, we show that decreased ICU admissions were not due to uneven distribution of comorbidities or other prognostic indicators, to imperfect blinding, or to chance, but were instead associated with the calcifediol intervention. We conclude that the Córdoba study provides sufficient evidence to warrant immediate, well-designed pivotal clinical trials of calcifediol in a broader cohort of inpatients and outpatients with COVID-19, and to consider broad adoption of calcifediol treatment for vitamin-D-deficient hospitalized COVID-19 patients*. [**note: this is a reanalysis using a different statical approach on a Spanish Vitamin D study.**] https://www.medrxiv.org/content/10.1101/2020.11.08.20222638v1
- Here, we characterize the time-dependent progression of COVID-19 through different stages of the disease, by measuring 86 accredited diagnostic parameters and plasma proteomes at 687 sampling points, in a cohort of 139 patients during hospitalization. We report that the time-

resolved patient molecular phenotypes reflect an initial spike in the systemic inflammatory response, which is gradually alleviated and followed by a protein signature indicative of tissue repair, metabolic reconstitution and immunomodulation. Further, we show that the early host response is predictive for the disease trajectory and gives rise to proteomic and diagnostic marker signatures that classify the need for supplemental oxygen therapy and mechanical ventilation, and that predict the time to recovery of mildly ill patients. In severely ill patients, the molecular phenotype of the early host response predicts survival, in two independent cohorts and weeks before outcome. We also identify age-specific molecular response to COVID-19, which involves increased inflammation and lipoprotein dysregulation in older patients. Our study provides a deep and time resolved molecular characterization of COVID-19 disease progression, and reports biomarkers for risk-adapted treatment strategies and molecular disease monitoring. Our study demonstrates accurate prognosis of COVID-19 outcome from proteomic signatures recorded weeks earlier. [**note: here is a proteomic approach to characterizing COVID-19 disease progression and outcome. Interesting stuff here!**]
https://www.medrxiv.org/content/10.1101/2020.11.09.20228015v1

- The present study investigated blood metabolites in order to elucidate how infection with severe acute respiratory syndrome coronavirus 2 can lead to such a variety of pathologies and what common ground they share. COVID-19 patient blood samples were taken at hospital admission in two Belgian patient cohorts, and a third cohort that included longitudinal samples was used for additional validation (total n=581). A total of 251 blood metabolite measures and ratios were assessed using nuclear magnetic resonance spectroscopy and tested for association to disease severity. In line with the varied effects of severe COVID-19, *the range of severity-associated biomarkers was equally broad and included increased inflammatory markers (glycoprotein acetylation), amino acid concentrations (increased leucine and phenylalanine), increased lipoprotein particle concentrations (except those of very low density lipoprotein, VLDL), decreased cholesterol levels (except in large HDL and VLDL), increased triglyceride levels (only in IDL and LDL), fatty acid levels (decreased poly-unsaturated fatty acid, increased mono-unsaturated fatty acid) and decreased choline concentration, with association sizes comparable to those of routine clinical chemistry metrics of acute inflammation. Our results point to systemic metabolic biomarkers for COVID-19 severity that make strong targets for further fundamental research into its pathology (e.g. phenylalanine and omega-6 fatty acids*). [**note: this is from Belgium and looks at metabolic markers for COVID-19 severity.**]
https://www.medrxiv.org/content/10.1101/2020.11.09.20228221v1
- There are currently no effective treatments for outpatients with coronavirus disease 2019 (COVID-19). Interferon-lambda-1 is a Type III interferon involved in the innate antiviral response with activity against respiratory pathogens. Methods: In this double-blind, placebo-controlled trial, outpatients with laboratory-confirmed COVID-19 were randomized to a single subcutaneous injection of peginterferon-lambda 180mcg or placebo within 7 days of symptom onset or first positive swab if asymptomatic. The primary endpoint was proportion negative for SARS-CoV-2 RNA on Day 7 post-injection. Findings: There were 30 patients per arm, with median baseline SARS-CoV-2 viral load of 6.71 (IQR 1.3-8.0) log copies/mL. *The decline in SARS-CoV-2 RNA was greater in those treated with peginterferon-lambda than placebo (p=0.04). On Day 7, 24 participants (80%) in the peginterferon-lambda group had an undetectable viral load compared to 19 (63%) in the placebo arm (p=0.15). After controlling for baseline viral load,*

peginterferon lambda treatment resulted in a 4.12-fold (95CI 1.15-16.7, p=0.029) higher likelihood of viral clearance by Day 7. Of those with baseline viral load above 10E6 copies/mL, 15/19 (79%) in the peginterferon-lambda group were undetectable on Day 7 compared to 6/16 (38%) in the placebo group (p=0.012). Adverse events were similar between groups with only mild reversible transaminase elevations more frequently observed in the peginterferon-lambda group. Interpretation: Peginterferon-lambda accelerated viral decline in outpatients with COVID-19 resulting in a greater proportion with viral clearance by Day 7, particularly in those with high baseline viral load. Peginterferon-lambda may have potential to prevent clinical deterioration and shorten duration of viral shedding. (NCT04354259) [**note: here are the results from a Canadian trial of peginterferon lambda. We need more patients to confirm this result.**] https://www.medrxiv.org/content/10.1101/2020.11.09.20228098v1

