Science's response to CoVID-19

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Abstract: CoVID-19 is a multi-symptomatic disease which has made a global impact due to its ability to spread rapidly, and its relatively high mortality rate. Beyond the heroic efforts to develop vaccines, which we will not discuss, the response of scientists and clinicians to this complex problem has reflected the need to detect CoVID-19 rapidly, to diagnose patients likely to show adverse symptoms, and to treat severe and critical CoVID-19. Here we aim to encapsulate these varied and sometimes conflicting approaches and the resulting data in terms of chemistry and biology. In the process we highlight emerging concepts, and potential future applications that may arise out of this immense effort.

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Introduction

Between the dawn of the new millennium and today, several viral diseases have emerged due to zoonotic processes. Such events occur when a virus that infects an animal becomes able to infect humans. Until recently, the most well-known of these diseases were: Middle Eastern Respiratory Syndrome coronavirus (MERS-CoV; arrived in the Middle East circa 2012), with a mortality of ~35%; and Sudden Acute Respiratory Syndrome coronavirus (SARS-CoV; arrived circa 2002 from [Guangdong, China](#)), which had a mortality of ~15%.¹ Such mortality data are a function of ability to precisely assign the number of people infected and those that died from the disease, both of which can be underestimated. The number infected is often a severe underestimate: e.g., during the CoVID-19 pandemic, 10-times more people were infected than the number of people formally declared as suffers in the USA.² Thus, these and similar mortalities are approximations, and often overestimates. Nevertheless, in the case of MERS-CoV, mortality lies close to that of the bubonic plague.³ Other milder diseases, such as HCoV-229E, likely arose via a similar route. More intriguingly, all these events have actually been linked, to some extent, to coronaviruses (CoVs; principally the β - and α -variants) endemic to bats.

Indeed, bats may be a common link to all zoonotic CoV events.⁴ In the case of MERS-CoV and SARS-CoV, these diseases have been principally ascribed to β -CoVs from horseshoe bats, of the genus *Rhinolophus*. This group of sedentary bats (i.e., bats that do not stray far from their roosts)⁵ is widely distributed across temperate and tropical areas, including China, and other parts of Eurasia all the way to Africa. As a result of MERS- and SARS-CoVs, zoonosis from bats was perceived as a clear and present threat to global health in the early years of the new millennium.⁶ Further stoking these worries, our understanding of CoVs endemic in bats was, and remains, overall poor. Thousands of endemic CoVs are hitherto unknown. Thus, several initiatives were established to understand and map CoVs in bats since 2000. These efforts were enacted most extensively in bats in China,⁷ but also in other groups of bats in Asia⁸ and Africa.⁹ This research unearthed numerous new CoVs,¹⁰ several of which appear to be pathogenic to humans. Despite remonstrations, many of these initiatives were either halted or coming to an end as of 2019.

In December 2019, almost a year to the day of writing this piece, a new respiratory virus surfaced in Wuhan, the capital city of Hubei province, China. This virus can spread between humans and is also believed to have derived from transfer from β -CoVs in horseshoe bats,¹¹ although the precise route is disputed. Estimates have varied, but the currently quoted mortality of this virus, that came to be known as SARS-CoV-2, causing a disease/pandemic referred to as CoVID-19, is ~0.5-2%.¹² CoVID-19's mortality is significantly less than MERS-CoV and SARS-CoV; nonetheless, the mortality of CoVID-19 is significantly higher than common flu, and at the low end of estimates of the mortality of the Spanish flu of 1918, which killed ~50 million people worldwide¹³ (equivalent to quadruple that number in today's figures).¹⁴ [SARS-CoV-2](#) shows a reproductivity (R) number ~3,¹⁵ although estimates vary considerably¹⁶ and interpretation of R needs careful consideration. Indeed, R values vary as a function of method and circumstances:¹⁷ e.g., some methods show that the R value of CoVID-19 aboard the Diamond Princess cruise ship was ~15. Nevertheless, the average R value for CoVID-19 is significantly higher than MERS-CoV¹⁸ (R 0.9, for most estimates¹⁹) but is quite similar to that of SARS-CoV.^{20,21} Critically, SARS-CoV is spread most effectively when patients are manifesting symptoms,²² which made management/containment of SARS-CoV relatively simple. By contrast, CoVID-19-infected people are

infectious prior to onset of symptoms.²³ Most studies agree that asymptomatic CoVID-19 patients (1 in 5 of those infected)²⁴ can spread the disease.²⁵ However, estimates vary as to the importance of spreading by totally asymptomatic people.²⁶

Although this is not uncommon for viral infections,²⁷ CoVID-19 presents an astounding number of pathologies, with growing evidence that there are specific phenotypes associated with severity²⁸ and timing post infection/progression (**Fig. 1**). Many symptoms are related to respiratory issues and lung damage.²⁹ Consistent with these observations, viral proteins bind host proteins abundant in the lungs. Many other reported CoVID-19-associated pathologies are associated with the immune response and blood clotting: cytokine storms leading to hyperinflammation (elevated inflammatory markers including ferritin, interleukins 1 and 6, and C-reactive protein have been reported);³⁰ oxidative stress, potentially leading to ferroptosis in several tissues;³¹ changes in immune cells;^{32,33} thrombotic microangiopathy (blood clots forming in capillaries and arterioles);³⁴ and a host of other issues, including neurological problems.³⁵ Immune evasion is one of the hallmarks separating CoVID-19 from SARS-CoV, although how these observations link to the clinical outcomes is unclear. Intriguingly, reports of self-harm in infected patients, due to, e.g., consumption of ethanol-based hand cleaners have also surfaced. This has also been met with a general increase in calls to poison control centers concerning ingestion of household cleaners.³⁶

After early reports from China, the Western world watched as daily news updates surfaced of the escalating troubles that began to permeate from the epicenter of this crisis to other parts of China and later reverberate to the rest of the world. This spread was doubtless fanned by the frequency of global travel, and the season during which these events occurred, among other variables. Due to an unprecedented response, China started to flatten the progression of the disease in the proceeding months. Huge hospitals were built in days; whole cities were locked down; mandatory quarantine was introduced;³⁷ and interaction between people/towns/cities was minimized. However, it would not be until April of 2020 that Wuhan would be lifted from lockdown.³⁸ By February 2020, the first CoVID-19-related death was recorded in Europe. Shockingly, despite advanced warning, by March, hospitals in many Western countries were overrun, leading to mass shutdowns of government and businesses as well as border closures. By June 2020, almost half of the global population had experienced quarantine/confinement of some description,³⁹ including in India,⁴⁰ and Africa.⁴¹ However, the global response was overall quite varied.⁴² Over the summer of 2020, many countries that had instigated lockdown measures reopened and a semblance of normality returned in much of the Western world, at least. However, this hiatus was in turn met with an autumn and winter in which the number of cases spiked again, spurring “new waves” of CoVID-19. More recently and into 2021, new strains of the virus have been identified which are more infectious than the original strain(s), including the UK strain,⁴³ and the South African strain.⁴⁴ These have started to dominate in some populations. Thus, blanket lockdown procedures and curfews have been reinstated in numerous countries. Intriguingly, in China, and even Wuhan, SARS-CoV-2 was almost totally under control by September 2020. This reflects differences in the ways governments dealt with the crisis,⁴⁵ but likely also cultural differences in the ways governments and citizens interact.⁴⁶

All the above responses were taken from a playbook dating at least as far back as the 12th-16th centuries.⁴⁷ Indeed, the word quarantine derives from “quaranta” referring to the 40 day isolation imposed on outsiders wishing to enter European cities during the plague around that period.⁴⁸

Bolstering this tried-and-tested response, were novel technological approaches that reflect a modern intervention to a disease presenting myriad symptoms and with an unnerving ability to spread. These approaches constitute two different aspects: (i) initiatives aimed at diagnosing infected people, which required rapid, reliable and innovative testing initiatives and protocols to be developed that could be field (and even “home”) ready; (ii) continuations of huge chemical and biological counteroffensive that was launched to identify and exploit weak points in SARS-CoV-2. Arguably, this two-pronged response represents a focused and coordinated effort across a huge number of disciplines, perhaps rivaling the war on cancer and the space program. The current apogee of this drive is the record-breaking approval of several SARS-CoV-2 vaccines, including those from Pfizer and Moderna that were approved for use in the USA before 2021. Work toward vaccine development was backed up by doctors globally developing new treatment regimens in real-time, investigations of new medications, and basic science approaches aimed at identifying chemical weak points in the virus’ armor and in the patients’ themselves. Many reviews have covered in depth deployment and development of advanced technical paraphernalia. However, it is crucial to note that one of the largest divergences between the response to the plague in the 12th-16th centuries and SARS-CoV-2 today is that the latter leveraged global communications that allowed the passage of data, personnel, and equipment to guide and coordinate responses. In the wake of CoVID-19, maintaining,⁴⁹ and honing this network will be crucial.⁵⁰ Of course, the human, economic, and social impact of this epidemic cannot be understated. More than 2 million people have died; falls in GDP, e.g., in the UK, have been the highest since records were maintained; numerous patients with non-CoVID-19-related diseases have suffered,⁵¹ either indirectly and/or due to be sensitized to the disease;⁵² and the mental and physical health of the population will have been impacted for years to come.⁵³

Our review specifically focuses on knowledge and ideas that arose out of the two forementioned scientific responses to the disease, and how that knowledge-base can be deployed for our future benefit. We discuss some take home lessons that the community could draw from this difficult period, and suggest some improvements. Likely both the issues raised and their remedies we discuss will require refining through individual reflection and also debate. Finally, although our review encompasses most of the key emerging concepts, the rapidity of changes in treatments, screens, etc., effectively prohibits being able to include all relevant work; we thus apologize to everyone whose work is not covered by this piece.

A brief, guided tour of SARS-CoV-2

A brief outline of what the virus is and how it enters the cell is clearly important for understanding and treating the disease. Much of this information is derived from analogy to previous CoV’s, but also through the use of innovative work based around reverse genetics,⁵⁴ viral genome sequencing strategies,⁵⁵ as well as multi-omics.⁵⁶ We will also refer to many of these aspects in subsequent sections. SARS-CoV2 contains a 30 kbp RNA genome (**Fig. 2A**). This is very large by virus standards.⁵⁷ SARS-CoV2’s genome has high (>87%) identity with three SARS-like bat viruses BatCoV-RaTG13, Bat-SL-CoV-ZC45 and Bat-SL-CoV-ZXC21.⁵⁸ It also has ~80% pairwise identity to SARS-CoV and 50% identity to MERS-CoV (**Fig. 2B**).^{59,60} The amino acid sequence of the largest ORF, ORF1ab, in SARS-CoV and SARS-CoV-2 are ~95% identical, indicating that these viruses belong to the same species. The SARS-CoV-2 genome is unusually AT rich in protein-coding regions and contains CG-rich internal ribosomal entry sites in the 5’-UTRs of several genes, both of which may promote viral replication.⁶¹

At the 5'-end of the genome lies the largest ORF, comprising ORF1a and ORF1b, which is two-thirds the length of the whole genome (**Fig. 2A**).⁶¹ This ORF encodes 16 proteins, called non-structural proteins (NSPs1-16), comprising two cysteine proteases [NSP3, also known as (aka) papain-like protease, PL^{PRO}; and NSP5, aka 3-chymotrypsin-like, 3CL^{PRO}], an endonuclease (NSP15),⁶² an exonuclease⁶³/N7-methyltransferase⁶⁴ (NSP14) that bolsters fidelity of the relatively-inaccurate RNA-dependent RNA polymerase (RdRp),⁶⁵ a 2'-O-methyltransferase (NSP16),⁶⁶ a helicase (NSP13), and RdRp (NSP12).⁶⁷ All the above proteins have clear (and likely essential) roles in the viral lifecycle.⁶⁸ Compromising function of many of these gene products can strongly lower virulence and elevate sensitivity to anti-viral drugs:⁶⁹ mutations in some of these proteins in [SARS-CoV-2](#) may be important for virulence.⁷⁰

Interspersed across the 3'-end of the genome, SARS-CoV-2 contains several structural genes. Arguably the most commonly discussed is the spike protein (S) that is a transmembrane glycoprotein required for host-cell entry. This is a site of relatively high mutational frequency⁷¹ [and several high throughput methods have mapped sites essential for/enhancing function on this protein.](#)⁷² There are also the envelope (E) and membrane (M) proteins, that along with the S-protein form the viral envelope. Finally, there is the nucleocapsid protein (N), aka ORF9a, that encases the genome in the mature virus.

The 3'-region contains several ancillary proteins, whose encoding genes sometimes overlap with structural proteins or each other (**Table 1, Fig. 2**). These proteins are overall less well understood than the other protein components of the virus, and seem to be mostly virulence factors whose requirements for infectivity vary. The principal proteins are: ORF3a (a (largest) accessory protein that assists virus synthesis/escape also linked to cell death),⁷³ several other proteins have been proposed to lie in this ORF (in alternative reading frames);⁷⁴ ORF6,⁷⁵ involved in inhibiting type 1⁷⁶ interferon response;⁷⁷ ORF7a, a transmembrane protein that assists virulence;⁷⁸ ORF7b that localizes to the Golgi apparatus, may suppress interferon signaling,⁷⁹ and may be a structural component of the virion;⁸⁰ ORF8, likely defective in a strain from Singapore that eventually died out,⁸¹ and likely unnecessary for [SARS-CoV-2](#) persistence,⁸² (ORF8 is a known target of human antibodies, and evidence indicates selection against this protein as a function of disease progression from both SARS-CoV and CoVID-19);⁸³ ORF9b, a dimeric all β -sheet membrane protein housing a tunnel that can incorporate hydrophobic molecules⁸⁴ (lies within ORF9a and is likely linked to evading the innate immune response);⁸⁵ and ORF9c, a transmembrane protein linked to impairing antiviral response.⁸⁶ These regions have high to moderate (in the case of ORF8) homology with SARS-CoV. Two other ORFs possess limited homology with SARS-CoV. The first is ORF3b is a suppressor of interferon activity that is more efficient than its SARS-CoV ortholog. More active versions of this protein are found in severe [SARS-CoV-2](#) variants,⁷⁷ despite it being lost in some strains.⁸⁷ The second is ORF10. This protein is not necessary for infection in humans,⁸³ and likely not a bona fide protein. The SARS-CoV ORF8b protein (that triggers ER stress and autophagy, and stimulates cell death) is missing from SARS-CoV-2.⁸⁹

Table 1: the accessory proteins and their roles in SARS-CoV and SARS-CoV-2

Name	Protein identity with SARS-CoV (Frankfurt 1)	Function in SARS-CoV-2
ORF3a	73% identity (divergence in N-terminus)	ion channel activity; inflammasome activation; replication and pathogenesis; possible links to cell death. ⁹⁰ Viruses lacking ORF3a (but not ORF8a) and E are not viable. ⁹¹
ORF3b	Minimal (ORF3b is located in different regions of Orf3a in SARS-CoV1 and 2)	Not necessarily a genuine ORF. ⁹² Potential interferon antagonist. ⁹³ Lost in population. ⁸⁷
ORF3c	Not present in SARS-CoV	Predicted to be a transmembrane protein. ⁷⁴
ORF3d	Not present in SARS-CoV	A potential novel gene product that is poorly characterized (overlaps partly with ORF3c). ⁹⁴
ORF6	69% (variation in body and C terminus)	Antagonizes interferon signaling. ⁷⁵
ORF7a	85%	Deletions in the viral population have been identified. ⁹⁵ This is a transmembrane protein regulating host/virus interplay. ⁹⁶ May also regulate cell death.
ORF7b	81% (only 43 amino acids long)	May be incorporated into the virus; may suppress interferon signaling. ⁷⁹
ORF8	weak versus both ORF8a and ORF8b	SARS-CoV-2 ortholog can form high order oligomers that cannot occur in SARS-CoV. ⁹⁷ Antagonizes interferon signaling, ⁹⁸ and downregulates MHC-1. ⁹⁹
ORF8b	Not present in SARS-Cov-2	SARS-CoV protein is prone to aggregation. Can trigger cell death and activate inflammasome. Triggers ER stress and activates autophagy/lysosomes. ⁸⁹
ORF9b	72% (divergence mainly in N-terminus)	This is also a suppressor of interferon responses. ¹⁰⁰ Antibodies have been detected in SARS-CoV-2 patients. ¹⁰¹
ORF9c	Not present in SARS-CoV	Linked to avoiding the immune response. This is a transmembrane protein. ⁸⁶
ORF10	Not present in SARS-CoV	Unknown/potentially not translated

SARS-CoV and SARS-CoV-2 (although not MERS-CoV and several other CoVs) enter cells via interaction with angiotensin-converting enzyme 2 (ACE2) (**Fig. 2C**).¹⁰² [There is evidence that newly emerged SARS-CoV-2 strains show higher affinity between the S-protein and ACE2, testifying to the importance of this interaction.](#)¹⁰³ Genomewide association (GWAS) analysis of 1980 Spanish and Italian severe COVID-19 patients showed that a cluster of 6 genes on chromosome 3p21.31 is associated with genetic sensitivity to COVID-19.¹⁰⁴ Among other interesting genes, this cluster includes SLC6A20, which functionally interacts with ACE2. These results have been backed up by other experiments.¹⁰⁵ The receptor tyrosine

kinase, AXL, may also be involved in virus entry through a direct interaction with the S-protein; importantly these studies showed that mouse AXL did not bind CoVID-19 S-protein.¹⁰⁶ Such nuances are hugely important for planning and evaluating in vitro and cellular/animal model studies of infection.

Once anchored on the host cell, the virus requires S-protein cleavage at several sites (S1/S2/S2') by host cell proteases (TMPRSS2,¹⁰⁷ a serine protease, the principal cleavage enzyme in some cell lines in the case of SARS-CoV-2, although cathepsin B and cathepsin L can also be significant players in these events in some cell lines). S-protein cleavage appears to be particularly critical for the virus lifecycle. Addition of external proteases (i.e., in trans to the viral proteome) is sufficient to allow some MERS-CoV-like viruses that can dock with the membrane, but otherwise cannot infect human cells, to become infective.¹⁰⁸ Additionally, unlike SARS-CoV,⁵⁸ the SARS-CoV-2 S-protein can also be cleaved by the serine protease furin, in what has been described as a preprocessing step that may aid virulence¹⁰⁹ through promoting the TMPRSS2 cleavage step.¹¹⁰ It is possible that furin cleavage sites are positively selected in SARS-CoV-2.¹¹¹ Furin-mediated cleavage is inhibited by plasma from CoVID-19 sufferers.¹¹²

Upon entry to the cell, the viral genome needs to be translated.¹¹³ ORF1a/b is translated to give ORF1a (~500 kDa, comprising NSPs1-11). A -1 frameshift can occur during translation to give a longer polypeptide ORF1ab (800 kDa, comprising NSPs1-10 *and* 12-16). This frameshift is not 100% efficient, giving rise to different expression levels of gene products derived from ORF1a and ORF1ab (the amount of ORF1b-derived protein is ~50% of ORF1a-derived protein). Notably, subsequent proteolysis-mediated processing creates 16 proteins in total. This proteolysis is executed by the two viral cysteine proteases (PL^{PRO} and 3CL^{PRO}) (similar to how linear polyubiquitin is cleaved to ubiquitin monomers, for instance).¹¹⁴ 3CL^{PRO} (aka main protease, M^{PRO}) performs the bulk of this work, leading to M^{PRO} being proposed as a drug target (**Fig. 2A**).¹¹⁵ These proteases also cleave human proteins, potentially aiding virulence.¹¹⁶ The cleaved proteins so produced start to generate the viral genome. This process requires making a minus strand (reverse complement of the virus genome) to serve as a template to make copies of the coding RNA. These processes are performed by two different RNA-synthesis complexes.¹¹⁷ Once viruses are assembled, β -CoVs use an Arl8-dependent lysosome pathway to exit the cell. Intriguingly, lysosomes appear to be deacidified during coronavirus infection, perhaps to prevent protease cleavage (reported to be reduced by 40% in infected cells) that could occur during egress. This altering of lysosomal function also affects antigen presentation.¹¹⁸

New CoVID-19 diagnostic methods

One of the significant issues promoting spread of CoVID-19 has been the virus's prolonged incubation time, with often ~2 weeks post exposure being required to display symptoms coupled with a relatively early infective period.¹¹⁹ Thus, rapid development of sensitive and diagnostic testing¹²⁰ has been necessary to quell local eruptions of the disease and focus confinement, e.g., in the tier system introduced in the UK. Clearly how infected people were dealt with, and how this response was organized, has differed greatly amongst countries, and is in part responsible for differences in spread and management of the virus across different continents,¹²¹ which makes a general discussion difficult. This review will not dwell on those issues here. Instead, we will outline the technological approaches and how they differ. Numerous sampling regions on the patient are of course possible, including nasopharyngeal areas, eyes,¹²² and saliva.¹²³ The sampling region can affect fidelity of results, as a

function of different testing strategies, and manifest different likelihoods of detection based on the stage of infection, and impinge on administration of the test. The choice of sampling region can in turn bring additional discomfort to patients, and change the resulting danger to healthcare workers who may be needed to take the samples.¹²⁴ Thus, when evaluating diagnostic methodologies, such factors must be taken into consideration.¹²⁵ Indeed, the end goal of testing, or use case, also needs to be considered.¹²⁶

We will [discuss](#) each method based on typical parameters, e.g., cost, turnaround time, and accuracy (see below).¹²⁵ These variables provide numerical data that are deceptively easy to interpret: in terms of cost, the lower, the better; in terms of accuracy, the higher the better. However, it has been concluded that these arguments require contextualization. E.g., >90% accuracy may not be necessary for effective population-based screening when coupled with other active countermeasures, especially among predominantly younger populations.¹²⁷ Conversely, false positives can potentially have severe ramifications for individual patients, e.g., mandatory hospitalization and potential exposure to CoVID-19 patients¹²⁸ in many Asian countries, and may convey other risks in terms of loss of capital and isolation elsewhere. On the other hand, simple probability laws have been used to show how tests with ~70% accuracy administered in series can lead to >90% accuracy when two such tests are run in series, and a patient is only considered negative if both tests give a negative result.¹²⁹ This sort of sequential amplification is known in numerous fields of science.¹³⁰ However, increasing sampling necessarily increases cost and time. Furthermore, given as this protocol mandates that we consider opposing outputs from the two tests as a negative by default, this strategy could lead to lowering confidence among the general public. Thus, from the perspective of an individual buying the test-kit(s), or a researcher trying to accurately understand the disease, it is critical that we seek optimal performance. Our review will [thus discuss assays in terms of individual experiments , for standardization. We will not directly compare these procedures, as in effect, the different variables require different considerations for given purposes. However, this has been done elsewhere, and we refer readers to such papers.](#)¹³¹

Reverse transcription polymerase chain reaction (RT-PCR)

Real time RT-PCR screening is the gold standard test for CoVID-19, and numerous other CoVs (**Fig. 3A**).¹³² The basic protocol is common to the majority of RT-PCR procedures. Critically, each step is an important variable, and can affect fidelity of testing. The general scheme involves, lysis, RNA purification, RT of the RNA, amplification of specific stretches of the product cDNA using carefully designed primers¹³³ by PCR, and detection of these DNA products by probes¹³⁴ (e.g., by TAQ-MAN, molecular beacon). Several amplification regions have been suggested for this procedure, including a two-step process that examines the E-gene as a screen, followed by the RdRp region of ORF1b as a second confirmatory step;¹³⁵ and a two-step RT-PCR analysis using RdRp and N-genes from Abbot that is approved for use on nasopharyngeal and oropharyngeal swabs. These two-step testing regimens can also be supplemented to include control sequences to account for competence or sample degradation.¹²⁶ Self-collected saliva and nasopharyngeal swabs are potentially viable for testing.¹³⁶ All RT-PCR methods have the benefit of testing based on the viral genome, and hence can show that a patient is presented with a viral load at the time of testing. RT-PCR also appears to be able to detect viral loads the earliest out of the traditionally-used methods, and gives the highest accuracy.¹³⁷ Variants

of RT-PCR, e.g., digital PCR, may be more sensitive¹³⁸ than RT-PCR with lower false-positive rates,¹³⁹ although such improvements, are likely not necessary for routine applications. Nevertheless, pooling of samples from different patients for mass testing has been suggested as an effective strategy to reduce workloads and increase efficiency. In this scenario, high accuracy and sensitivity are critically important factors.^{140,141}

Although, deservedly often considered the gold standard, RT-PCR strategies suffer from several issues.¹⁴² Perhaps the greatest issue with RT-PCR is data analysis itself, which can be subjective and requires experience and standardization.¹⁴³ There are various schools of thought to define if the products so derived are due to specific amplification or off target behaviors, and these are often not standardized.¹⁴⁴ Furthermore, the method is complex and prone to experimental-induced artifacts, particularly during collection and processing:¹⁴⁵ several steps are required, RNA extraction and handling steps are a must, specialized equipment is needed, and high technical skill is mandatory. Turnaround times can be long, mandating patient isolation. Mitigating some of these considerations, protocols side-stepping extraction and minimizing screening time are being optimized.¹⁴⁶ Perhaps counterintuitively, the sensitivity of RT-PCR is, in some contexts, reportedly too high, meaning that this assay catches people who are not in the peak of infectivity. This could be an issue since SARS-CoV-2 viral load takes a long time to return to 0, post infection.¹⁴⁷ As with all homology-based amplification strategies, mutation to the priming sequences, or the detection sequences will hamper sensitivity and could lead to false negatives. Thus, it is important to choose loci with relatively low mutagenesis rates and it is preferable to screen several loci at a time. It is also important to note that although CoVs are considered relatively stable, potentially assuaging these worries, there are numerous circulating mutants although overall variation remains small.¹⁴⁸ It is likely that variation in SARS-CoV-2 strains will increase with the introduction of new vaccines and increasing deployment of CoVID-19-targeting drugs.¹⁴⁹

RT-Loop-mediated isothermal amplification (LAMP) screening

LAMP¹⁵⁰ is a multiple primer/multi-homology strategy to perform PCR to generate multi-concatenated amplicons that can be detected by several simple methods (Fig. 3C). This isothermal amplification method has been applied to detection of numerous viruses, including MERS-CoV.¹⁵¹ It is noteworthy that other isothermal amplification techniques (some of which will be discussed below) are also in existence and have been used for viral detection, for instance: recombinase polymerase amplification (RPA);¹⁵² rolling cycle amplification (RCA);¹⁵³ and nucleic acid sequence-based amplification (NASBA).¹⁵⁴ We will discuss LAMP as an exemplar. One key benefit of this method over traditional PCR is that it is an isothermal reaction, and hence can be run in simple setups without the need for a thermocycler. However, the use of isothermal conditions may increase the chance of false positives.^{155,156} Although LAMP requires multiple sequential PCR reactions, it is also reportedly of very high fidelity, low background and very sensitive: down to 6 copies of the target have been successfully detected, even when coupled to RT-PCR in mock blood samples.¹⁵⁷ It is particularly critical to ensure that a strand displacing polymerase be used for LAMP. Given the need for strand displacement, this method works best on short stretches of nucleic acid (<300 bp).¹⁵⁸

RT-LAMP (where RT is coupled to LAMP, either separately, or in the same pot)¹⁵⁷ has proven to be a particularly versatile platform on which to build CoVID-19 testing. Indeed, this procedure is also

believed to be particularly robust to inhibitors of the PCR process, rendering it ideal for field use/one-step procedures.¹⁵⁹ Protocols amplifying the N gene and the S-gene, or portions of ORF1ab have been reported.¹⁶⁰ These and similar methods take less than one hour.¹⁶¹ Several of these have proven to be useful on primary clinical samples. This was also extended to a direct CoVID-19 test (i.e., that did not require prior RNA isolation: this was faster than RT-PCR, although less sensitive).¹⁶² Barcoded LAMP reactions are also possible to allow multiplexing of samples with individual-level specificity.¹⁶³ Obviously, continued development of these processes will enable better evaluation of how RT-LAMP holds up relative to other strategies, but initial reports are encouraging.¹⁶⁴ However, the low technical requirements render this strategy applicable to low technology level areas that are in need of rapid and cheap tests that are simple to run. It should be noted that one issue with such strategies is product contamination across samples, although some approaches have been proposed to minimize this issue.¹⁶⁵

Crispr-Cas-based screening

SHERLOCK screening

Cas-13 enzymes are RNA-guided RNA-cleaving proteins that show an unusual property often referred to as collateral activation (**Fig. 3D**). This means that when a guide RNA is loaded into the Cas13 nuclease and that complex recognizes its target RNA, the activity of Cas13 to cleave RNA non-specifically is increased, leading to non-specific cleavage of nearby sequences (i.e., in a sequence-independent manner). This activity-amplification pathway, which could be linked to a bacterial cell-death pathway.¹⁶⁶ In vitro, this activity-promoting mechanism can be used for signal amplification, leading to sensitive sequence-detection strategies. This method is typically referred to as **SHERLOCK**, and has been applied to numerous virus detection stratagems.¹⁶⁶

The basic steps of **SHERLOCK** involve, RT-PCR of the sequence to be detected; transcription, typically using an engineered T7 site; addition of Cas13 and an RNA sequence for detection and a self-quenched single-stranded RNA probe, that can be cleaved by activated Cas13. Sherlock-based methods manifest attomolar sensitivity.¹⁶⁷ Readouts are made by measuring fluorescence increase, often in relation to positive and negative controls, and are applicable to test strips. Generally speaking, these assays are rapid, accurate, do not require complex equipment (e.g., thermocyclers), and are reliable. This reliability has been extended to the testing kit stage that has been trialed in hospitals. Single step assays have been reported.¹⁶⁸ Several loci within the SARS-CoV-2 genome have been tested and shown to function well; these include: Orf1ab;¹⁶⁹ S-protein;¹⁷⁰ and the N gene.¹⁵⁵ More recently massively multiplexed assays using Cas13 have also arisen.¹⁷¹ A potential drawback of Cas13 methods is that they use RNA-based reporters. Clearly this renders adventitious cleavage and RNAase contamination a worry. The use of standards certainly help assuage these errors.¹⁵⁵

Similar detection stratagems using Cas12 collateral activity have also been reported.^{172, 173} In one instance, called “DETECTR” amplification of cDNA (encoding viral E- and N-genes, and human RNase P (included as a positive control) was performed using RT-LAMP (which has also been used for SHERLOCK).¹⁷⁴ Then a viral cDNA sequence was detected using a specific guide RNA that triggered

cleavage of a self-quenched ssDNA fluorescent probe. Tests were rapid (~1 hour) and showed similar sensitivity to RT-PCR. One pot methods have been shown to work on limited numbers of patient samples with good accuracy.¹⁵⁶ A Cas12a-based approach has also been used to develop a glucose-meter-coupled SARS-CoV-2 assay for simple field use.¹⁷⁵

Obviously, all the above methods require more field testing, larger scale manufacturing, and more robust testing, especially in challenging environments. Initial results are promising for high throughput applications in real-world settings¹⁶⁴ and they promise to be effective and accurate tests, which may come to maturity in the near future and even if not deployed directly against CoVID-19, could be useful in future applications as outlined below. One potential issue is that these tests are accurate down to the single-nucleotide level,¹⁷⁶ which means highly stable loci need to be taken. Developing several detection loci and methods to deal with conflicting results then becomes important.

Antigen testing

Antigen testing has been approved for use in the USA (**Fig. 3B**). This has also been approved as the first over the counter CoVID-19 test in the USA;¹⁷⁷ data are reported to be available in as little as 20 minutes. This test directly detects a SARS-CoV-2 antigen (e.g., S-protein) using immunochromatography. Apparently, production test kit capacity will reach 50 million per month.¹³⁷ The Panbio version of this test has almost 90% accuracy, especially when nasopharyngeal swabs are used. Throat and saliva samples are not as effective.¹⁷⁸

Obviously, this test fares particularly well against others in terms of accessibility (no laboratory is required), rapidity, and affordability (\$5). Such considerations are particularly important variables for deployment in developing countries. The accuracy and threshold of detection, are impressive and offer detection prior to onset of symptoms with good accuracy. However, they are not as accurate/sensitive as PCR-based strategies. Some studies indicate sensitivity of some kits may be prohibitively low.¹⁷⁹ Hence it is critical that users research the specific kit used and evaluate how those data were obtained. Nevertheless, it is still debated if the level of accuracy and sensitivity of RT-PCR is really necessary¹⁴⁷ so opinions on what constitutes “a good test” may change. As antigen testing requires detection of a viral gene product, this strategy is also susceptible to false negatives due to mutation. There is no hard and fast rule on how nucleic acid-based detection strategies versus antibody-based strategies differ in terms of their susceptibility to mutagenesis.