DRUG DEVELOPMENT

- We evaluated a new PARP inhibitor, stenoparib, which was recently advanced to Stage II clinical trials for treatment of ovarian cancer. This is an initial report on the activity of stenoparib against human respiratory coronaviruses, including SARS-CoV-2, in vitro. Stenoparib exhibits dose-dependent suppression of SARS-CoV-2 multiplication and spread in Vero E6 monkey kidney and Calu-3 human lung adenocarcinoma cells. Stenoparib was also strongly inhibitory to multiplication of the HCoV-NL63 human seasonal respiratory coronavirus. Compared to remdesivir, which inhibits the viral replicon subsequent to cell entry, stenoparib is inhibitory to virus entry and post-entry processes as determined by time-of-addition (TOA) experiments. Moreover, a 10 µM dosage of stenoparib, which is far below its 25.5 µM half-maximally effective concentration (EC50), when combined with 0.5 µM remdesivir suppressed coronavirus growth by 90.7%, indicating a potentially synergistic effect for this drug combination. Thus, stenoparib as a standalone or as a component of combinatorial therapy with remdesivir may be a valuable addition to the arsenal against COVID-19. [**note: this is an experimental drug developed by Allarity Pharma.]** https://www.biorxiv.org/content/10.1101/2020.11.12.380394v1
- In a search for a drug against COVID-19, we have performed a massive X-ray crystallographic screen of repurposing drug libraries containing 5953 individual compounds against the SARS-CoV-2 main protease (Mpro), which is a potent drug target as it is essential for the virus replication. In contrast to commonly applied X-ray fragment screening experiments with molecules of low complexity, our screen tested already approved drugs and drugs in clinical trials. *From the three-dimensional protein structures, we identified 37 compounds binding to Mpro. In subsequent cell-based viral reduction assays, one peptidomimetic and five non-peptidic compounds showed antiviral activity at non-toxic concentrations. Interestingly, two compounds bind outside the active site to the native dimer interface in close proximity to the S1 binding pocket. Another compound binds in a cleft between the catalytic and dimerization domain of Mpro. Neither binding site is related to the enzymatic active site and both represent attractive targets for drug development against SARS-CoV-2. This X-ray screening approach thus has the potential to help deliver an approved drug on an accelerated time-scale for this and future pandemics*. [**note: there are a large number of authors on this paper from Hamburg. There may be some alternate binding sites on the Mpro that can be targeted for pharmaceutical intervention. As one who worked on allosteric enzymes in the far ago past, this paper was of great interest to me.**] https://www.biorxiv.org/content/10.1101/2020.11.12.378422v1

- The Covid-19 pandemic has highlighted the importance of aerosolized droplets inhaled into the nose in the transmission of respiratory viral disease. Inactivating pathogenic viruses at the nasal portal of entry may reduce viral loads, thereby reducing transmission and contagion. We have developed an oil-in-water nanoemulsion (nanodroplet) formulation containing the potent antiseptic 0.13% Benzalkonium Chloride (NE-BZK) which demonstrates safe and broad anti-viral activity. While The Centers for Disease Control and Prevention (CDC) have reported that BZK may have less reliable activity than ethyl alcohol against certain viruses, including coronaviruses, we have demonstrated that NE-BZK exhibits broad-spectrum, long-lasting antiviral activity with >99.99% in vitro killing of enveloped viruses including SARS-CoV-2, human coronavirus, RSV and influenza B. Furthermore, in vitro studies demonstrated that NE-BZK continues to kill >99.99% of human coronavirus even when diluted 20-fold, while 0.13% aqueous BZK solution (AQ-BZK) did not. Ex vivo studies of NE-BZK on human cadaver skin demonstrated persistent >99.99% killing of human coronavirus for at least 8 hours after application. AQ-BZK failed to demonstrate durable antimicrobial activity on skin over time. The repeated application of NE-BZK, twice daily for 2 weeks on to rabbit nostrils indicated safety with no irritation. These findings demonstrate that formulating BZK on the surface of proprietary nanodroplets offers a safe and effective antiviral as a significant addition to strategies to combat the spread of respiratory viral infectious diseases. [**note: here is a study on nano-droplets containing benzalkonium chloride that has antiviral activity. This is a totally unsurprising finding as the compound is a potent surfactant and is present in many disinfectant cleaners. The paper comes from Blue Willow Biologics.}** https://www.biorxiv.org/content/10.1101/2020.11.12.377598v1
- Coronaviruses have caused three major outbreaks of infectious disease since the beginning of 21st century. Broad-spectrum strategies that can be utilized in both current and future coronavirus outbreaks and mutation-tolerant are sought after. *Here we report a monoclonal antibody 3E8 targeting human angiotensin-converting enzyme 2 (ACE2) neutralized pseudo-typed coronaviruse SARS-CoV-2, SARS-CoV-2-D614G, SARS-CoV and HCoV-NL63, without affecting physiological activities of ACE2 or causing toxicity in mouse model. 3E8 also blocked live SARS-CoV-2 infection in vitro and in a mouse model of COVID-19. Cryo-EM studies revealed the binding site of 3E8 on ACE2 and identified Histone 34 of ACE2 as a critical site of anti-viral epitope. Overall, our work has provided a potential pan coronavirus management strategy and disclosed a pan anti-coronavirus epitope on human ACE2 for the first time*. [**note: this is from China and shows that an ACE2 targeted monoclonal antibody might be a good approach to therapy.**] https://www.biorxiv.org/content/10.1101/2020.11.11.375972v1
- A well-known strategy to identify molecules with inhibitory potential against SARS-CoV-2 proteins is the repurposing of clinically developed drugs, e.g., anti-parasitic drugs. The results described in this study demonstrate the inhibitory potential of quinacrine and suramin against SARS-CoV-2 main protease ($3CL^{pro}$). Quinacrine and suramin molecules present a competitive and non-competitive mode of inhibition, respectively, with IC_{50} and K_D values in low µM range. Using docking and molecular dynamics simulations we identified a possible binding mode and the amino acids involved in these interactions. Our results suggested that suramin in combination with quinacrine showed promising synergistic efficacy to inhibit SARS-CoV-2 $3CL^{pro}$. The identification of effective, synergistic drug combinations could lead to the design of better treatments for the COVID-19 disease. Drug repositioning offers hope to the SARS-CoV-2 control.

[**note: lots of things work *in vitro***]
https://www.biorxiv.org/content/10.1101/2020.11.11.378018v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Measuring the adaptive immune response after SARS-CoV-2 infection may improve our understanding of COVID-19 exposure and potential future protection or immunity. We analyzed T-cell and antibody signatures in a large population study of over 2,200 individuals from the municipality of Vo', Italy, including 70 PCR-confirmed COVID cases (24 asymptomatic, 37 symptomatic, 9 hospitalized). Blood samples taken 60 days after PCR diagnosis demonstrated 97% (68/70) of the latter subjects had a positive T-cell test result, higher than an antibody serology assay (77%; 54/70 of subjects) performed on the same samples. *The depth and breadth of the T-cell response was associated with disease severity, with symptomatic and hospitalized COVID cases having significantly higher response than asymptomatic cases. In contrast, antibody levels at this convalescent time point were less informative as they did not correlate with disease severity. 45 additional suspected infections were identified based on T-cell response from the 2,220 subjects without confirmatory PCR tests. Among these, notably, subjects who reported symptoms or had household exposure to a PCR-confirmed infection presented a higher T-cell test positive rate. Taken together, these results establish that T cells are a sensitive, reliable and persistent measure of past SARS-CoV-2 infection*. [**note: here is a study on the diagnoses and tracking of viral infection in an area of Italy using T-cell receptor sequencing.**]
https://www.medrxiv.org/content/10.1101/2020.11.09.20228023v1
- Interindividual clinical variability in the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is vast. We report that at least 101 of 987 patients with life-threatening coronavirus disease 2019 (COVID-19) pneumonia had neutralizing immunoglobulin G (IgG) autoantibodies (auto-Abs) against interferon-ω (IFN-ω) (13 patients), against the 13 types of IFN-α (36), or against both (52) at the onset of critical disease; a few also had auto-Abs against the other three type I IFNs. The auto-Abs neutralize the ability of the corresponding type I IFNs to block SARS-CoV-2 infection in vitro. These auto-Abs were not found in 663 individuals with asymptomatic or mild SARS-CoV-2 infection and were present in only 4 of 1227 healthy individuals. Patients with auto-Abs were aged 25 to 87 years and 95 of the 101 were men. A B cell autoimmune phenocopy of inborn errors of type I IFN immunity accounts for life-threatening COVID-19 pneumonia in at least 2.6% of women and 12.5% of men. [**note: this is a large multi-national study showing that severe COVID-19 can be caused by auto-antibodies to interferon. The New Yorker article I noted above discusses some of this. It's a rare occurrence and appears to impact men more than women.**]
https://science.sciencemag.org/content/370/6515/eabd4585
- Background. There are conflicting results about the duration of antibodies induced by SARS-CoV-2, but several studies show a rapid decay in a few months after infection. To evaluate antibody decline, we re-evaluated the presence of anti-SARS-CoV-2 antibodies among individuals found seropositive in a first population survey conducted 4 months before. Methods. All individuals above ten years of age resident in 5 municipalities of the Autonomous Province of Trento, northern Italy, who resulted IgG positive for anti-SARS-CoV-2 nucleocapsid (NC) antibodies in a serosurvey conducted on May 2020 were retested after 4 months. Anti-SARS-CoV-2 antibodies were detected using the Abbott SARS-CoV-2 IgG assay (Abbott Diagnostics, USA) detecting anti-

NC antibodies. Samples that gave a negative result were re-tested using the same test plus Liaison SARS-CoV-2 IgG assay (DiaSorin, Italy) to assess anti-spike (S) S1/S2 IgG antibodies. Seroprevalence was calculated as the proportion of positive people on the total number of tested. A neutralizing assay was performed on a subgroup of formerly positives sera using fifty-percent tissue culture infective dose (TCID50) as endpoint dilution to produce a cytopathic effect in 50% of inoculated Vero E6 cells culture. In all the analyses a p value < 0.05 were considered statistically significant. Statistical analysis was performed by STATA version 16.1 (STATA Corp., College Station, Texas, USA). Findings. *Overall, 1159 out of 1402 initially anti-NC seropositive participants were enrolled in the study. Of them, 480 (41.1%) became seronegative for anti-NC IgG antibodies. When 479 negative sera were tested for anti-S IgG, 373 samples (77.9%) resulted positives. A functional neutralization assay was performed on 106 sera showing high concordance with anti-S antibodies positivity. Interpretation. A decline of anti-NC IgG values was recorded 4 months after the first evaluation. Worth of note, a high proportion of anti-NC seronegative individuals were positive for anti-spike IgG antibodies, which appear to persist longer and to better correlate with neutralization activity*. [**note: this study of antibody persistence is from Italy and looks at convalescents four months out.**] https://www.medrxiv.org/content/10.1101/2020.11.11.20229062v1