Antibody testing

This test detects whether a patient is producing antibodies to a specific viral protein. Presence of such antibodies are inferred to mean the patient has been exposed to the virus, although some patients do not mount a strong or persistent antibody response to SARS-CoV-2.¹⁸⁰ The antibody response to SARS-CoV is mediated principally IgM¹⁰¹ production (for early stage) and IgG¹⁸¹ production for later/post recovery stages. These can be detected by a range of methods including ELISA, immunofluorescence, and immunochromatographic assays. Numerous tests (probing for IgGs binding to either the N or S-

proteins) have been given approval for use by the FDA;¹⁸² other tests for IgM are available and several tests assay for both IgM and IgG.^{183,184} Although outputs are varied, these tests do seem to be rapid, accurate and sensitive.¹⁸⁵ Unlike RT-PCR and other virus detection methods, the antibody test has the added benefit in that it can indicate if a patient has been exposed to the virus, even if they have recovered. Antibodies have been detected circulating in CoVID-19 patients 8 months after infection.¹⁸⁵ Conversely, for this reason, the antibody test does not inform on whether a patient is infectious at the instance of testing.¹⁸⁶ It further has a relatively long latency (two commercially-available kits show positives 14-15¹⁸⁴ days post onset of symptoms), that could lead to the most infectious periods being missed.

As cases are typically significantly underestimated during outbreaks, the use of antibody testing can be important for population studies to inform on the actual spread of the virus. Such testing must be performed in a window where antibodies are present, although antibody production in patients is quite long lasting.¹⁸⁵ One potential use for antibody testing is to inform a patient on whether they *may* have acquired immunity. However, it is also not fully known how the presence of antibodies due to CoVID-19 infection confers protection against reinfection: both human IgG and IgM bind to important viral proteins including the N and S1 proteins;^{187,101} there is a statistically significant reduction (although not complete protection) in [SARS-CoV-2](#) infection in people who have already [suffered from](#) CoVID-19 up to 6 months post infection.¹⁸⁸ comorbidities may promote reinfection.¹⁸⁹ Nevertheless, second infections are known.¹⁹⁰ Furthermore, there are also no real indication as to whether second infections of [SARS-CoV-2](#) are similar or worse than the first infections or how different mutants are able to evade an existing immune response due to exposure to an earlier strain. It should also be noted that high levels of IgG may indicate poor response to severe CoVID-19,¹⁹¹ whereas asymptomatic patients tend to mount a poor antibody response.¹⁸⁰ Thus there are potential diagnostic uses of the antibody test. This method shows overall low accuracy to different CoV-based human infections (e.g. SARS-CoV and MERS-CoV).

One final note on immunity: the importance of antibodies has somewhat dwarfed the T-cell mediated response.¹⁹² However, T-cell response in patients who had recovered from SARS-CoV almost 20 years ago, was strong against [SARS-CoV-2](#).²⁷ Given the prevalence of CoVs in the broader population, it is likely that T-cell immunity to [SARS-CoV-2](#) exists in fractions of the population through various routes. However, it is not entirely clear how such acquired immunity would affect CoVID-19 patients, and how effective it would be.¹⁹³ Significant careful research needs to be done in this area. Such research is particularly critical for the protection of young exposed to [SARS-CoV-2](#) as we outline later.

Other diagnostic methods

Other methods have been used to detect [SARS-CoV2](#). In many cases, these have been compared to other diagnostic procedures, typically RT-PCR. [New molecular tests are in development, such as development of protein sensors¹⁹⁴ and direct RNA detection strategies,¹⁹⁵ as well as novel methods for product detection, such as through aptamers.¹⁹⁶](#) Screening for temperature, i.e., looking for signs of fever, is a common strategy deployed in shops and other commercial centers. This strategy is clearly

not diagnostic for CoVID-19 and requires that the person be symptomatic at the time of screening. Thus, many people who are contagious will be missed. Other issues with this strategy have been reported, namely that faulty or poorly-designed equipment may be incapable of detecting fever.¹⁹⁷ Molecular noses, and dogs have also been proposed as a means to identify CoVID-19 sufferers, although these seem a long way off deployment.¹⁹⁸ Several nanotechnology-based strategies have also been reported,¹⁹⁹ although again these seem a long way from even entering trials. Chest computed tomography (CCT) procedures were shown to be 97% accurate, 56% specific and 72% accurate by a group from Italy,²⁰⁰ which agrees with previous data from a group from China.²⁰¹ Some studies claim CTC procedures may be more accurate than RT-PCR.²⁰² These studies have also incorporated artificial intelligence to standardize and rigidify protocols.²⁰³ Clearly these methods require specialized equipment and specialists to interpret, and are unlikely to be used as routine screening procedures.

Putting these screening methods into action post CoVID-19

Changing geopolitical profiles and climate variations are causing shifts in numerous animal habitats, including bats (horseshoe bats, are sedentary; disturbing their habitats is unduly stressful). Such stressors, in conjunction with increases in bat population densities, are often causes of viral outbreaks²⁰⁴ and can promote virus mutations in bats, and cause human/bat conflicts. All these factors can elevate the threat of zoonotic events and are potentially relevant to former, current, and future zoonotic outbreaks. Given the unprecedented levels of SARS-CoV-2 in the community, we should further not underestimate the potential risk of spilling back of SARS-CoV-2 into bat and other animal populations,²⁰⁵ which could introduce new complications²⁰⁶/mutations.

Fortunately, similarly to how the ramifications of efforts to respond to MERS-CoV and SARS-CoV facilitated development of vaccines,²⁰⁷ as discussed above, there now exists an arsenal of accurate screening and rapid response tests for CoVID-19. Critically, due to the huge imperative set by CoVID-19, many of these tests function in clinical settings. To meet the demand and market competitiveness, the cost and intricacy of the technology has overall drastically fallen as well. Further consistent with a global disease, local knowledge and production ability of test kits have risen, in many parts of the world.^{208, 209} In other words, we are once again “woke” to the dangers of zoonoses, while at the same time, we are globally in a better position than ever, to screen for CoV infection at point of care, or in the field, even in developing countries. It is vital that this confluence of awareness and technological advancement be harnessed to developing a screening net for understanding the movement and genetic changes of animal CoVs across areas believed to be at high risk of zoonotic events and to monitor their impact on human populations. Clearly such a mechanism needs to incorporate local farmers and laborers both in regular health checks, and in establishing a reporting system for potential dangers. Such a system thus mandates development of sustainable local involvement and education and building of trust between research scientists and people in the front line of animal/human contact, in a large part of Asia and Africa. It further mandates global collaboration between disease research laboratories and hospitals, which is important for future scientific development and pandemic detection. The varied needs and networks currently in place may necessitate several distinct (county/canton or state-localized) efforts in the early years post CoVID-19. However, these efforts should be encouraged to unite as early as possible: critically, this net, and subsequent screening efforts centered around early mapping of potential zoonotic events and characterization of associated strains, must strive to be

externally coordinated, and run by an overseeing committee with rapid and equal access to all data.²² Logistical, legal, and language barriers will all serve to mount huge obstacles to establishing such a system.

It is also critical to note that bats are of huge importance to all countries' economies (billions of dollars to the US economy alone,²¹⁰ and proportionally more to developing nations).²¹¹ Bat Guano is also a common commodity used and traded in Asia, and this is also a source of CoVs.²¹² Thus, developing commitments between farmers and scientists to sustain bat populations in a healthy way are critical to reinitializing agribusiness post CoVID-19. It is certainly true that local bat populations have been targeted in numerous countries throughout the world²¹³ as a result of CoVID19, meaning that screening, education, and improved local involvement/complicity (requiring close collaboration with and coordination by conservationists)²¹⁴ will be even more critical in years to come.

Research into CoVID-19 treatments

In parallel to screening efforts, those who were diagnosed, especially patients showing severe symptoms, needed treatment. This was initially a difficult task, as no drugs were approved to treat CoVID-19 or other SARS-like infections. This led to a huge number of clinical and exploratory investigations that we outline below. In line with the broadening of drug-discovery platforms beyond traditional small-molecule-based therapies, a large number of therapeutic options have been and are being investigated [to treat](#) CoVID-19. Although we will discuss mainly small-molecule-based approaches in detail here, it is noteworthy that several other options have been investigated and may be effective. One of the early treatments was convalescent plasma, i.e., plasma from patients who had had CoVID-19. Such an approach is an established therapy that has been used for over 100 years to treat many infectious diseases,²¹⁵ and was the subject of the first Nobel Prize in Physiology and Medicine.²¹⁶ This approach for CoVID-19 has been met with checkered results,^{217,218} although recent data indicate that high titers and early administration is important for efficacy, especially in older adults.²¹⁹ These observations underscore that context dependence—particularly in terms of timing post infection, but also in terms of symptom presentation—is a crucial factor determining success of trials and treatments.

Clinical (and clinically-orientated) research has focused on evaluating the efficacies of approved drugs against CoVID-19, in a bid to repurpose. We will evaluate this approach further subsequently. Given time constraints facing clinicians, and being met with what appeared to be at some stretches a losing battle, this approach was on the surface logical: it leverages known information on drug mechanism/targets, doses, contraindications and general toxicity to help pinpoint potential clinically-validated molecules. Conversely, reapplication of approved drugs brings several unknowns that were not well-controlled in early tests. These include effective doses, timing of administration, and how side effects can synergize with drug symptoms.

Assessment of clinical reports on potential CoVID-19-treatment options reveals that most data are often far from clear cut. The importance of side effects of administered drugs can also be subject to debate,²²⁰ and how much drug-drug interactions were considered in planning is not always clear.²²¹ Dosing, and other simple factors are often not standardized.²²² This confusion is further hindered

because especially in the early days of CoVID-19, investigations were not conducted with sufficient sample size (patient number) to achieve “statistical significance”.²²² Given the many years spent carefully honing drug development, these loose ends may seem surprising. This is perhaps further disappointing as these investigations were often functioning as Phase-II trials, which routinely take hundreds of people precisely to ensure adequate statistical power. Nevertheless, such controversies are not uncommon,²²³ even in data that have been contested for many years, especially among context-specific diseases. Indeed, efficacies of CoV treatments in general also seem to be somewhat prone to being controversial;²²⁴ in the case of CoVID-19 perhaps even more controversy may be expected given the complex relation of mortality to age, and numerous unknown factors and the variations in pathophysiology as a function of severity, and time post contraction (many of which may not have even been considered as variables in early studies). Coupling the disease complexity with the extreme circumstances under which the CoVID-19 clinical data have been collected, different practices across different countries, clinical standards/routines, and the rapidly-shifting circumstances that occurred during the months from February 2020 to even today, discrepancies were likely. Faced with such realities, interpretations of clinical data could change in the future. Deciding on treatment options requires discussion with an expert to make informed treatment decisions; as of today, there are around 3500 CoVID-19-associated clinical trials either underway or completed.

CoVID-19 treatments

One of the early screens, performed in cell culture models of infection, published in March 2020 identified from a pool of approved drugs remdesivir and chloroquine (both known anti-virals)^{225,226} as potential CoVID-19 therapeutics (**Fig. 4**).²²⁷ Notably, the SARS-CoV/MERS-CoV-active drug, ribiravirin, was very weakly effective in this assay (although it later reemerged as a molecule of interest); nafamostat, a potent MERS-CoV inhibitor¹⁰⁷ (likely through TMPRSS2 inhibition), was also only marginally effective (**Fig. 4**). However, the efficacy of these drugs against SARS-CoV/MERS-CoV was not assessed, making the origins of these failures far from clear. We begin our discussion of clinical data on the hits that came out of this screen.

Targeting transcription: Remdesivir

Remdesivir,²²⁸ an ATP analog, is an inhibitor of viral RdRp, displaying activity against SARS-CoV, MERS-CoV and SARS-CoV-2.²²⁹ It appears to be active in the regimen post viral entry, consistent with this mechanism. Numerous clinical trials have shown that this molecule is well tolerated in humans,²³⁰ although some negative effects were reported.²³¹ A treatment regimen of 200 mg followed by daily 100 mg for up to 9 days is currently considered to be effective at dropping recovery times by around one-third, although survival was not affected.²³² That being said, a study by the World Health Organization has published results to the contrary, with a final conclusion that there was no improvement on mortality, initiation of ventilation and hospital-stay duration.²³³ The reason for these two trials (featuring similar dosing regimens) giving disparate results is unclear.

Notably, remdesavir is a nucleoside analog, like many widely-used approved drugs (e.g., the anticancer drugs chlofarabine and gemcitabine).²³⁴ These molecules require activation to the di/triphosphate in

order to be active and in many instances, monophosphorylation of the nucleoside can be rate limiting. To mitigate these concerns, remdesavir is essentially a protected monophosphate allowing bypassing of [monophosphorylation](#), in a bid to normalize patient response. How prodrug activation differs across patients is less well known. Similar to its dNTP-mimicking cousins such as clofarabine and gemcitabine, remdesivir is also incorporated into the viral genome, leading to genomic-replication stall, and viral genomic stress.²³⁵ The viral protein, NSP14, is protective against such effects for remdesivir and numerous nucleoside analogs sharing a similar mechanism.⁶⁵ It will be interesting to map NSP14 mutations as remdesavir is more widely used. More importantly, the observation that NSP14 is required to protect from remdesavir implies that NSP14 is a good drug target, although no NSP14 inhibitors are approved.²³⁶ Some molecules like ritonavir (below, although these studies are very preliminary)²³⁷ are possible candidates and could be considered for combination therapies. No drug-drug interactions have been reported for remdesivir.²³⁸

Inhibitors of other viral RdRps, e.g., sofosbuvir (another nucleoside-monophosphate prodrug),²³⁹ approved for use against hepatitis-C virus, although only in conjunction with other drugs, have also entered trials.²⁴⁰ Recent reports have indicated that sofosbuvir/daclatasvir may help reduce the number of patients with fatigue 1-month post CoVID-19 infection, although it did not significantly relieve virus-induced early symptoms.²⁴¹

(hydroxy)Chloroquine

Aside from identification from the aforementioned screen, (hydroxy)chloroquine was also evaluated as a potential anti-CoVID-19 drug based off a CoVID-19/human-proteome-interaction screen. This screen showed that the Sigma-2 receptor (part of a family of proteins implicated in viral infection)²⁴² interacts with the CoVID-19 ORF9c. [Knockout](#) of Sigma-2 receptor reduces SARS-CoV-2 replication,²⁴³ indicating that modulation of this protein may help treat CoVID-19 patients. In retrospect, two issues stand in the way of this logic. Firstly, ORF9c has been reported to contain premature stop codons in a subset of viral isolates, arguing that ORF9c may not be a particularly important protein for [SARS-CoV-2 propagation](#) in humans. Secondly, the mode of action of (hydroxy)chloroquine has been linked to effecting receptor activity, although this evidence is weak, as far as we know, relying upon drug combinations and comparisons with other drugs of presumed similar mechanism.²⁴⁴

Early reports²⁴⁵ (often in combination with the antibiotic azithromycin)²⁴⁶ on clinical use of hydroxychloroquine, that were admittedly later questioned,²⁴⁷ were promising. Hydroxychloroquine was given limited emergency use authorization by the FDA in combination with azithromycin, which was subsequently revoked.²⁴⁸ More recently, (hydroxy)chloroquine has been declared ineffective in numerous CoVID-19 trials.²³³ Analysis across numerous publications indicates little beneficial effect of hydroxychloroquine on CoVID-19. The use of hydroxychloroquine with azithromycin may actually increase mortality²⁴⁹ and negative symptoms associated with both drugs.²⁵⁰ Some interest remains in (hydroxy)chloroquine serving as a prophylactic treatment against CoVID-19,²⁵¹ although recent reports are not encouraging.²⁵² Nevertheless, this is one of the most-trialed drugs to treat CoVID-19. As of writing, no other potential CoVID-19 drug is more emblematic of the disagreement among experts (and

politicians) as to what the appropriate therapies of CoVID-19 should be than (hydroxy)chloroquine.²⁵³ There is evidence that gravitation towards certain compounds may have been detrimental to the global response.²⁵³

Targeting inflammation: Glucocorticoids

The logic for the use of dexamethasone and other glucocorticoids (**Fig. 4**), that are now recommended for use in extreme cases of CoVID-19, is the goal of reducing extreme inflammation and ensuing elevated immune response that can be a key cause of CoVID-19 morbidity.²⁵⁴ Although there have been debates about the general utility of corticoid steroids as therapies for CoVID-19,²⁵⁵ dexamethasone at the time of writing, the only treatment approved to lower mortality of CoVID-19.²⁵⁶ Treatments of over 10 days are considered only for the worst cases.²⁵⁷ These results were backed up by several other studies.²⁵⁸

Several issues remain from clinical studies. From a practical standpoint, these include unclear exclusion criteria and the scant information in terms of levels of oxygen support given to patients during these trials. Admittedly, as noted above, these issues have not been unusual in CoVID-19 trials.²⁵⁹ Furthermore, several detractors have voiced their disagreement with dexamethasone deployment and improvements to standard protocols have been suggested.²⁶⁰ It has also been pointed out that glucocorticoids can lead to a pro-coagulatory environment, which could exacerbate the thrombotic aspects of CoVID-19.²⁵⁰ SARS-CoV Viral loads post survival may be more persistent in patients treated with dexamethasone,²⁶¹ meaning that clinicians have a difficult choice to make about implicating dexamethasone as part of the treatment regimen.