DIAGNOSTIC DEVELOPMENT

- The emergence and quick spread of SARS-CoV-2 has pointed at a low capacity response for testing large populations in many countries, in line of material, technical and staff limitations. The traditional RT-qPCR diagnostic test remains the reference method and is by far the most widely used test. These assays are limited to a couple of probe sets, require large sample PCR reaction volumes, along with an expensive and time-consuming RNA extraction steps. Here we describe a quantitative nanofluidic assay that overcomes some of these shortcomings, based on the Biomark instrument from Fluidigm. This system offers the possibility of performing 4608 qPCR end-points in a single run, equivalent to 192 clinical samples combined with 12 pairs of primers/probe sets in duplicate, thus allowing the monitoring in addition to SARS-CoV-2 probes of other pathogens and/or host cellular responses (virus receptors, response markers, microRNAs). Its 10 nL range volume is compatible with sensitive and reproducible reactions that can be easily and cost-effectively adapted to various RT-qPCR configurations and sets of primers/probe. Finally, we also evaluated the use of inactivating lysis buffers composed of various detergents in the presence or absence of proteinase K to assess the compatibility of these buffers with a direct reverse transcription enzymatic step and we propose several procedures, bypassing the need for RNA purification. *We advocate that the combined utilization of an optimized processing buffer and a high-throughput real-time PCR device would contribute to improve the turn-around-time to deliver the test results to patients and increase the SARS-CoV-2 testing capacities*. [**note: this is the first diagnostics paper in some time that I found interesting. This approach can speed up the diagnostic process without losing much sensitivity.**] https://www.medrxiv.org/content/10.1101/2020.11.09.20228437v1
- Currently available rapid diagnostic tests (RDTs) for SARS-CoV-2 nucleocapsid antigens (Ag) have sensitivity well below PCR. The correlation of Ag and RNA quantities in clinical nasopharyngeal (NP) samples is unknown. Methods: An ultrasensitive, quantitative electrochemiluminescence immunoassay for SARS-CoV-2 nucleocapsid (the MSD S-PLEX CoV-2 N assay) was used to

measure Ag in clinical NP samples from adults and children previously tested by PCR. Results: The S-PLEX Ag assay had a limit of detection (LOD) of 0.16 pg/mL and a cutoff of 0.32 pg/mL. Ag concentrations measured in clinical NP samples (collected in 3.0 mL media) ranged from less than 160 fg/mL to 2.7 ug/mL. Log-transformed Ag concentrations correlated tightly with Ct values. In 35 adult and 101 pediatric PCR-positive samples, sensitivity was 91% (95% CI, 77-98%) and 79% (70-87%), respectively. In samples with Ct < or = 35, sensitivity was 100% (88-100%) and 96% (88-99%), respectively. In 50 adult and 40 pediatric PCR-negative specimens, specificity was 100% (93-100%) and 98% (87-100%), respectively. Conclusions: Nucleocapsid concentrations in clinical NP samples span 8 orders of magnitude and correlate closely with RNA concentrations (Ct values). The S-PLEX Ag assay had 96-100% sensitivity in samples from children and adults with Ct values < or = 35, and 98-100% specificity. These results clarify Ag concentration distributions in clinical samples, providing insight into the performance of Ag RDTs and offering a new approach to diagnosis of COVID-19. [**note: here is an interesting new diagnostic approach showing the correlation between nucleocapsid antigen and RNA concentrations in nasopharyngeal samples.**]
https://www.medrxiv.org/content/10.1101/2020.11.10.20227371v1

- We here introduce a modular nanobody-organic electrochemical transistor architecture that enables the fast and specific detection and quantification of single-molecule to nanomolar protein antigen concentrations in complex bodily fluids. The sensor combines a new solution-processable organic semiconductor material in the transistor channel with the high-density and orientation-controlled bioconjugation of nanobody fusion proteins on disposable gate electrodes. It provides results after a 10 minutes exposure to 5 µL of unprocessed samples, maintains high specificity and single-molecule sensitivity in human saliva or serum, and is rapidly reprogrammed towards any protein target for which nanobodies exist. We demonstrate the use of this highly modular platform for the detection of green fluorescent protein, SARS-CoV-1/2, and MERS-CoV spike proteins and validate the sensor for COVID-19 screening in unprocessed clinical nasopharyngeal swab and saliva samples. [**note: here is a cool use of nanobodies from Saudi Arabia. They created an organic electrochemical transistor that can detect viral antigens at the physical limit.**] https://www.medrxiv.org/content/10.1101/2020.11.12.20228874v1

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

2020-11-14

Let's wrap up Tchaikovsky week with his Symphonie #1, 'Winter Dreams.' This is perhaps my favorite of the symphonies, followed by the 'Pathetique.' Again we have the Frankfurt Radio Symphony, this time conducted by Paavo Järvi: https://www.youtube.com/watch?v=oclajFkPwyU I often feature this orchestra because the video quality is excellent as is the playing. They also tend to put up many performances on YouTube.