Other glucocorticoid steroids have been used to treat CoVID-19. The most common alternative is methylprednisolone, which improved clinical outcome in several studies,²⁶² although not all.²⁶³ The mode of action of methylprednisolone appears to be, at least in part, through reduction of inflammatory response.²⁶⁴ Recently dexamethasone and methylprednisolone were compared head to head. In a relatively small study (100 people in total, split 33% in terms of dexamethasone and 66% to methylprednisolone) there were no differences observed between the twain.²⁶⁵ It should be noted that no control group was included in this study, meaning that it is difficult to assess how effective these drugs were.

Targeting infection and harnessing fortuitous antiviral effects: Antibiotics

As was mentioned above, several antibiotics have been shown to have anti-viral behaviors and have been trialed either alone, or in combination to treat CoVID-19. It should be stressed that although antibiotics are designed to target bacterial infections, and are often cautioned against being used in people with viral infections, this is very much a rule of thumb and it can be ignored upon advice from a suitably-qualified medical professional, [for instance in cases where the antibiotic in question has antiviral activity, or where an adventitious bacterial infection has occurred](#). Aside from azithromycin, the glycopeptide antibiotic teicoplanin, used to treat staphylococcal infections, has been used as a

treatment for CoVID-19,²⁶⁶ and was previously used to treat MERS-CoV.²⁶⁷ The mode of action is believed to occur through inhibition of S-protein cleavage by cathepsin L in late endosomes,²⁶⁸ although this is not believed to be the only route by which S-protein cleavage can occur (**Fig. 2**).¹⁰⁷ Intriguingly, it has been reported that blood stream infections can be elevated in CoVID-19 patients.²⁶⁹ It has been suggested that the use of teicoplanin may assist lowering viral loads and preventing adventitious gram-positive bacterial infections.²⁷⁰ Clearly more work is needed in this area, but in a disease where bacterial infections are a common side effects, dual acting drugs that ideally synergize with point-of-care antivirals is a neat solution that warrants investigation.

Targeting papain-like and 3C-like proteases: lopinavir and natural products

The viral life cycle necessitates cleavage of the gene products of ORF1a and ORF1ab into active component proteins to allow replication. As discussed above (**Fig. 2**), CoVs have two proteases that perform this function, PL^{PRO}, and 3CL^{PRO} (or M^{PRO}). In many viruses, these proteases have unusual canonical cleavage sequences (many are used as bioorthogonal proteases in chemical biology)²⁷¹ and hence are good drug targets. Nevertheless, these are both cysteine proteases (**Fig. 5**). Unlike serine²⁷²/threonine proteases²⁷³ and aspartate proteases,²⁷⁴ cysteine proteases have proven particularly difficult to drug selectively and successfully,²⁷⁵ despite being a deceptively simple class of proteases to target through blanket inhibition strategies.²⁷⁶

Lopinavir, an inhibitor of Type-I HIV aspartate protease, typically combined with the inhibitor of cytochrome p450, ritonavir, to increase lopinavir's half-life, is active against SARS-CoV (**Fig. 4**).²⁷⁷ The mode of action of these molecules against CoVs is unclear. We will use this as example of some of the *potential* errors of logic that can crop up during drug repurposing. Lopinavir appears to be active against MERS-CoV,²⁷⁸ and indeed inhibits purified SARS-CoV 3CL^{PRO}, albeit at high concentrations.²⁷⁹ Such data do not prove that 3CL^{PRO} is the anti-CoVID-19 target of lopinavir, but they are at least indicative that an interaction with lopinavir that can lead to suppression of 3CL^{PRO} activity. Lopinavir's poor inhibitor of 3CL^{PRO} *in vitro* is unsurprising. Aspartate proteases and cysteine proteases are mechanistically dissimilar: the former use aspartates to promote water to directly hydrolyze the scissile peptide bond of the target; the latter use a nucleophilic cysteine to form an enzyme-substrate covalent intermediate which is hydrolyzed by water (**Fig. 5**). Inhibitor scaffolds targeting these two protease families are typically different. Indeed, lopinavir contains a hydroxymethine group that is a stable analog of the peptide hydrolysis transition state facilitating reversible inhibition of aspartate proteases. Such a motif is not ideal for targeting cysteine proteases;²⁸⁰ reactive Michael acceptors, ketones (which form a hemithioacetal as part of the mechanism), or other electrophiles that can harness the nucleophilicity of the reactive cysteine to create a covalent bond to the target are often used. Indeed, a screen for SARS-CoV M^{PRO} inhibitors identified mostly electrophilic compounds as hits from a screening set of 50,000 compounds.²⁸¹ However, molecular dynamics simulations appear to support that lopinavir can bind in the active site of SARS CoV-2 M^{PRO}. *Clearly an off-active site binding mode is also possible.* Although some have found some promise in the reported clinical trial data,²⁸² lopinavir/ritonavir was concluded to have no significant efficacy in hospitalized adults with CoVID-19 by two trials.²⁸³ Further studies may change these conclusions, and should that be the case, it is important that the real target(s) of lopinavir/ritonavir be identified, as such insights could inform on new drug screens in the future.

With the publication of the crystal structure of SARS-CoV-2 PL^{PRO} and 3CL^{PRO},²⁸⁴ more work has been done to identify potential inhibitors of these proteases.²⁸⁵ It is worth noting that in the wake of SARS-CoV, numerous other inhibitors of PL^{PRO} or 3CL^{PRO} were released. Indeed, natural products inhibiting PL^{PRO} have been reported from a host of species, including *P. corylifolia*,²⁸⁶ *P. tomentosa*,²⁸⁷ *T. terrestris*,²⁸⁸ and others. Despite being interesting, and although several trials using traditional Chinese medicines to treat CoVID-19 were initiated, little has come from these outputs so far.²⁸⁹

Other hypotheses

The pathology of CoVID-19 has indicated that acute hyper inflammation and cytokine storm is a critical contributing component in severe CoVID-19 cases; this is queried by some authors as cytokine levels are lower than in patients with sepsis and acute respiratory distress.²⁹⁰ Nevertheless, IL-6 inhibitors are being trialed; several of these also may have inherent antiviral activity.²⁹¹ Several other treatments have been proposed to suppress the principal symptoms of the disease, such as aspirin (shown to have little clinical effect in a study of 183 patients in China,²⁹² although pre-existing aspirin prescription was found to decrease mortality),²⁹³ tocilizumab,²⁹⁴ an immunosuppressive, and colchicine, an anti-inflammatory molecule used to treat gout²⁵⁴ (initial patient data indicate a positive response in mild to severe CoVID-19 cases).²⁹⁵ Oxidative stress is an important contributing factor to serious CoVID-19 cases and there are reports that CoVID-19 infection may suppress cellular antioxidant response.²⁹⁶ In these respects, there has been proposed to be a link between multiple sclerosis (MS) and severe CoVID-19 pathologies.³² One interesting hypothesis to arise from this, albeit slightly tenuous, link is that dimethylfumarate (DMF; [Tecfidera](#)), a drug approved to treat relapsing MS, may be active against CoVID (**Fig. 5**). This is because DMF is a known immunosuppressive drug that also stimulates cellular antioxidant response (likely not as part of its primary mechanism).²⁹⁷ DMF is known to lower infiltrating neutrophil levels²⁹⁸ and impede their function,²⁹⁹ which could also help to suppress hyper inflammation.³⁰⁰

Oxygen/ozone therapy (another treatment known to promote antioxidant response) has also been proposed to help protect against ischemia-reperfusion injury and other pathological manifestations of severe CoVID-19.³⁰¹ Some reports on small cohorts of patients have reported numerical, but not statistically-significant effects of ozone on CoVID-19;³⁰¹ others, with similarly small numbers of people have reported positive effects.³⁰²

A recent study showed numerous pathways rewired upon CoVID-19 infection, including processing mRNA and several metabolic elements, including nucleic acid metabolism pathways. Inhibition of proteins identified in such pathways (albeit often by compounds not in clinical use) appeared to inhibit viral proliferation, although the effects on host cells were not thoroughly investigated. One very interesting observation is that approved inhibitors targeting inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in guanine metabolism and a key immune-suppressive target, could suppress [SARS-CoV-2 proliferation](#) in cell culture models.³⁰³ This observation is clearly worthy of further investigation, and ribavirin, a pleiotropic IMPDH-focused inhibitor with known antiviral properties, has entered clinical trials against CoVID-19.³⁰⁴ Early reports were not encouraging.³⁰⁵ Most recent reports

indicate that ribavirin may affect CoVID-19 through lowering of ACE2 expression,³⁰⁶ i.e., through an off-target effect or collateral damage. Nonetheless, several other IMPDH inhibitors exist,³⁰⁷ and they are at different levels of development (see also below).

An analog of nafamostat,³⁰⁸ camostat, an approved covalent inhibitor of TMPRSS2,¹⁰⁷ which cleaves the S-protein on cell entry, has also been proposed as an anti-CoVID-19 therapeutic. In early trials, admittedly with few participants, camostat decreased CoVID-19 severity; hydroxychloroquine was shown not to have similar effects in the same paper.³⁰⁹ Further trials are ongoing.³¹⁰ Recently, avoralstat, an orphan drug against hereditary angioedema, was proposed to inhibit TMPRSS2. The quoted IC₅₀'s of avoralstat and [camostat](#) for purified TMPRSS2 (presumably derived by fitting to a tight-binding equation) were similar (single-digit nanomolar). Efficacy of the two drugs appears to be similar in mouse models,³¹¹ although these may not be ideal for screening, given that the Axl-mediated import pathway is inactive.

Finally, early in the pandemic, it was proposed that some seemingly-unrelated viruses may share some similarity to CoVID-19 (e.g., 29% homology between the Macro domain of rubella and CoVID-19). Based on this, albeit relatively weak homology, and anticorrelation between mumps IgG titers and CoVID-19-related symptoms, the MMR vaccine was proposed to potentially help protect against CoVID-19.³¹²

Choice of compounds to be evaluated in rapid screening clinical trials

Most studies above have focused on approved drugs, although some included molecules in “preclinical” evaluation. Given the situation, preclinical molecules were unlikely to lead to a timely solution, but, we believe that focusing solely on approved drugs was likely too narrow.²³³ To explain this logic, we breakdown the trials process. Phase-0 (not a mandatory step, but becoming increasingly common in cancer research) is designed to validate that the intended target can be hit or that a blood can enter the blood stream in humans at low dose.³¹³ Phase-I usually focuses on gross tolerance/side effects and pharmacokinetics/pharmacodynamics. These two stages have little interest on drug efficacy, although if present, efficacy is recorded. Although side effects and toxicity etc., continue to be monitored, Phases-II and -III start to home in on the clinical benefits of the drug-candidate, in increasingly large cohorts. These trials are often first conducted vis-à-vis a control group of untreated or placebo patients (Phase-II), and later against approved therapies (Phase-III). Thus, in many cases, passing Phase-I clinical trials tells us a drug-candidate is in principal, safe, at least for acute exposure. Failure beyond this point indicates, in many instances, more about the application of the drug in a specific context, or contexts. In this way, looking at candidates that have entered Phase-II trials (incidentally, where the majority of drugs fail) and above (and even those withdrawn if not due to adverse side effects, or with manageable contraindications)³¹⁴ can help to broaden the scope of screening programs. Such experiments can be performed with knowledge of toxicity and having ideas about, e.g., tolerated doses, pharmacokinetics, in humans.³¹⁵ Such data and resources are highly exploitable.

Drug polypharmacology of effective compounds from clinical trials

It is intriguing that many of the molecules discussed above function through pleiotropic mechanisms. This property is certainly not uncommon in drug discovery. Even supposedly targeted drugs, like [gleevec](#) and Dasatinib, display multiple targets.³¹⁶ Nevertheless, the use of, for instance, repurposed IL-6 inhibitors, antibiotics or immunosuppressive drugs that *also* kill viruses is an appealing idea to use as treatment for a viral disease that is prone to causing pneumonia and immune catastrophies. Perhaps this is a hidden benefit of drug repurposing that deserves further investigation³¹⁷ and *could* stem from the necessary application of suboptimal drugs or just be serendipitous. Nevertheless, this concept could also be potentially incorporated into new drug design.³¹⁷

The pipeline

With an average cost of >1 billion USD and an average time of 12 years from preclinical testing to approval,³¹⁸ and a vaccine in the works, there seems to be little advantage in setting about a CoVID-19 drug *de novo*. However, many questions remain about vaccine efficacy, duration, and the effect that vaccination may have on CoVID-19 mutability, especially if the global vaccination response be uneven. Furthermore, there is the worrying trend of (retro)zoonotic events that have become more widespread and more deadly recently. Furthermore, given the similarity between zoonotic CoVs in general, it is possible that a drug targeting CoVID-19 may be active against future viruses (note: drugs active against much less homologous viruses and bacteria were deployed above). Thus, funding bodies, drug companies, and philanthropic organizations/communities seem to be set to provide sustained support to basic science research into CoVID-19 drugs post the pandemic. To sustain this interest, it is important that such drug studies focus on compounds targeting proteins in SARS-CoV-2 that are also general β -CoV drug targets with relatively low mutagenicity, i.e., targets that are likely to be retained amongst CoVs, rather than being quirks of SARS-CoV-2. Obviously one way to achieve this is to target human proteins necessary for the virus lifecycle,³¹⁹ although as we have seen the same human proteins are not always hijacked by different CoVs and some CoVs may be able to hijack several proteins redundantly. Either way, seeking broad-spectrum anti-CoV-drugs will give a better chance of developing drugs that are useful for the future. We accordingly suggest that future drug screening programs should be broad, focusing on screening several zoonotic CoVs, minimally MERS-CoV, SARS-CoV, and SARS-CoV2, for instance. This work must also gravitate to find medicinally relevant/realistic molecules that can provide results that allow hits to be moved forward quickly into trials. We cast a critical eye over the early fruits of such efforts. Obviously, some of the discussion in the section below is speculative, and [is intended](#) for discussion among researchers versed in the art, and to stimulate their ideas only.

Obviously, it is critical that important targets be identified and screened for inhibitors. Drugging targets shown to be actively inhibited by effective repurposed compounds is one route towards new approved drugs. One alternative approach is to choose proteins or other functions that are not currently drugged but are necessary to the viral lifecycle. NSP14 is a likely candidate, especially, as noted above, its inhibition could synergize with other CoVID-19 drugs.³²⁰ It should be noted that there are differences between NSP14 catalytic behaviors in terms of single- and double-stranded RNA cleavage between even very closely related CoV strains, which could affect inhibitor generality, although that is unknown.³²¹ Screens for inhibitors are already underway.³²²

One other common, and indeed unusual feature of several different viral translation programs is the -1-translation frameshift that occurs to create the ORF1ab gene product. Inhibitors of this process are

also under development, fueled by a cell-based assay for translation slippage. One interesting lead from this screen is merafloxacin, a fluoroquinolone antibiotic. This molecule also shows inhibition of viral proliferation at low micromolar concentrations, albeit only in one cell culture model, which admittedly weakens the impact. Nevertheless, this is a particularly innovative and interesting approach³²³ which should be expanded to more industry-level screens.