As all my readers are aware, outbreaks of SARS-CoV-2 are happening all over the US. I'm not going to try to provide links to all the stories on this as they are easy to find. I will continue to look for the unusual and first-person accounts.

According to the Washington Post, it is not a good time to be a member of the Secret Service. The Governor of West Virginia tightens the state mask mandate. Distance learning is straining parent-teacher relationships. This is useful advice for President-Elect Biden for additions to the COVID-19 task force.

The New York Times has a clip about Russia and North Korea attempted hacking of companies engaged in COVID-19 research. This is not playing nice, but I guess it is expected. This cruise ship's return to service was short lived as passengers tested positive for the virus. Rural students in Internet dead zones cannot attend virtual classes.

Ed Yong writes about the plight of the healthcare workers as more people are hospitalized with COVID-19. For me this is the principal case for taking public health precautions.

The Lancet have a letter about researchers experience with cyber-bullying following publication of a meta-analysis showing HCQ with or without azithromycin did not work.

JAMA have an article regarding the estimated loss of life expectancy in children resulting from school closings during the pandemic. I believe that loss of in school learning is potentially injurious to children, I am not sure that I believe the thesis of this work. We need more and better testing (one of the authors of this viewpoint is a potential FDA Commissioner in the Biden administration)!! Here is a viewpoint on hospital-acquired SARS-CoV-2 infection.

Nature have an article on the mutations in SARS-CoV-2 found in the Denmark mink population. The mutation appears not have impacted the morbidity or mortality of the virus. Here is a nice piece on why COVID-19 death rates are falling.

MODELING

- Most of the western nations have been unable to suppress the COVID-19 and are currently experiencing second or third surges of the pandemic. *Here, we analyze data of incidence by age groups in 25 European countries, revealing that the highest incidence of the current second wave is observed for the group comprising young adults (aged 18-29 years old) in all but 3 of the countries analyzed. We discuss the public health implications of our findings*. [**note: young adults are more commonly infected in this new wave.**]
  https://www.medrxiv.org/content/10.1101/2020.11.11.20230177v1

NEWLY REGISTERED CLINICAL TRIALS

- Adapted from 'Annie' - The new clinical trials will come out tomorrow
  Bet your bottom dollar that tomorrow
  There'll be a report in this section

CLINICAL TRIAL RESULTS

- Double-blind, randomized, fully remote (contactless) clinical trial of fluvoxamine vs placebo. Participants were community-living, nonhospitalized adults with confirmed severe acute

respiratory syndrome coronavirus 2 infection, with COVID-19 symptom onset within 7 days and oxygen saturation of 92% or greater. One hundred fifty-two participants were enrolled from the St Louis metropolitan area (Missouri and Illinois) from April 10, 2020, to August 5, 2020. The final date of follow-up was September 19, 2020. Of 152 patients who were randomized (mean [SD] age, 46 [13] years; 109 [72%] women), 115 (76%) completed the trial. Clinical deterioration occurred in 0 of 80 patients in the fluvoxamine group and in 6 of 72 patients in the placebo group (absolute difference, 8.7% [95% CI, 1.8%-16.4%] from survival analysis; log-rank *P* = .009). The fluvoxamine group had 1 serious adverse event and 11 other adverse events, whereas the placebo group had 6 serious adverse events and 12 other adverse events. *In this preliminary study of adult outpatients with symptomatic COVID-19, patients treated with fluvoxamine, compared with placebo, had a lower likelihood of clinical deterioration over 15 days. However, the study is limited by a small sample size and short follow-up duration, and determination of clinical efficacy would require larger randomized trials with more definitive outcome measures*. [**note: OK, the numbers in this trial are small but think about the ramifications if this does work. We can level out people's moods and control COVID-19 at the same time!!! Add it to the water supply.]** https://jamanetwork.com/journals/jama/fullarticle/2773108

DRUG DEVELOPMENT

- Development of effective therapies against SARS-CoV-2 is urgently needed. Here, we evaluated the antiviral activity of a remdesivir parent nucleotide analog, GS441524, which targets the coronavirus RNA-dependent RNA polymerase enzyme, and a feline coronavirus prodrug, GC376, which targets its main protease, using a mouse-adapted SARS-CoV-2 infected mouse model. Our results showed that GS441524 effectively blocked the proliferation of SARS-CoV-2 in the mouse upper and lower respiratory tracts via combined intranasal (i.n.) and intramuscular (i.m.) treatment. However, the ability of high-dose GC376 (i.m. or i.n. and i.m.) was weaker than GS441524. Notably, low-dose combined application of GS441524 with GC376 could effectively protect mice against SARS-CoV-2 infection via i.n. or i.n. and i.m. treatment. *Moreover, we found that the pharmacokinetic properties of GS441524 is better than GC376, and combined application of GC376 and GS441524 had a synergistic effect. Our findings support the further evaluation of the combined application of GC376 and GS441524 in future clinical studies*. [**note: this drug discovery paper is from China. It uses both a proteas and RNA polymerase inhibitor in combination. This is the same approach as has been taken with HIV therapies.**] https://www.biorxiv.org/content/10.1101/2020.11.12.380931v1
- The ability of widely-available mouthwashes to inactivate SARS-CoV-2 in vitro was tested using a protocol capable of detecting a 5-log10 reduction in infectivity, under conditions mimicking the naso/oropharynx. During a 30 second exposure, two rinses containing cetylpyridinium chloride and a third with ethanol/ethyl lauroyl arginate eliminated live virus to EN14476 standards (>4-log10 reduction), while others with ethanol/essential oils and povidone-iodine (PVP-I) eliminated virus by 2-3-log10. Chlorhexidine or ethanol alone had little or no ability to inactivate virus in this assay. Studies are warranted to determine whether these formulations can inactivate virus in the human oropharynx in vivo, and whether this might impact transmission. [**note: here is one for all you mouthwash fans!**] https://www.biorxiv.org/content/10.1101/2020.11.13.381079v1

- Antivirals against coronaviruses do not exist, hence global drug re-purposing efforts have been carried out to identify agents that may provide clinical benefit to patients with COVID-19. Itraconazole, an antifungal agent, has been reported to have potential activity against animal coronaviruses. Methods: Using cell-based phenotypic assays, the in vitro antiviral activity of itraconazole and 17-OH itraconazole was assessed against clinical isolates from a German and Belgian patient infected with SARS-CoV-2. Results: Itraconazole demonstrated antiviral activity in human Caco-2 cells (EC50 = 2.3 µM; MTT assay). Similarly, its primary metabolite, 17-OH itraconazole, showed inhibition of SARS-CoV-2 activity (EC_{50} = 3.6 µM). Remdesivir inhibited viral replication with an EC_{50} = 0.4 µM. Itraconazole and 17-OH itraconazole resulted in a viral yield reduction in vitro of approximately 2-log_{10} and approximately 1-log_{10}, as measured in both Caco-2 cells and VeroE6-eGFP cells, respectively. The viral yield reduction brought about by remdesivir or GS-441524 (parent nucleoside of the antiviral prodrug remdesivir; positive control) was more pronounced, with an approximately 3 log_{10} drop and >4 log_{10} drop in Caco-2 cells and VeroE6-eGFP cells, respectively. *Discussion: Itraconazole and 17-OH itraconazole exert in vitro low micromolar activity against SARS-CoV-2. Despite the in vitro antiviral activity, itraconazole did not result in a beneficial effect in hospitalized COVID-19 patients in a clinical study (EudraCT Number: 2020-001243-15).* [**note: this is from Janssen Pharma and shows that *in vitro* data on viral inhibition don't always translate into *in vivo* activity.**]
  https://www.biorxiv.org/content/10.1101/2020.11.13.381194v1

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- The SARS-CoV-2 viral spike (S) protein mediates attachment and entry into host cells and is a major target of vaccine and drug design. Potent SARS-CoV-2 neutralizing antibodies derived from closely related antibody heavy chain genes (IGHV3-53 or 3-66) have been isolated from multiple COVID-19 convalescent individuals. These usually contain minimal somatic mutations and bind the S receptor-binding domain (RBD) to interfere with attachment to the cellular receptor angiotensin-converting enzyme 2 (ACE2). We used antigen-specific single B cell sorting to isolate S-reactive monoclonal antibodies from the blood of a COVID-19 convalescent individual. The seven most potent neutralizing antibodies were somatic variants of the same IGHV3-53-derived antibody and bind the RBD with varying affinity. We report X-ray crystal structures of four Fab variants bound to the RBD and use the structures to explain the basis for changes in RBD affinity. *We show that a germline revertant antibody binds tightly to the SARS-CoV-2 RBD and neutralizes virus, and that gains in affinity for the RBD do not necessarily correlate with increased neutralization potency, suggesting that somatic mutation is not required to exert robust antiviral effect. Our studies clarify the molecular basis for a heavily germline-biased human antibody response to SARS-CoV-2*. [**note: this is from Harvard and shows the molecular basis for germline-biased neutralizing antibodies.**]
  https://www.biorxiv.org/content/10.1101/2020.11.13.381533v1

DIAGNOSTIC DEVELOPMENT

- Nothing new

>>

2020-11-15

For reflection Sunday, we have a delightful send up of Mozart's 'Cosi fan Tutte' (alert readers will know this is my favorite of the three operas by Mozart and da Ponte). Here is the Finnish National Opera in a pandemic take of entlitled 'Covid fan Tutte'! The music is all Mozart other than a little bit of Wagner at the beginning as a performance of Die Walkure is cancelled. The words are changed for our time. The staging and singing are exceptional from this all Finnish cast under the direction of Esa-Pekka Salonen. There are English subtitles. Enjoy this one!! We all need to have some fun with the pandemic in so far as is possible. I certainly had some good laughs: https://www.youtube.com/watch?v=LwNz8C33JOc