Branching the gap between naïve cell-culture models (poor mimics of CoV-mediated diseases) and human trials, especially as the population's immunity increases and case numbers drop will be important. Organoid models could fulfill this role. One study has shown proof of concept of organoid models for CoVID-19 (other SARS-like viruses were not examined) and performed preliminary screening. These models express the (standard) CoVID19-susceptibility markers.³²⁴ One (of several) interesting compounds emerging from an ensuing drug screen was mycophenolic acid (MPA), a non-nucleoside IMPDH inhibitor. In this instance, MPA was proposed to inhibit Furin expression (contrary to data from another IMPDH inhibitor, ribavirin, above). Such varied proposed mechanisms from two different IMPDH-inhibiting drugs is worrying. However, little work has been done to probe even if IMPDH activity is being hit by these compounds, e.g., by examining how exogenous guanosine can suppress viral inhibition. Nevertheless, MPA and ribavirin affect the interaction between NSP14 and IMPDH2 (one of the two isoforms of human IMPDH),³²⁵ offering a potential IMPDH-related common mode of action for both drugs. However, it is unclear to us if such an interaction would even be beneficial for the virus; similar interactions between deoxynucleotide synthesis machinery and proofreading and damage-response enzymes have been proposed for the human replisome,³²⁶ but functional evidence is weak. Of course, NSP14 could affect non-canonical modes of IMPDH or have an unexpected mechanism, which is also worthy of interest.³²⁷ Finally, MPA selectively targets lymphocytes,³²⁸ which could be an issue as lymphopenia is a marker for severe CoVID19 and could contribute to cytokine storm.³²⁹

Nanobodies:

Nanobodies, small, antibody-like molecules that are soluble and stable and can be readily engineered and mass produced, are a growing area of research. Several research efforts have been directed at discovering CoVID-19 neutralizing nanobodies. As the interaction between the nanobody and the virus is likely to be in blood, these studies have focused on blocking the first step of viral infection, through interaction with the S-protein, or blocking viral escape. Several of llama-derived nanobodies (binding to different epitopes) were reported to bind with below 500 nM affinity to the S-protein. A few of these nanobodies bound with picomolar affinity to an inactive (down) conformation of the S-protein at the receptor-binding domain, a region of high conservation believed to be a good target for prophylactics.³³⁰ These nanobodies were shown to be effective in a cell-culture infection assay, although disappointingly no animal model data, or other advanced testing results, were shown. Such experiments are important as the immunogenicity/tolerability of the nanobodies is unknown and cell-based assays have not been great predictors of CoVID-19 treatment efficacies. The report points out that identifying multiple epitopes as targets for nanobodies may help overcome resistance, which is an important consideration. Similar nanobodies were discovered from a yeast display screen.³³¹

Natural products

Natural-product screening has also been undertaken. Natural products are no less easy to get approved as a drug than a man-made one, and oftentimes, efficacies of plant extracts used in traditional

medicines are not necessarily ascribable to a single molecule alone.³³² Nevertheless, despite a recent downturn,³³³ this group of molecules, and those that nature has inspired, makes up around 30% of all approved drugs.³³² One study reported that several cardiac glycosides (e.g. digoxin) exhibited nanomolar inhibition of CoVID-19 infection in a cell-culture model.³³⁴ These are inhibitors of Na⁺/K⁺-ATPase, which is a target of many drugs and natural products, including cardiotonic steroids, some of the most widely used molecules in medicine.³³⁵ These molecules also have anti-inflammatory properties along with several other complex behaviors that render them worth bearing in consideration in screening programs.³³⁶

Other screening efforts have included the use of DNA-encoded libraries;³³⁷ [cyclic peptides](#);³³⁸ [peptide aldehyde inhibitors of M^{PRO} or PL^{PRO}](#),³³⁹ and large-scale fragment screening by combined MS and crystallography. One such screen identified the N-chloroacetyl-N'sulfonamido piperazine or N-chlorocetylaniline motifs as frequent hits.³⁴⁰ It also unveiled a 3-bromopropargyl pharmacophore as an M^{PRO} inhibiting motif. This unit is reminiscent of the propargyl reactive pharmacophore in some deubiquitinating enzyme inhibitors³⁴¹ although it is likely more electrophilic and generally more reactive. Nevertheless, based on this observation (and the fact that SARS-CoV-2 PL^{PRO} can bind to ubiquitin propargylamide),³⁴² propargyl-functionalized peptides that bind with high affinity to the M^{PRO} active site, could be worth investigating as low-reactivity irreversible M^{PRO} inhibitors. Despite opening some leads, this study unfortunately overall provided little in the way of toxicity studies, off-target effects, and focused on warheads that have not traditionally been viewed positively [due to their high reactivity and proclivity to create permanently modified states semi-indiscriminately](#).³⁴³

Using patients to predict therapies

The flip side of the CoVID-19 drug-discovery pipeline examines genetic or expression-level-based causes of pathologies to predict novel drug usage. Such an approach is an extension, or perhaps inversion, of the concept of personalized medicine.³⁴⁴ Of course, these data cannot aid in design of drugs *de novo*, but can aid repurposing, or guide drug-discovery efforts in the future. In some respects, such studies give more contextual data than what could be found from traditional drug-screening data, which are usually conducted in naïve models. We already discussed above how mutations in 3p21.31 leads to sensitization to CoVID-19. Other studies have uncovered other genetic mutations that predispose patients to CoVID-19, such as mutations impinging on Type-1 interferon signaling.³⁴⁵

Recent GWAS studies have elucidated new regions of patient susceptibility linked to severe CoVID-19. These studies were in part enabled by the large number of CoVID-19 cases, and the homogeneity observed amongst the most severe CoVID-19 cases.³⁴⁶ One recent study analyzed data from 1676 individuals (of European descent) from across the UK suffering from severe CoVID-19 and sought to identify genetic signatures leading to susceptibility using GWAS. These individuals were matched to a control cohort of ancestry-matched individuals from the UK Biobank (five controls were examined per test subject, with controls known to have had a positive CoVID-19 test excluded).³⁴⁷ These data identified 15 independent regions with significance at the $p < 5 \times 10^{-8}$ level. Several of the genes in these regions have been implicated in either early or severe CoVID-19. Intriguingly, high expression of interferon receptor subunit IFNAR2 conferred protection against severe CoVID-19. However, exogenous treatment with interferon did not reduce mortality in clinical trials.³⁴⁸ This failure could

reflect incorrect timing of dose, but nonetheless highlights that GWAS, despite being derived from data collected in a medical context, lack the ability to inform on the stage of the disease at which mutations confer predisposition/protection to the disease. This analysis was also backed up by analysis of transcription-wide associations (TWAS), which identified 5 transcripts, including several located around 3p21.31, which was also identified in the GWAS analysis, consistent with previous studies.¹⁰⁴ Such studies should of course incorporate/be incorporated into GWAS from SARS-CoV/MERS-CoV patients to broaden the scope of this research.

A commitment to introspective science reporting and collaborative discussion

When we look back on science coming out of 2020/2021, and likely subsequent years, it will be dominated by [CoVID-19-related](#) research. This is not unsurprising nor without precedent: there was indeed a spike in PL^{PRO} inhibitor design and other aspects of SARS-CoV after the first SARS infection; similar research initiatives have happened in the wake of new drug approvals, as occurred post the approval of taxol.³⁴⁹ What was different during this epidemic, in part because of the confluence of confinement measures, the general fear about CoVID-19 contraction, and the necessary blending of politicians and scientists to combat the disease, was that research actively played out almost entirely in public in real time. Such events are not without precedent,³⁵⁰ but the scale and temporal contraction was on the whole at a new level.³⁵¹ Indeed, swathes of scientific data were released through open-access journals and even group websites that rapidly became news headlines. Of course, such trying times are, in fact, when peer reviewing, correct use of trial design/statistics/planning³⁵² and our faith in the scientific process and the drug approval pipeline should be highest. These lessons should be learned by scientists, and indeed also politicians and journalists alike.³⁵³ Better education of non-scientists in the scientific method and logic may help to limit these issues.

Obviously, with current practices and standards (many of which promote release of research material prior to peer review, possibly to allow scientists to rapidly elaborate on data),³⁵⁴ it is difficult perhaps even impossible to prevent release of unreviewed work globally. However, the onus is on all people involved in scientific research (including doctors, field scientists, laboratory researchers etc), to solicit the opinion of our peers prior to releasing information. Such checkpoints are critical because in situations such as a pandemic, the traditional scientific validation process may also lag behind the public's desire to act on published data. Certainly, scientists must be (re)trained to be more self-critical, and as reviewers and mentors we must encourage open criticism amongst our mentees *and* papers we review. In such a way, non-experts reading preprints etc can have more context. One additional means to promote more introspective practices could be by establishing tangible ties (e.g. *scheduled* regular joint seminars, prereview meetings prior to major publications, and sharing common databases) between different departments, across different regions and countries in core response units (e.g. infectious diseases; bioweapons; emergency medicine). These links could in turn be used to orchestrate and standardize point of care responses, treatments, and trials in response to global or more widespread situations, more effectively than ad hoc initiatives, while being more flexible than larger governmental schemes. Such networks could be triggered early in emergency situations to ensure coordination of research etc. We see that such multilateral collaborations are emerging, and further think that the ties so formed must be maintained and broadened.³⁵⁵ However, it is noteworthy that

construction of so-called Fangcang hospitals in China, required a collaboration between a huge number of disciplines. Our considerations of what scientific collaboration means thus needs to be reconsidered.⁴⁵

CoVID-19 targeting protection strategies?

In terms of mortality, CoVID-19 is a disease that targets the elderly highly selectively (89% of deaths in the over 65 age group). This trend holds up more or less independent of R value, geography,³⁵⁶ and other factors.³⁵⁷ In other words, for the vast majority of global working age people, little danger was posed from CoVID-19. Although established early in our understanding of the virus, this observation appears to have curiously been leveraged little in terms of guiding government or state responses. Although a strategy targeted towards shielding the elderly was advocated by a series of academics in the UK,³⁵⁸ it was described as age-based apartheid by one National Health Service official.³⁵⁹ It is true that the elderly are significantly prone to mental and physical issues associated with extreme isolation,³⁶⁰ a situation referred to as the CoVID-19 Connectivity Paradox.³⁶¹

Age-related mortality is very common amongst respiratory diseases, often with large swathes of the populous seemingly untouched by the disease. Hence such planning likely needs to be seriously considered in the future. We appreciate that the following discussion is weighted to a more Western perspective. However, the considerations could readily apply, in some respects, to all countries. High mortality in the elderly is common in many respiratory diseases. This means that we need to consider foundations for future confinement protocols (which are likely to remain the bulk of response to epidemics and pandemics) by providing resources for the elderly. These could include promoting education into the use of the internet, including government incentives to promote elderly people to go online (and use internet resources safely), as well as promoting engagement in remote access to clubs, religious/social meetings, and the like for the elderly. Contrary to CoVID-19, many respiratory diseases show substantial child mortality, with young to middle aged adults being spared.³⁶² Thus, provision certainly needs to be made for the protection of children from such pandemics as well; this could include ensuring that all children continue to have mandatory access to remote learning post the pandemic, and implementing remote learning as a necessary part of the curriculum.

On the other hand, The Spanish Flu of 1918 exhibited a trefoil or so-called w-shaped mortality. The three principal peaks of mortality centered on the young, the very old *and those around 28 years of age*. The precise reasons for the susceptibility of young adults are not absolutely clear, but it is critical to appreciate that should such a profile have been displayed by CoVID-19, it would have likely pushed the impact of coronavirus on medical and other response teams way beyond breaking point.³⁶³ This would have been particularly acute in more developing countries where the population is younger than in the Western world. One growing hypothesis for the unusual susceptibility of young adults to the Spanish Flu is original antigenic sin. In some respects this hypothesis is like vaccination, only its effect is a misguided immune response, leading to a weakening to exposure to a pathogen: once young people who had been affected by Russian Flu (another global virus that infected many people around 28 years prior to Spanish Flu) at a particular stage early in their lives, were exposed to the Spanish Flu they

created a large number of T-cells targeted to Russian Flu and whose efficacy against Spanish Flu was low. This patterned response allowed the Spanish Flu to overwhelm these conditioned subjects, promoting their death. This is not an unusual behavior to different virus strains.³⁶⁴ Given the severity and huge scale of the current pandemic, these considerations should be born in mind in planning: the CoVID-19 pandemic should not become the “Russian Flu”. It should be noted that children can show positive tests for CoVID-19³⁶⁵ and appear to be able to mount a strong immune response to CoVID-19³⁶⁶ and that an outbreak of a CoV-related disease has arisen 3 times in the past 20 years. We encourage the readers to consider how department and governmental policies may have differed as a function of these different morbidity profiles, if at all.

Epilog

CoVID-19 was and continues to be a huge challenge. The huge research effort across a huge number of disciplines, as witnessed by this review, and many others, shows the scientific community’s great positive impact on this disease. Armed with this research, and lessons we can learn from the way the community responded, we should be better prepared for future issues.

Figure 1. Some of the most common symptoms of CoVID-19, grouped by typical, i.e. less severe (on left) and critical (on right). For typical symptoms parenthesis indicates percentages in one reported

study of >24000 people;³⁶⁷ bolding indicates that these are associated with more severe CoVID19, if symptoms are particularly severe. Severe symptoms taken from several sources.^{368,369}

Figure 2. A brief guided tour of CoV biology. (A) SARS-CoV2 genome and proteolytic processing. In the genome, light blue denotes ORF1ab; green denotes structural proteins; red ancillary proteins. Shading denotes conservation; dotted borders indicates variants lacking this gene are known (truncations in ORF7b are known, but not shown here)³³¹. In the protein, Pp1a and pp1ab denotes different gene products derived from ORF1ab. Height of boxes denotes translation rate. Note NSP11 is only 13 amino acids in length; other NSPs range from 180 amino acids (NSP1) to 1945 amino acids (NSP3).³⁷⁰ For NSPs, red boxes indicate proteases; Green down arrow indicates a step catalyzed by PL^{PRO}; purple downwards arrow indicates cleavage by 3CL^{PRO} (faded arrows indicate not necessary for replication in some CoVs, but can lead to attenuation).³⁷¹ Red line indicates genomic positions. (B) Genomes of SARS-CoV and MERS-CoV for comparison. (C) SARS-CoV-2 infection occurs when the S-protein binds to an acceptor protein on the cell surface. This protein is believed to be ACE-2 or AXL (except in mice). Upon formation of the complex, the S-protein is cleaved by several different proteases (Furin and TMPRSS2 or

cathepsins L or B). Other proteins, such as neuropilin-1 are required for viral entry.³⁷² Upon cleavage the activated S-protein can orchestrate fusion with the plasma membrane and delivery of the genome to the cell.

Figure 3. Detection strategies for CoVID-19. A. qRT-PCR, arguably the gold standard. B. Antigen testing, a rapid and cheap alternative, that is less sensitive than rt-RT-PCR. C. RT-LAMP, a modern method using multiple primers that generates concatenated products. D. **SHERLOCK**, another modern method using Cas enzymes (Cas13 is shown here) that gain activity dependent on a specific interaction with an amplicon derived from the viral genome. All methods could be harnessed for new screening technologies against zoonotic diseases; only some are apposite for deployment in at risk areas.

Figure 4. Structures and functions of compounds discussed above, highlighting bioactivities and potential behaviors.

Figure 5. Top panel discusses similarities between aspartate and cysteine proteases. Below, other compounds discussed and their possible functions. Note in **camostat**, the red moiety becomes bound to the target protease (see red mechanistic arrow), and this moiety is identical to that in **nafamostat** (above).

1. WHO | Update 49 - SARS case fatality ratio, incubation period. *WHO*
https://www.who.int/csr/sarsarchive/2003_05_07a/en/.
2. Wu, S. L. *et al.* Substantial underestimation of SARS-CoV-2 infection in the United States. *Nature Communications* **11**, 4507 (2020).
3. Plague. <https://www.who.int/news-room/fact-sheets/detail/plague>.
4. Woo, P. C. Y., Lau, S. K. P., Huang, Y. & Yuen, K.-Y. Coronavirus Diversity, Phylogeny and Interspecies Jumping. *Exp Biol Med (Maywood)* **234**, 1117–1127 (2009).
5. Afonso, E. *et al.* Is the lesser horseshoe bat (*Rhinolophus hipposideros*) exposed to causes that may have contributed to its decline? A non-invasive approach. *Global Ecology and Conservation* **8**, 123–137 (2016).
6. Kupferschmidt Oct. 30, K., 2013 & Pm, 2:15. Bats May Be Carrying the Next SARS Pandemic. *Science / AAAS* <https://www.sciencemag.org/news/2013/10/bats-may-be-carrying-next-sars-pandemic> (2013).
7. Lin, X.-D. *et al.* Extensive diversity of coronaviruses in bats from China. *Virology* **507**, 1–10 (2017).
8. Valitutto, M. T. *et al.* Detection of novel coronaviruses in bats in Myanmar. *PLOS ONE* **15**, e0230802 (2020).
9. Nziza, J. *et al.* Coronaviruses Detected in Bats in Close Contact with Humans in Rwanda. *Ecohealth* **17**, 152–159 (2020).
10. Qiu, J. How China's 'Bat Woman' Hunted Down Viruses from SARS to the New Coronavirus. *Scientific American* <https://www.scientificamerican.com/article/how-chinas-bat-woman-hunted-down-viruses-from-sars-to-the-new-coronavirus1/>
doi:10.1038/scientificamerican0620-24.
11. Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C. & Garry, R. F. The proximal origin of SARS-CoV-2. *Nature Medicine* **26**, 450–452 (2020).