The Washington Post has an interesting story about stolen polio vaccine in Montreal back in 1959. Visitors to Lake Tahoe during the pandemic didn't follow park etiquette. The virus doesn't care who won the Presidential election. What happens if you have a positive COVID-19 antibody test? There is truth in this op-ed that COVID-19 is driving a wedge between us. Libertarians should recognize the pandemic doesn't care about your personal freedom. A lead editorial notes the virus origins continue to be a mystery and we need to investigate this. Here is the linked PNAS paper by Stanford's David Relman on this topic that is very useful reading. [**note: this also raises some very uncomfortable points that need to be addressed regarding laboratory safety and what types, if any, genetic manipulations were being done on this class of coronaviruses. I don't think I linked to this before, but The Lancet has established a commission that is offering help. More details on that are HERE. I cannot emphasize enough how important this is.**]

The New York Times notes that states may not be prepared to distribute the COVID-19 vaccines when they are ready. I'm curious to see how Pfizer plans to handle distribution of their vaccine which requires super low temperature storage. Doctors are pleading with Americans to take the virus surge seriously. I don't understand the court decision in El Paso that prevents the town from locking down certain businesses. Do they know that the virus does not understand their decision and won't just go away?

Derek Lowe covers the Eli Lilly monoclonal antibody EUA.

There are some good immunology papers today but little else of interest. As promised, the newly registered trials were updated.

MODELING

- Nothing today.

NEWLY REGISTERED CLINICAL TRIALS

- Currently, SARS-CoV-2 the novel member of the corona virus family, affecting the world leading to COVID-19 disease. It can result life-threatening condition by developing severe acute respiratory distress syndrome (ARDS). Based on previous evidence a group of patients with severe COVID-19 develop a cytokine storm syndrome which leads to hyper-inflammation lung tissue damage. Supportive care is the current management of COVID-19 is and management of ARDS as a main cause of mortality has been remained challenging. Therefore, an urgent effective treatment of COVID-19 regarding hyper-inflammation mechanism is required. Currently, development of novel anti-viral agents and vaccines are the main issues. However, it needs long time, from months to years, until suitable new medications and vaccines have been developed. An immune-modulatory tetra deca peptide (14-mer peptide) named Human Ezrin Peptide 1 (HEP-1) (trade name Gepon) was introduced by the group of Ataullakhanov in Russia.

Regarding its proved anti-viral and anti-inflammatory effect, Russian authorities approved Gepon for treatment of ulcerative colitis treatment and Hepatitis -C. [**note: this is an Irani clnical trial of a peptide first identified by Russian investigators several years ago.**] NCT04627233

- Both mindfulness meditation and expectancy effects are known to reduce anxiety, stress and catastrophizing, but it is unknown whether and how expectancy effects contribute to the overall effect of mindfulness meditation on these outcomes, especially during significant global events such as the coronavirus pandemic. This study includes four interrelated aims that will probe these effects and interactions. [**note: I look forward to the results of this Australian study as mindfulness meditation is what I am after!!!**] NCT04602312

CLINICAL TRIAL RESULTS

- Nothing today.

DRUG DEVELOPMENT

- Nothing today.

VIRUS BIOCHEMISTRY & IMMUNOLOGY

- Several efforts to control SARS-CoV-2 dissemination are still running including vaccines and drug treatments. The effectiveness of these procedures depends, in part, that the regions to which these treatments are directed do not vary considerably. Although, it is known that the mutation rate of SARS-CoV-2 is relatively low it is necessary to monitor the adaptation and evolution of the virus in the different stages of the pandemic. Thus, identification, analysis of the dynamics, and possible functional and structural implication of mutations are relevant. Here, we first estimate the number of COVID-19 cases with a virus with a specific mutation and then calculate its global relative frequency (NRFp). *Using this approach in a dataset of 100 924 genomes from GISAID, we identified 41 mutations to be present in viruses in an estimated number of 750 000 global COVID-19 cases (0.03 NRFp). We classified these mutations into three groups: high-frequent, low-frequent non-synonymous, and low-frequent synonymous. Analysis of the dynamics of these mutations by month and continent showed that high-frequent mutations appeared early in the pandemic, all are present in all continents and some of them are almost fixed in the global population. On the other hand, low-frequent mutations (non-synonymous and synonymous) appear late in the pandemic and seems to be at least partially continent-specific. This could be due to that high-frequent mutation appeared early when lockdown policies had not yet been applied and low-frequent mutations appeared after lockdown policies. Thus, preventing global dissemination of them. Finally, we present a brief structural and functional review of the analyzed ORFs and the possible implications of the 25 identified non-synonymous mutations*. [**note: this is a nice paper from Brazil that looks the dynamics and distribution of SARS-CoV-2 mutations. Thus far it seems none of the mutations will impact the vaccines currently under development.**] https://www.biorxiv.org/content/10.1101/2020.11.13.381228v1
- The evolution of SARS-CoV-2 humoral response in infected individuals remains poorly characterized. Here, we performed a longitudinal study of sera from 308 RT-qPCR+ individuals with mild disease, collected at two time-points, up to 6 months post-onset of symptoms (POS). We performed two anti-S and one anti-N serology assays and quantified neutralizing antibodies