12. Yang, W. *et al.* Estimating the infection-fatality risk of SARS-CoV-2 in New York City during the spring 2020 pandemic wave: a model-based analysis. *The Lancet Infectious Diseases* **0**, (2020).
13. Johnson, N. P. A. S. & Mueller, J. Updating the accounts: global mortality of the 1918-1920 'Spanish' influenza pandemic. *Bull Hist Med* **76**, 105–115 (2002).
14. Kolata, G. Coronavirus Is Very Different From the Spanish Flu of 1918. Here's How. *The New York Times* (2020).
15. Di Domenico, L., Pullano, G., Sabbatini, C. E., Boëlle, P.-Y. & Colizza, V. Impact of lockdown on COVID-19 epidemic in Île-de-France and possible exit strategies. *BMC Medicine* **18**, 240 (2020).
16. Barber, A. *et al.* The basic reproduction number of SARS-CoV-2: a scoping review of available evidence. *medRxiv* 2020.07.28.20163535 (2020) doi:10.1101/2020.07.28.20163535.
17. Billah, M. A., Miah, M. M. & Khan, M. N. Reproductive number of coronavirus: A systematic review and meta-analysis based on global level evidence. *PLOS ONE* **15**, e0242128 (2020).
18. Liu, J. *et al.* A comparative overview of COVID-19, MERS and SARS: Review article. *Int J Surg* **81**, 1–8 (2020).
19. Choi, S., Jung, E., Choi, B. Y., Hur, Y. J. & Ki, M. High reproduction number of Middle East respiratory syndrome coronavirus in nosocomial outbreaks: mathematical modelling in Saudi Arabia and South Korea. *J Hosp Infect* **99**, 162–168 (2018).
20. Liu, Y., Gayle, A. A., Wilder-Smith, A. & Rocklöv, J. The reproductive number of COVID-19 is higher compared to SARS coronavirus. *Journal of Travel Medicine* **27**, (2020).
21. Petersen, E. *et al.* Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics. *The Lancet Infectious Diseases* **20**, e238–e244 (2020).
22. Gates, B. Responding to Covid-19 — A Once-in-a-Century Pandemic? *New England Journal of Medicine* **382**, 1677–1679 (2020).
23. Cevik, M. *et al.* SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *The Lancet Microbe* **2**, e13–e22 (2021).

24. Nogrady, B. What the data say about asymptomatic COVID infections. *Nature* **587**, 534–535 (2020).
25. Gandhi, M., Yokoe, D. S. & Havlir, D. V. Asymptomatic Transmission, the Achilles' Heel of Current Strategies to Control Covid-19. *New England Journal of Medicine* (2020) doi:10.1056/NEJMe2009758.
26. Pollock, A. M. & Lancaster, J. Asymptomatic transmission of covid-19. *BMJ* **371**, m4851 (2020).
27. Le Bert, N. *et al.* SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature* **584**, 457–462 (2020).
28. Millar, J. E. *et al.* Robust, reproducible clinical patterns in hospitalised patients with COVID-19. *medRxiv* 2020.08.14.20168088 (2020) doi:10.1101/2020.08.14.20168088.
29. Xu, Z. *et al.* Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet Respiratory Medicine* **8**, 420–422 (2020).
30. Ruan, Q., Yang, K., Wang, W., Jiang, L. & Song, J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* **46**, 846–848 (2020).
31. Jacobs, W. *et al.* Fatal lymphocytic cardiac damage in coronavirus disease 2019 (COVID-19): autopsy reveals a ferroptosis signature. *ESC Heart Failure* **7**, 3772–3781 (2020).
32. Timpani, C. A. & Rybalka, E. Calming the (Cytokine) Storm: Dimethyl Fumarate as a Therapeutic Candidate for COVID-19. *Pharmaceuticals* **14**, 15 (2021).
33. Immunologic perturbations in severe COVID-19/SARS-CoV-2 infection | bioRxiv. <https://www.biorxiv.org/content/10.1101/2020.05.18.101717v1>.
34. Ghebrehiwet, B. & Peerschke, E. I. Complement and coagulation: key triggers of COVID-19–induced multiorgan pathology. *J Clin Invest* **130**, 5674–5676 (2020).
35. Wood, H. New insights into the neurological effects of COVID-19. *Nature Reviews Neurology* **16**, 403–403 (2020).

36. Chary, M. A., Overbeek, D. L., Papadimoulis, A., Sheroff, A. & Burns, M. M. Geospatial correlation between COVID-19 health misinformation and poisoning with household cleaners in the Greater Boston Area. *Clinical Toxicology* **0**, 1–6 (2020).
37. China Has Built Over 20 Mass Quarantine Centers For Coronavirus Patients In Wuhan. *NPR.org*
<https://www.npr.org/2020/02/24/808995258/china-has-built-over-20-mass-quarantine-centers-for-coronavirus-patients-in-wuha>.
38. ‘Liberation’ as Wuhan’s coronavirus lockdown ends after 76 days. *the Guardian*
<http://www.theguardian.com/world/2020/apr/07/liberation-as-wuhans-coronavirus-lockdown-ends-after-76-days> (2020).
39. Sandford, A. Coronavirus: Half of humanity on lockdown in 90 countries. *euronews*
<https://www.euronews.com/2020/04/02/coronavirus-in-europe-spain-s-death-toll-hits-10-000-after-record-950-new-deaths-in-24-hou> (2020).
40. The Lancet. India under COVID-19 lockdown. *The Lancet* **395**, 1315 (2020).
41. Haider, N. *et al.* Lockdown measures in response to COVID-19 in nine sub-Saharan African countries. *BMJ Global Health* **5**, e003319 (2020).
42. Hallal, P. C. SOS Brazil: science under attack. *The Lancet* **397**, 373–374 (2021).
43. Leung, K., Shum, M. H., Leung, G. M., Lam, T. T. & Wu, J. T. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Eurosurveillance* **26**, 2002106 (2021).
44. Tang, J. W., Toovey, O. T. R., Harvey, K. N. & Hui, D. D. S. Introduction of the South African SARS-CoV-2 variant 501Y.V2 into the UK. *Journal of Infection* **0**, (2021).
45. Wang, K.-W. *et al.* Fangcang shelter hospitals are a One Health approach for responding to the COVID-19 outbreak in Wuhan, China. *One Health* **10**, 100167 (2020).
46. Burki, T. China’s successful control of COVID-19. *The Lancet Infectious Diseases* **20**, 1240–1241 (2020).

47. Angelerio, Q. T. *Quincti Tyberii Angelerii Ectypa pestilentis status Algeriae Sardiniae ...* (typis haeredum ... D.D. Nicolai Canelles, 1588).
48. Mackowiak, P. A. & Sehdev, P. S. The Origin of Quarantine. *Clinical Infectious Diseases* **35**, 1071–1072 (2002).
49. Subba, D. R. COLLECTIVE INTELLIGENCE AND INTERNATIONAL COORDINATION: ANTIDOTE FOR THE NOVEL BIOLOGICAL ZERO-DAY EXPLOIT #COVID-19. 4.
50. Da'ar, O. B., Haji, M. & Jradi, H. Coronavirus Disease 2019 (COVID-19): Potential implications for weak health systems and conflict zones in the Middle East and North Africa region. *The International Journal of Health Planning and Management* **35**, 1240–1245 (2020).
51. Tilli, M. *et al.* Neglected tropical diseases in non-endemic countries in the era of COVID-19 pandemic: the great forgotten. *Journal of Travel Medicine* **28**, (2021).
52. Al-Quteimat, O. M. & Amer, A. M. The Impact of the COVID-19 Pandemic on Cancer Patients. *Am J Clin Oncol* **43**, 452–455 (2020).
53. Amendola, S., Spensieri, V., Hengartner, M. P. & Cerutti, R. Mental health of Italian adults during COVID-19 pandemic. *British Journal of Health Psychology* **n/a**.
54. Xie, X. *et al.* Engineering SARS-CoV-2 using a reverse genetic system. *Nature Protocols* 1–24 (2021) doi:10.1038/s41596-021-00491-8.
55. Chiara, M. *et al.* Next generation sequencing of SARS-CoV-2 genomes: challenges, applications and opportunities. *Briefings in Bioinformatics* (2020) doi:10.1093/bib/bbaa297.
56. Tárnok, A. Machine Learning, COVID-19 (2019-nCoV), and multi-OMICS. *Cytometry Part A* **97**, 215–216 (2020).
57. Lai, M. M., Liao, C. L., Lin, Y. J. & Zhang, X. Coronavirus: how a large RNA viral genome is replicated and transcribed. *Infect Agents Dis* **3**, 98–105 (1994).
58. Jaimes, J. A., André, N. M., Chappie, J. S., Millet, J. K. & Whittaker, G. R. Phylogenetic Analysis and Structural Modeling of SARS-CoV-2 Spike Protein Reveals an Evolutionary Distinct and Proteolytically Sensitive Activation Loop. *Journal of Molecular Biology* **432**, 3309–3325 (2020).

59. Lu, R. *et al.* Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *The Lancet* **395**, 565–574 (2020).
60. Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273 (2020).
61. Wang, Y. *et al.* Human SARS-CoV-2 has evolved to reduce CG dinucleotide in its open reading frames. *Scientific Reports* **10**, 12331 (2020).
62. Ricagno, S. *et al.* Crystal structure and mechanistic determinants of SARS coronavirus nonstructural protein 15 define an endoribonuclease family. *PNAS* **103**, 11892–11897 (2006).
63. Minskaia, E. *et al.* Discovery of an RNA virus 3'→5' exoribonuclease that is critically involved in coronavirus RNA synthesis. *PNAS* **103**, 5108–5113 (2006).
64. Chen, Y. *et al.* Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel cap N7 methyltransferase. *PNAS* **106**, 3484–3489 (2009).
65. Ferron, F. *et al.* Structural and molecular basis of mismatch correction and ribavirin excision from coronavirus RNA. *PNAS* **115**, E162–E171 (2018).
66. Bouvet, M. *et al.* In Vitro Reconstitution of SARS-Coronavirus mRNA Cap Methylation. *PLOS Pathogens* **6**, e1000863 (2010).
67. Snijder, E. J. *et al.* Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J Mol Biol* **331**, 991–1004 (2003).
68. Angeletti, S. *et al.* COVID-2019: The role of the nsp2 and nsp3 in its pathogenesis. *Journal of Medical Virology* **92**, 584–588 (2020).
69. Smith, E. C., Blanc, H., Vignuzzi, M. & Denison, M. R. Coronaviruses Lacking Exoribonuclease Activity Are Susceptible to Lethal Mutagenesis: Evidence for Proofreading and Potential Therapeutics. *PLOS Pathogens* **9**, e1003565 (2013).
70. Angeletti, S. *et al.* COVID-2019: The role of the nsp2 and nsp3 in its pathogenesis. *Journal of Medical Virology* **92**, 584–588 (2020).

71. Preliminary genomic characterisation of an emergent SARS-CoV-2 lineage in the UK defined by a novel set of spike mutations - SARS-CoV-2 coronavirus / nCoV-2019 Genomic Epidemiology. *Virological* <https://virological.org/t/preliminary-genomic-characterisation-of-an-emergent-sars-cov-2-lineage-in-the-uk-defined-by-a-novel-set-of-spike-mutations/563> (2020).
72. Starr, T. N. *et al.* Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **182**, 1295-1310.e20 (2020).
73. Freundt, E. C. *et al.* The open reading frame 3a protein of severe acute respiratory syndrome-associated coronavirus promotes membrane rearrangement and cell death. *J Virol* **84**, 1097–1109 (2010).
74. Firth, A. E. A putative new SARS-CoV protein, 3c, encoded in an ORF overlapping ORF3a. *Journal of General Virology*, **101**, 1085–1089 (2020).
75. Miorin, L. *et al.* SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. *PNAS* **117**, 28344–28354 (2020).
76. Activation and evasion of type I interferon responses by SARS-CoV-2 | Nature Communications. <https://www.nature.com/articles/s41467-020-17665-9>.
77. Cagliani, R., Forni, D., Clerici, M. & Sironi, M. Coding potential and sequence conservation of SARS-CoV-2 and related animal viruses. *Infect Genet Evol* **83**, 104353 (2020).
78. Nizamudeen, Z. A. *et al.* Structural assessment of SARS-CoV2 accessory protein ORF7a predicts LFA-1 and Mac-1 binding potential. *Biosci Rep* **41**, (2021).
79. Xia, H. *et al.* Evasion of Type I Interferon by SARS-CoV-2. *Cell Reports* **33**, 108234 (2020).
80. Schaecher, S. R., Mackenzie, J. M. & Pekosz, A. The ORF7b Protein of Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Is Expressed in Virus-Infected Cells and Incorporated into SARS-CoV Particles. *Journal of Virology* **81**, 718–731 (2007).
81. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study - The Lancet. [https://www.thelancet.com/article/S0140-6736\(20\)31757-8/fulltext](https://www.thelancet.com/article/S0140-6736(20)31757-8/fulltext).

82. Pereira, F. Evolutionary dynamics of the SARS-CoV-2 ORF8 accessory gene. *Infect Genet Evol* **85**, 104525 (2020).
83. Su, Y. C. F. *et al.* Discovery and Genomic Characterization of a 382-Nucleotide Deletion in ORF7b and ORF8 during the Early Evolution of SARS-CoV-2. *mBio* **11**, (2020).
84. Meier, C. *et al.* The Crystal Structure of ORF-9b, a Lipid Binding Protein from the SARS Coronavirus. *Structure* **14**, 1157–1165 (2006).
85. Shi, C.-S. *et al.* SARS-Coronavirus Open Reading Frame-9b Suppresses Innate Immunity by Targeting Mitochondria and the MAVS/TRAF3/TRAF6 Signalosome. *The Journal of Immunology* **193**, 3080–3089 (2014).
86. Andres, A. D. *et al.* SARS-CoV-2 ORF9c Is a Membrane-Associated Protein that Suppresses Antiviral Responses in Cells. *bioRxiv* 2020.08.18.256776 (2020) doi:10.1101/2020.08.18.256776.
87. Lam, J.-Y. *et al.* Loss of orf3b in the circulating SARS-CoV-2 strains. *Emerging Microbes & Infections* **9**, 2685–2696 (2020).
88. Pancer, K. *et al.* The SARS-CoV-2 ORF10 is not essential in vitro or in vivo in humans. *PLOS Pathogens* **16**, e1008959 (2020).
89. Shi, C.-S., Nabar, N. R., Huang, N.-N. & Kehrl, J. H. SARS-Coronavirus Open Reading Frame-8b triggers intracellular stress pathways and activates NLRP3 inflammasomes. *Cell Death Discovery* **5**, 1–12 (2019).
90. Severe acute respiratory syndrome Coronavirus ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC - Siu - 2019 - The FASEB Journal - Wiley Online Library.
<https://faseb.onlinelibrary.wiley.com/doi/10.1096/fj.201802418R>.
91. Castaño-Rodríguez, C. *et al.* Role of Severe Acute Respiratory Syndrome Coronavirus Viroporins E, 3a, and 8a in Replication and Pathogenesis. *mBio* **9**, (2018).
92. Michel, C. J., Mayer, C., Poch, O. & Thompson, J. D. Characterization of accessory genes in coronavirus genomes. *Virology Journal* **17**, 131 (2020).

93. Konno, Y. SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist Whose Activity Is Increased by a Naturally Occurring Elongation Variant. *OPEN ACCESS* 21.
94. Nelson, C. W. *et al.* Dynamically evolving novel overlapping gene as a factor in the SARS-CoV-2 pandemic. *eLife* **9**, e59633 (2020).
95. Identification of eight SARS-CoV-2 ORF7a deletion variants in 2,726 clinical specimens | bioRxiv. <https://www.biorxiv.org/content/10.1101/2020.12.10.418855v1>.
96. Taylor, J. K. *et al.* Severe Acute Respiratory Syndrome Coronavirus ORF7a Inhibits Bone Marrow Stromal Antigen 2 Virion Tethering through a Novel Mechanism of Glycosylation Interference. *Journal of Virology* **89**, 11820–11833 (2015).
97. Flower, T. G. *et al.* Structure of SARS-CoV-2 ORF8, a rapidly evolving immune evasion protein. *PNAS* **118**, (2021).
98. Li, J.-Y. *et al.* The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Research* **286**, 198074 (2020).
99. Zhang, Y. *et al.* The ORF8 Protein of SARS-CoV-2 Mediates Immune Evasion through Potently Downregulating MHC-I. *bioRxiv* 2020.05.24.111823 (2020) doi:10.1101/2020.05.24.111823.
100. Jiang, H. *et al.* SARS-CoV-2 Orf9b suppresses type I interferon responses by targeting TOM70. *Cellular & Molecular Immunology* **17**, 998–1000 (2020).
101. Jiang, H. *et al.* SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses. *Nature Communications* **11**, 3581 (2020).
102. V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology* 1–16 (2020) doi:10.1038/s41579-020-00468-6.
103. Zahradník, J. *et al.* SARS-CoV-2 RBD in vitro evolution follows contagious mutation spread, yet generates an able infection inhibitor. *bioRxiv* 2021.01.06.425392 (2021) doi:10.1101/2021.01.06.425392.

104. Genomewide Association Study of Severe Covid-19 with Respiratory Failure. *New England Journal of Medicine* **383**, 1522–1534 (2020).
105. The COVID-19 Host Genetics Initiative. The COVID-19 Host Genetics Initiative, a global initiative to elucidate the role of host genetic factors in susceptibility and severity of the SARS-CoV-2 virus pandemic. *European Journal of Human Genetics* **28**, 715–718 (2020).
106. Wang, S. *et al.* AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. *Cell Research* 1–15 (2021) doi:10.1038/s41422-020-00460-y.
107. Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271-280.e8 (2020).
108. Menachery, V. D. *et al.* Trypsin Treatment Unlocks Barrier for Zoonotic Bat Coronavirus Infection. *Journal of Virology* **94**, (2020).
109. Walls, A. C. *et al.* Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **181**, 281-292.e6 (2020).
110. Meng, T. *et al.* The insert sequence in SARS-CoV-2 enhances spike protein cleavage by TMPRSS. *bioRxiv* 2020.02.08.926006 (2020) doi:10.1101/2020.02.08.926006.
111. Jin, X. *et al.* Virus strain from a mild COVID-19 patient in Hangzhou represents a new trend in SARS-CoV-2 evolution potentially related to Furin cleavage site. *Emerging Microbes & Infections* **9**, 1474–1488 (2020).
112. Farrera-Soler, L. *et al.* Identification of immunodominant linear epitopes from SARS-CoV-2 patient plasma. *PLOS ONE* **15**, e0238089 (2020).
113. Finkel, Y. *et al.* The coding capacity of SARS-CoV-2. *Nature* **589**, 125–130 (2021).
114. Grou, C. P., Pinto, M. P., Mendes, A. V., Domingues, P. & Azevedo, J. E. The de novo synthesis of ubiquitin: identification of deubiquitinases acting on ubiquitin precursors. *Scientific Reports* **5**, 12836 (2015).

115. V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology* 1–16 (2020) doi:10.1038/s41579-020-00468-6.
116. Moustaqil, M. *et al.* SARS-CoV-2 proteases cleave IRF3 and critical modulators of inflammatory pathways (NLRP12 and TAB1): implications for disease presentation across species and the search for reservoir hosts. *bioRxiv* 2020.06.05.135699 (2020) doi:10.1101/2020.06.05.135699.
117. Sawicki, S. G. Coronavirus Genome Replication. in *Viral Genome Replication* (eds. Raney, K. D., Gotte, M. & Cameron, C. E.) 25–39 (Springer US, 2009). doi:10.1007/b135974_2.
118. Ghosh, S. *et al.* β -Coronaviruses Use Lysosomes for Egress Instead of the Biosynthetic Secretory Pathway. *Cell* **183**, 1520-1535.e14 (2020).
119. He, X. *et al.* Author Correction: Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine* **26**, 1491–1493 (2020).
120. Orooji, Y. *et al.* An Overview on SARS-CoV-2 (COVID-19) and Other Human Coronaviruses and Their Detection Capability via Amplification Assay, Chemical Sensing, Biosensing, Immunosensing, and Clinical Assays. *Nano-Micro Lett.* **13**, 18 (2020).
121. Han, E. *et al.* Lessons learnt from easing COVID-19 restrictions: an analysis of countries and regions in Asia Pacific and Europe. *The Lancet* **396**, 1525–1534 (2020).
122. Güemes-Villahoz, N. *et al.* Detecting SARS-CoV-2 RNA in conjunctival secretions: Is it a valuable diagnostic method of COVID-19? *Journal of Medical Virology* **93**, 383–388 (2021).
123. Leung, E. C., Chow, V. C., Lee, M. K. & Lai, R. W. Deep throat saliva as an alternative diagnostic specimen type for the detection of SARS-CoV-2. *Journal of Medical Virology* **93**, 533–536 (2021).
124. Frazee, B. W. *et al.* Accuracy and Discomfort of Different Types of Intranasal Specimen Collection Methods for Molecular Influenza Testing in Emergency Department Patients. *Annals of Emergency Medicine* **71**, 509-517.e1 (2018).

125. Jayamohan, H. *et al.* SARS-CoV-2 pandemic: a review of molecular diagnostic tools including sample collection and commercial response with associated advantages and limitations. *Anal Bioanal Chem* 1–23 (2020) doi:10.1007/s00216-020-02958-1.
126. Vandenberg, O., Martiny, D., Rochas, O., van Belkum, A. & Kozlakidis, Z. Considerations for diagnostic COVID-19 tests. *Nature Reviews Microbiology* 1–13 (2020) doi:10.1038/s41579-020-00461-z.
127. Paltiel, A. D., Zheng, A. & Walensky, R. P. Assessment of SARS-CoV-2 Screening Strategies to Permit the Safe Reopening of College Campuses in the United States. *JAMA Netw Open* **3**, e2016818 (2020).
128. Ogawa, T. *et al.* Another false-positive problem for a SARS-CoV-2 antigen test in Japan. *Journal of Clinical Virology* **131**, 104612 (2020).
129. Ramdas, K., Darzi, A. & Jain, S. ‘Test, re-test, re-test’: using inaccurate tests to greatly increase the accuracy of COVID-19 testing. *Nature Medicine* **26**, 810–811 (2020).
130. Tu, H.-F., Yang, P., Lin, Z.-H., Zheng, C. & You, S.-L. Time-dependent enantiodivergent synthesis via sequential kinetic resolution. *Nature Chemistry* **12**, 838–844 (2020).
131. Subsoontorn, P., Lohitnavy, M. & Kongkaew, C. The diagnostic accuracy of isothermal nucleic acid point-of-care tests for human coronaviruses: A systematic review and meta-analysis. *Scientific Reports* **10**, 22349 (2020).
132. Shen, M. *et al.* Recent advances and perspectives of nucleic acid detection for coronavirus. *Journal of Pharmaceutical Analysis* **10**, 97–101 (2020).
133. How to choose the right real-time RT-PCR primer sets for the SARS-CoV-2 genome detection? (2020) doi:10.21203/rs.3.rs-36512/v1.
134. Comparative Analysis of Primer–Probe Sets for RT-qPCR of COVID-19 Causative Virus (SARS-CoV-2) | ACS Infectious Diseases. <https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00464>.
135. Hong, K. H. *et al.* Guidelines for Laboratory Diagnosis of Coronavirus Disease 2019 (COVID-19) in Korea. *Annals of Laboratory Medicine* **40**, 351–360 (2020).

136. Braz-Silva, P. H. *et al.* Performance of at-home self-collected saliva and nasal-orpharyngeal swabs in the surveillance of COVID-19. *Journal of Oral Microbiology* **13**, 1858002 (2021).
137. Guglielmi, G. Fast coronavirus tests: what they can and can't do. *Nature* **585**, 496–498 (2020).
138. Suo, T. *et al.* ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens. *Emerging Microbes & Infections* **9**, 1259–1268 (2020).
139. Dong, L. *et al.* Highly accurate and sensitive diagnostic detection of SARS-CoV-2 by digital PCR. *Talanta* **224**, 121726 (2021).
140. Verdun, C. M. *et al.* Group testing for SARS-CoV-2 allows for up to 10-fold efficiency increase across realistic scenarios and testing strategies. *medRxiv* 2020.04.30.20085290 (2020)
doi:10.1101/2020.04.30.20085290.
141. Procop, G. W. *et al.* Asymptomatic Patient Testing After 10:1 Pooling Using the Xpert Xpress SARS-CoV-2 Assay. *American Journal of Clinical Pathology* (2021) doi:10.1093/ajcp/aqaa273.
142. Tahamtan, A. & Ardebili, A. Real-time RT-PCR in COVID-19 detection: issues affecting the results. *Expert Review of Molecular Diagnostics* **20**, 453–454 (2020).
143. Kuang, J., Yan, X., Genders, A. J., Granata, C. & Bishop, D. J. An overview of technical considerations when using quantitative real-time PCR analysis of gene expression in human exercise research. *PLOS ONE* **13**, e0196438 (2018).
144. Bustin, S. A. & Nolan, T. Pitfalls of quantitative real-time reverse-transcription polymerase chain reaction. *J Biomol Tech* **15**, 155–166 (2004).
145. Oliveira, B. A. *et al.* SARS-CoV-2 and the COVID-19 disease: a mini review on diagnostic methods. *Revista do Instituto de Medicina Tropical de São Paulo* **62**, (2020).
146. El-Kafrawy, S. A. *et al.* A Direct Method for RT-PCR Detection of SARS-CoV-2 in Clinical Samples. *Healthcare* **9**, 37 (2021).
147. Mina, M. J., Parker, R. & Larremore, D. B. Rethinking Covid-19 Test Sensitivity — A Strategy for Containment. *New England Journal of Medicine* **383**, e120 (2020).
148. Callaway, E. The coronavirus is mutating — does it matter? *Nature* **585**, 174–177 (2020).

149. Phan, T. Genetic diversity and evolution of SARS-CoV-2. *Infection, Genetics and Evolution* **81**, 104260 (2020).
150. Thompson, D. & Lei, Y. Mini review: Recent progress in RT-LAMP enabled COVID-19 detection. *Sensors and Actuators Reports* **2**, 100017 (2020).
151. Shirato, K. *et al.* Detection of Middle East respiratory syndrome coronavirus using reverse transcription loop-mediated isothermal amplification (RT-LAMP). *Virology Journal* **11**, 139 (2014).
152. Nassir, A. A. *et al.* RPA-Based Method For The Detection Of SARS-COV2. *medRxiv* 2020.09.17.20196402 (2020) doi:10.1101/2020.09.17.20196402.
153. Chaibun, T. *et al.* Rapid electrochemical detection of coronavirus SARS-CoV-2. *Nature Communications* **12**, 802 (2021).
154. Wu, Q. *et al.* INSIGHT: a scalable isothermal NASBA-based platform for COVID-19 diagnosis. *bioRxiv* 2020.06.01.127019 (2020) doi:10.1101/2020.06.01.127019.
155. Patchsung, M. *et al.* Clinical validation of a Cas13-based assay for the detection of SARS-CoV-2 RNA. *Nature Biomedical Engineering* **4**, 1140–1149 (2020).
156. Joung, J. *et al.* Detection of SARS-CoV-2 with SHERLOCK One-Pot Testing. *New England Journal of Medicine* (2020) doi:10.1056/NEJMc2026172.
157. Wang, D. One-pot Detection of COVID-19 with Real-time Reverse-transcription Loop-mediated Isothermal Amplification (RT-LAMP) Assay and Visual RT-LAMP Assay. *bioRxiv* 2020.04.21.052530 (2020) doi:10.1101/2020.04.21.052530.
158. Notomi, T. *et al.* Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* **28**, e63–e63 (2000).
159. Comparison of fluorescent intercalating dyes for quantitative loop-mediated isothermal amplification (qLAMP) | BioTechniques. https://www.future-science.com/doi/10.2144/000114432?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed.

160. Huang, W. E. *et al.* RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microbial Biotechnology* **13**, 950–961 (2020).
161. Lamb, L. E., Bartolone, S. N., Ward, E. & Chancellor, M. B. Rapid Detection of Novel Coronavirus (COVID-19) by Reverse Transcription-Loop-Mediated Isothermal Amplification. *medRxiv* 2020.02.19.20025155 (2020) doi:10.1101/2020.02.19.20025155.
162. Thi, V. L. D. *et al.* A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Science Translational Medicine* **12**, (2020).
163. Schmid-Burgk, J. L. *et al.* LAMP-Seq: Population-Scale COVID-19 Diagnostics Using Combinatorial Barcoding. *bioRxiv* 2020.04.06.025635 (2020) doi:10.1101/2020.04.06.025635.
164. Crone, M. A. *et al.* A role for Biofoundries in rapid development and validation of automated SARS-CoV-2 clinical diagnostics. *Nature Communications* **11**, 4464 (2020).
165. Ma, C. *et al.* A novel method to control carryover contamination in isothermal nucleic acid amplification. *Chem. Commun.* **53**, 10696–10699 (2017).
166. Gootenberg, J. S. *et al.* Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* **356**, 438–442 (2017).
167. Gootenberg, J. S. *et al.* Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science* **360**, 439–444 (2018).
168. Arizti-Sanz, J. *et al.* Streamlined inactivation, amplification, and Cas13-based detection of SARS-CoV-2. *Nature Communications* **11**, 5921 (2020).
169. Hou, T. *et al.* Development and evaluation of a rapid CRISPR-based diagnostic for COVID-19. *PLOS Pathogens* **16**, e1008705 (2020).
170. Zhang, F., Abudayyeh, O. O. & Gootenberg, J. S. A protocol for detection of COVID-19 using CRISPR diagnostics. **8**.
171. Ackerman, C. M. *et al.* Massively multiplexed nucleic acid detection with Cas13. *Nature* **582**, 277–282 (2020).

172. Broughton, J. P. *et al.* CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology* **38**, 870–874 (2020).
173. Lucia, C., Federico, P.-B. & Alejandra, G. C. An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12. *bioRxiv* 2020.02.29.971127 (2020) doi:10.1101/2020.02.29.971127.
174. Joung, J. *et al.* Point-of-care testing for COVID-19 using SHERLOCK diagnostics. *medRxiv* 2020.05.04.20091231 (2020) doi:10.1101/2020.05.04.20091231.
175. Huang, D. *et al.* A CRISPR-Cas12a-Derived Biosensor Enabling Portable Personal Glucose Meter Readout for Quantitative Detection of SARS-CoV-2. *Biotechnology and Bioengineering* **n/a**,.
176. Harrington, L. B. *et al.* Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. *Science* **362**, 839–842 (2018).
177. Commissioner, O. of the. Coronavirus (COVID-19) Update: FDA Authorizes Antigen Test as First Over-the-Counter Fully At-Home Diagnostic Test for COVID-19. *FDA* <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-antigen-test-first-over-counter-fully-home-diagnostic> (2020).
178. Stokes, W. *et al.* Real-World Clinical Performance of the Abbott Panbio with Nasopharyngeal, Throat and Saliva Swabs Among Symptomatic Individuals with COVID-19. *medRxiv* 2021.01.02.21249138 (2021) doi:10.1101/2021.01.02.21249138.
179. Nagura-Ikeda, M. *et al.* Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription–Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test To Diagnose COVID-19. *Journal of Clinical Microbiology* **58**, (2020).
180. Long, Q.-X. *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nature Medicine* **26**, 1200–1204 (2020).

181. Okba, N. M. A. *et al.* Severe Acute Respiratory Syndrome Coronavirus 2–Specific Antibody Responses in Coronavirus Disease Patients - Volume 26, Number 7—July 2020 - Emerging Infectious Diseases journal - CDC. doi:10.3201/eid2607.200841.
182. Commissioner, O. of the. Coronavirus (COVID-19) Update: FDA Authorizes First Test that Detects Neutralizing Antibodies from Recent or Prior SARS-CoV-2 Infection. *FDA* <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-first-test-detects-neutralizing-antibodies-recent-or> (2020).
183. Li, Z. *et al.* Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *Journal of Medical Virology* **92**, 1518–1524 (2020).
184. Prazuck, T. *et al.* Evaluation of performance of two SARS-CoV-2 Rapid IgM-IgG combined antibody tests on capillary whole blood samples from the fingertip. *PLoS One* **15**, (2020).
185. Choe, P. G. *et al.* Antibody Responses 8 Months after Asymptomatic or Mild SARS-CoV-2 Infection. *Emerg Infect Dis* **27**, (2020).
186. Petherick, A. Developing antibody tests for SARS-CoV-2. *The Lancet* **395**, 1101–1102 (2020).
187. Farrera-Soler, L. *et al.* Identification of immunodominant linear epitopes from SARS-CoV-2 patient plasma. *PLOS ONE* **15**, e0238089 (2020).
188. Griffin, S. Covid-19: Antibodies protect against reinfection for at least six months, study finds. *BMJ* **371**, m4961 (2020).
189. Murillo-Zamora, E., Mendoza-Cano, O., Delgado-Enciso, I. & Hernandez-Suarez, C. M. Predictors of severe symptomatic laboratory-confirmed SARS-COV-2 reinfection. *medRxiv* 2020.10.14.20212720 (2020) doi:10.1101/2020.10.14.20212720.
190. Tillett, R. L. *et al.* Genomic evidence for reinfection with SARS-CoV-2: a case study. *The Lancet Infectious Diseases* **21**, 52–58 (2021).
191. Zhao, J. *et al.* Antibody Responses to SARS-CoV-2 in Patients With Novel Coronavirus Disease 2019. *Clinical Infectious Diseases* **71**, 2027–2034 (2020).