(NAbs). At month 1 (M1), males, individuals > 50 years of age or with a body mass index (BMI) > 25 exhibited higher levels of antibodies. Antibody levels decreased over time. *At M3-6, anti-S antibodies persisted in 99% of individuals while anti-N IgG were measurable in only 59% of individuals. The decline in anti-S and NAbs was faster in males than in females, independently of age and BMI. Our results show that some serology tests are less reliable overtime and suggest that the duration of protection after SARS-CoV-2 infection or vaccination will be different in women and men*. [**note: here is a paper from France that looks at the sex-related differences in antibody decay. It appears to be faster in males than females.**] https://www.medrxiv.org/content/10.1101/2020.11.12.20230466v1

- A cohort of COVID-19 convalescent volunteers allowed the study of neutralizing (nAb) and ligand antibodies kinetics by providing sequential samples during a median of 100 days after onset of disease. Material and Methods: A cohort of previously RT-PCR+ve (detected by nasopharyngeal swab during the acute phase), male convalescent patients, all with mild symptoms, were enrolled on serial blood sample collection for evaluation of longitudinal nAb titers and anti-nucleocapsid (NP) antibodies (IgM, IgG and IgA). Nabs were detected by a cytopathic effect-based virus neutralization test (CPE-based VNT), carried out with SARS-CoV-2 (GenBank: MT350282) Results: *A total of 78 male volunteers provided 316 samples, spanning a total of 4820 days of study. Although only 25% of donors kept nAb titers ≥160, after a median of 100 days after the onset of disease, there was a high probability of sustaining nAB titers ≥160 in volunteers whose initial nAb titer was ≥1280, weight ≥ 90kg or BMI classified as overweight or obese, evidenced by Kaplan-Meier estimates and Cox hazard regression. There was no correlation between ABO group, ABO antibody titers and persistent high nAb titers. High IgG anti-NP (S/CO ≥5.0) is a good surrogate for detecting nAB ≥160, defined by ROC curve (sensitivity = 90.5%; CI95% 84.5-94.7%) Conclusion: Selection of CCP donors for multiple collections based on initial high nAb titers (≥1280) or overweight/obese (BMI) provides a simple strategy to achieve higher quality in CCP programs. High IgG anti-NP levels can also be used as surrogate markers for high nAb screening*. [**note: here is a longevity study from Brazil on 78 males.**] https://www.medrxiv.org/content/10.1101/2020.11.12.20230391v1
- COVID-19 is challenging healthcare preparedness, world economies, and livelihoods. The infection and death rates associated with this pandemic are strikingly variable in different countries. To elucidate this discrepancy, we analyzed 2431 early spread SARS-CoV-2 sequences from GISAID. We estimated continental-wise admixture proportions, assessed haplotype block estimation, and tested for the presence or absence of strains recombination. *Herein, we identified 1010 unique missense mutations and seven different SARS-CoV-2 clusters. In samples from Asia, a small haplotype block was identified; whereas, samples from Europe and North America harbored large and different haplotype blocks with nonsynonymous variants. Variant frequency and linkage disequilibrium varied among continents, especially in North America. Recombination between different strains was only observed in North American and European sequences. Additionally, we structurally modeled the two most common mutations D614G and P314L which suggested that these linked mutations may enhance viral entry and stability. Overall, we propose that COVID-19 virulence may be more severe in Europe and North America due to coinfection with different SARS-CoV-2 strains leading to genomic recombination which might be challenging for current treatment regimens and vaccine development. Furthermore, our study provides a possible explanation for the more severe second wave of COVID-19 that*

many countries are currently experiencing presented as higher rates of infection and death. [**note: here is a paper from Kuwait proposing that recombination between strains may have led to the mergence of possibly more virulent SARS-CoV-2. I don't agree with the conclusion based on the clinical observations I have seen in recent papers that have linked the emerging new phenotype.**] https://www.medrxiv.org/content/10.1101/2020.11.11.20229765v1

- Here, we analyzed antibody functions in 52 asymptomatic infected individuals, 119 mild and 21 hospitalized COVID-19 patients. We measured anti- Spike antibody levels with the S-Flow assay and mapped SARS-CoV-2 Spike- and N-targeted regions by Luminex. Neutralization, complement deposition and Antibody-Dependent Cellular Cytotoxicity (ADCC) were evaluated using replication-competent SARS-CoV-2 or reporter cell systems. *We show that COVID-19 sera mediate complement deposition and kill infected cells by ADCC. Sera from asymptomatic individuals neutralize the virus, activate ADCC and trigger complement deposition. Antibody levels and activities are slightly lower in asymptomatic individuals. The different functions of the antibodies are correlated, independently of disease severity. Longitudinal samplings show that antibody functions follow similar kinetics of induction and contraction, with minor variations. Overall, asymptomatic SARS-CoV-2 infection elicits polyfunctional antibodies neutralizing the virus and targeting infected cells. - Sera from convalescent COVID-19 patients activate the complement and kill infected cells by ADCC. - Asymptomatic and symptomatic SARS-CoV-2-infected individuals harbor polyfunctional antibodies. - Antibody levels and functions are slightly lower in asymptomatic individuals - The different antiviral activities of anti-Spike antibodies are correlated regardless of disease severity. - Functions of anti-Spike antibodies have similar kinetics of induction and contraction.* [**note: more work from France on antibody response. These investigators look at both symptomatic and asymptomatic patients and find both have polyfunctional antibodies. This is good news.**] https://www.medrxiv.org/content/10.1101/2020.11.12.20230508v1

DIAGNOSTIC DEVELOPMENT

- Nothing today.