192. Wise, J. Covid-19: T cell response lasts for at least six months after infection, study shows. *BMJ* **371**, m4257 (2020).
193. Karlsson, A. C., Humbert, M. & Buggert, M. The known unknowns of T cell immunity to COVID-19. *Science Immunology* **5**, (2020).
194. Quijano-Rubio, A. *et al.* De novo design of modular and tunable protein biosensors. *Nature* 1–6 (2021) doi:10.1038/s41586-021-03258-z.
195. Wang, D. *et al.* Rapid lateral flow immunoassay for the fluorescence detection of SARS-CoV-2 RNA. *Nature Biomedical Engineering* **4**, 1150–1158 (2020).
196. Woo, C. H., Jang, S., Shin, G., Jung, G. Y. & Lee, J. W. Sensitive fluorescence detection of SARS-CoV-2 RNA in clinical samples via one-pot isothermal ligation and transcription. *Nature Biomedical Engineering* **4**, 1168–1179 (2020).
197. Whelan, R. Covid-19 Temperature Scanners Have Accuracy Issues, Security Industry Says. *Wall Street Journal* (2020).
198. Jendryny, P. *et al.* Scent dog identification of samples from COVID-19 patients – a pilot study. *BMC Infectious Diseases* **20**, 536 (2020).
199. Narita, F. *et al.* A Review of Piezoelectric and Magnetostrictive Biosensor Materials for Detection of COVID-19 and Other Viruses. *Advanced Materials* **33**, 2005448 (2021).
200. Caruso, D. *et al.* Chest CT Features of COVID-19 in Rome, Italy. *Radiology* **296**, E79–E85 (2020).
201. Ai, T. *et al.* Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology* **296**, E32–E40 (2020).
202. Fang, Y. *et al.* Sensitivity of Chest CT for COVID-19: Comparison to RT-PCR. *Radiology* **296**, E115–E117 (2020).
203. Jin, C. *et al.* Development and evaluation of an artificial intelligence system for COVID-19 diagnosis. *Nature Communications* **11**, 5088 (2020).
204. Epstein, J. H. *et al.* Nipah virus dynamics in bats and implications for spillover to humans. *PNAS* **117**, 29190–29201 (2020).

205. Shi, J. *et al.* Susceptibility of ferrets, cats, dogs, and other domesticated animals to SARS–coronavirus 2. *Science* **368**, 1016–1020 (2020).
206. Olival, K. J. *et al.* Possibility for reverse zoonotic transmission of SARS-CoV-2 to free-ranging wildlife: A case study of bats. *PLOS Pathogens* **16**, e1008758 (2020).
207. Ball, P. The lightning-fast quest for COVID vaccines — and what it means for other diseases. *Nature* **589**, 16–18 (2020).
208. New Thai-made rapid Covid test to slash screening costs. <https://www.nationthailand.com>
<http://nationthailand/news/30397474>.
209. Inventor of made-in-Việt Nam COVID-19 quick test kits honoured as top inspirer - Society - Vietnam News | Politics, Business, Economy, Society, Life, Sports - VietNam News.
<https://vietnamnews.vn/society/828649/inventor-of-made-in-viet-nam-covid-19-quick-test-kits-honoured-as-top-inspirer.html>.
210. Bats are worth \$3.7bn to US agriculture. *the Guardian*
<http://www.theguardian.com/sustainable-business/2015/oct/14/bats-worth-37bn-to-us-agriculture-research-california-texas> (2015).
211. Sheherazade, Ober, H. K. & Tsang, S. M. Contributions of bats to the local economy through durian pollination in Sulawesi, Indonesia. *Biotropica* **51**, 913–922 (2019).
212. Wacharapluesadee, S. *et al.* Group C Betacoronavirus in Bat Guano Fertilizer, Thailand. *Emerg Infect Dis* **19**, 1349–1352 (2013).
213. Bats not the enemy in the fight against COVID-19. *Mongabay-India*
<https://india.mongabay.com/2020/04/bats-not-the-enemy-in-the-fight-against-covid-19/>
(2020).
214. Rocha, R. *et al.* Bat conservation and zoonotic disease risk: a research agenda to prevent misguided persecution in the aftermath of COVID-19. *Animal Conservation* **n/a**,.
215. Mair-Jenkins, J. *et al.* The Effectiveness of Convalescent Plasma and Hyperimmune Immunoglobulin for the Treatment of Severe Acute Respiratory Infections of Viral Etiology: A

- Systematic Review and Exploratory Meta-analysis. *The Journal of Infectious Diseases* **211**, 80–90 (2015).
216. MacNalty, A. S. Emil von Behring. *Br Med J* **1**, 668–670 (1954).
 217. Pathak, E. B. Convalescent plasma is ineffective for covid-19. *BMJ* **371**, m4072 (2020).
 218. Liu, S. T. H. *et al.* Convalescent plasma treatment of severe COVID-19: a propensity score–matched control study. *Nature Medicine* **26**, 1708–1713 (2020).
 219. Libster, R. *et al.* Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. *New England Journal of Medicine* **0**, null (2021).
 220. Bansal, P. *et al.* Hydroxychloroquine: a comprehensive review and its controversial role in coronavirus disease 2019. *Annals of Medicine* **53**, 117–134 (2021).
 221. Hodge, C. *et al.* Drug interactions: a review of the unseen danger of experimental COVID-19 therapies. *Journal of Antimicrobial Chemotherapy* **75**, 3417–3424 (2020).
 222. Shah, R. R. Chloroquine and hydroxychloroquine for COVID-19: Perspectives on their failure in repurposing. *Journal of Clinical Pharmacy and Therapeutics* **46**, 17–27 (2021).
 223. Perlmutter, A. S., Tran, V.-T., Dechartres, A. & Ravaud, P. Statistical controversies in clinical research: comparison of primary outcomes in protocols, public clinical-trial registries and publications: the example of oncology trials. *Annals of Oncology* **28**, 688–695 (2017).
 224. Zumla, A., Chan, J. F. W., Azhar, E. I., Hui, D. S. C. & Yuen, K.-Y. Coronaviruses — drug discovery and therapeutic options. *Nature Reviews Drug Discovery* **15**, 327–347 (2016).
 225. Sheahan, T. P. *et al.* Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *Science Translational Medicine* **9**, (2017).
 226. Savarino, A., Trani, L. D., Donatelli, I., Cauda, R. & Cassone, A. New insights into the antiviral effects of chloroquine. *The Lancet Infectious Diseases* **6**, 67–69 (2006).
 227. Wang, M. *et al.* Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* **30**, 269–271 (2020).

228. Eastman, R. T. *et al.* Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19. *ACS Cent. Sci.* **6**, 672–683 (2020).
229. Sheahan, T. P. *et al.* Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. *Nature Communications* **11**, 222 (2020).
230. Cao, Y., Deng, Q. & Dai, S. Remdesivir for severe acute respiratory syndrome coronavirus 2 causing COVID-19: An evaluation of the evidence. *Travel Medicine and Infectious Disease* **35**, 101647 (2020).
231. Wang, Y. *et al.* Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *The Lancet* **395**, 1569–1578 (2020).
232. Beigel, J. H. *et al.* Remdesivir for the Treatment of Covid-19 — Final Report. *New England Journal of Medicine* **383**, 1813–1826 (2020).
233. Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results. *New England Journal of Medicine* **0**, null (2020).
234. Long, M. J. C., Van Hall-Beauvais, A. & Aye, Y. The more the merrier: how homo-oligomerization alters the interactome and function of ribonucleotide reductase. *Current Opinion in Chemical Biology* **54**, 10–18 (2020).
235. Gordon, C. J. *et al.* Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. *J Biol Chem* **295**, 6785–6797 (2020).
236. Robson, F. *et al.* Coronavirus RNA Proofreading: Molecular Basis and Therapeutic Targeting. *Molecular Cell* **79**, 710–727 (2020).
237. Narayanan, N. & Nair, D. T. Ritonavir may inhibit exoribonuclease activity of nsp14 from the SARS-CoV-2 virus and potentiate the activity of chain terminating drugs. *International Journal of Biological Macromolecules* **168**, 272–278 (2021).

238. Jafari, A., Dadkhahfar, S. & Perseh, S. Considerations for interactions of drugs used for the treatment of COVID-19 with anti-cancer treatments. *Critical Reviews in Oncology/Hematology* **151**, 102982 (2020).
239. Sayad, B., Sobhani, M. & Khodarahmi, R. Sofosbuvir as Repurposed Antiviral Drug Against COVID-19: Why Were We Convinced to Evaluate the Drug in a Registered/Approved Clinical Trial? *Archives of Medical Research* **51**, 577–581 (2020).
240. Jockusch, S. *et al.* Sofosbuvir terminated RNA is more resistant to SARS-CoV-2 proofreader than RNA terminated by Remdesivir. *Scientific Reports* **10**, 16577 (2020).
241. Roozbeh, F. *et al.* Sofosbuvir and daclatasvir for the treatment of COVID-19 outpatients: a double-blind, randomized controlled trial. *Journal of Antimicrobial Chemotherapy* (2020) doi:10.1093/jac/dkaa501.
242. Vela, J. M. Repurposing Sigma-1 Receptor Ligands for COVID-19 Therapy? *Front. Pharmacol.* **11**, (2020).
243. Hashimoto, K. Repurposing of CNS drugs to treat COVID-19 infection: targeting the sigma-1 receptor. *Eur Arch Psychiatry Clin Neurosci* (2021) doi:10.1007/s00406-020-01231-x.
244. Hirata, Y. *et al.* Chloroquine inhibits glutamate-induced death of a neuronal cell line by reducing reactive oxygen species through sigma-1 receptor. *Journal of Neurochemistry* **119**, 839–847 (2011).
245. Lagier, J.-C. *et al.* Outcomes of 3,737 COVID-19 patients treated with hydroxychloroquine/azithromycin and other regimens in Marseille, France: A retrospective analysis. *Travel Med Infect Dis* **36**, 101791 (2020).
246. Gautret, P. *et al.* Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents* **56**, 105949 (2020).
247. El Bakkouri, K. Hydroxychloroquine (HCQ): real treatment or false hope? *International Journal of Antimicrobial Agents* **57**, 106170 (2021).

248. Commissioner, O. of the. Coronavirus (COVID-19) Update: FDA Revokes Emergency Use Authorization for Chloroquine and Hydroxychloroquine. *FDA* <https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-revokes-emergency-use-authorization-chloroquine-and> (2020).
249. Wang, G. *et al.* Mechanistic insights into ventricular arrhythmogenesis of hydroxychloroquine and azithromycin for the treatment of COVID-19. *bioRxiv* 2020.05.21.108605 (2020) doi:10.1101/2020.05.21.108605.
250. Fiolet, T. *et al.* Effect of hydroxychloroquine with or without azithromycin on the mortality of coronavirus disease 2019 (COVID-19) patients: a systematic review and meta-analysis. *Clinical Microbiology and Infection* **27**, 19–27 (2021).
251. Monti, M. *et al.* Hydroxychloroquine as Prophylaxis for COVID-19: A Review. *Front. Pharmacol.* **11**, (2020).
252. Lewis, K. *et al.* The efficacy and safety of hydroxychloroquine for COVID-19 prophylaxis: A systematic review and meta-analysis of randomized trials. *PLOS ONE* **16**, e0244778 (2021).
253. Idda, M. L., Soru, D. & Floris, M. Overview of the First 6 Months of Clinical Trials for COVID-19 Pharmacotherapy: The Most Studied Drugs. *Front. Public Health* **8**, (2020).
254. Reyes, A. Z. *et al.* Anti-inflammatory therapy for COVID-19 infection: the case for colchicine. *Annals of the Rheumatic Diseases* (2020) doi:10.1136/annrheumdis-2020-219174.
255. Russell, C. D., Millar, J. E. & Baillie, J. K. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. *The Lancet* **395**, 473–475 (2020).
256. Dexamethasone in Hospitalized Patients with Covid-19 — Preliminary Report. *New England Journal of Medicine* **0**, null (2020).
257. Villar, J., Confalonieri, M., Pastores, S. M. & Meduri, G. U. Rationale for Prolonged Corticosteroid Treatment in the Acute Respiratory Distress Syndrome Caused by Coronavirus Disease 2019. *Crit Care Explor* **2**, (2020).

258. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group *et al.* Association Between Administration of Systemic Corticosteroids and Mortality Among Critically Ill Patients With COVID-19: A Meta-analysis. *JAMA* **324**, 1330–1341 (2020).
259. Matthay, M. A. & Thompson, B. T. Dexamethasone in hospitalised patients with COVID-19: addressing uncertainties. *The Lancet Respiratory Medicine* **8**, 1170–1172 (2020).
260. Theoharides, T. C. & Conti, P. Dexamethasone for COVID-19? Not so fast. *J Biol Regul Homeost Agents* **34**, 1241–1243 (2020).
261. Lee, N. *et al.* Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. *Journal of Clinical Virology* **31**, 304–309 (2004).
262. Wang, Y. *et al.* A retrospective cohort study of methylprednisolone therapy in severe patients with COVID-19 pneumonia. *Signal Transduction and Targeted Therapy* **5**, 1–3 (2020).
263. Zhu, H.-M. *et al.* Effect of methylprednisolone in severe and critical COVID-19: Analysis of 102 cases. *World Journal of Clinical Cases* **8**, 5952–5961 (2020).
264. Administrator. Efficacy of methylprednisolone pulse therapy in patients with COVID-19. *KMAX* <http://cmac-journal.ru/en/publication/2020/2/cmac-2020-t22-n2-p088/> (2020).
265. Fatima, S. A., Asif, M., Khan, K. A., Siddique, N. & Khan, A. Z. Comparison of efficacy of dexamethasone and methylprednisolone in moderate to severe covid 19 disease. *Annals of Medicine and Surgery* **60**, 413–416 (2020).
266. Baron, S. A., Devaux, C., Colson, P., Raoult, D. & Rolain, J.-M. Teicoplanin: an alternative drug for the treatment of COVID-19? *Int J Antimicrob Agents* **55**, 105944 (2020).
267. Pan, T., Zhou, N. & Zhang, H. Use of Teicoplanin Anti-Middle East Respiratory Syndrome Coronavirus. (2016).
268. Zhou, N. *et al.* Glycopeptide Antibiotics Potently Inhibit Cathepsin L in the Late Endosome/Lysosome and Block the Entry of Ebola Virus, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). *J Biol Chem* **291**, 9218–9232 (2016).

269. Somers, E. C. *et al.* Tocilizumab for Treatment of Mechanically Ventilated Patients With COVID-19. *Clinical Infectious Diseases* (2020) doi:10.1093/cid/ciaa954.
270. Ceccarelli, G. *et al.* Superinfections in patients treated with Teicoplanin as anti-SARS-CoV-2 agent. *European Journal of Clinical Investigation* **51**, e13418 (2021).
271. Long, M. J. C., Rogg, C. & Aye, Y. An Oculus to Profile and Probe Target Engagement In Vivo: How T-REX Was Born and Its Evolution into G-REX. *Acc Chem Res* (2020) doi:10.1021/acs.accounts.0c00537.
272. Bowen, G. L. and J. P. Development of Trypsin-Like Serine Protease Inhibitors as Therapeutic Agents: Opportunities, Challenges, and their Unique Structure-Based Rationales. *Current Topics in Medicinal Chemistry* vol. 16 1506–1529 <https://www.eurekaselect.com/134955/article> (2016).
273. Cromm, P. M. & Crews, C. M. The Proteasome in Modern Drug Discovery: Second Life of a Highly Valuable Drug Target. *ACS Cent. Sci.* **3**, 830–838 (2017).
274. Eder, J., Hommel, U., Cumin, F., Martoglio, B. & Gerhartz, B. Aspartic proteases in drug discovery. *Curr Pharm Des* **13**, 271–285 (2007).
275. Long, M. J. C. *et al.* Diarylcarbonates are a new class of deubiquitinating enzyme inhibitor. *Bioorganic & Medicinal Chemistry Letters* **29**, 204–211 (2019).
276. Hedstrom, L. K., LONG, M. J. & LAWSON, A. P. Inhibitors of deubiquitinating proteases. (2016).
277. Chu, C. M. *et al.* Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* **59**, 252–256 (2004).
278. Wilde, A. H. de *et al.* Screening of an FDA-Approved Compound Library Identifies Four Small-Molecule Inhibitors of Middle East Respiratory Syndrome Coronavirus Replication in Cell Culture. *Antimicrobial Agents and Chemotherapy* **58**, 4875–4884 (2014).
279. Wu, C.-Y. *et al.* Small molecules targeting severe acute respiratory syndrome human coronavirus. *PNAS* **101**, 10012–10017 (2004).

280. Siklos, M., BenAissa, M. & Thatcher, G. R. J. Cysteine proteases as therapeutic targets: does selectivity matter? A systematic review of calpain and cathepsin inhibitors. *Acta Pharmaceutica Sinica B* **5**, 506–519 (2015).
281. Blanchard, J. E. *et al.* High-Throughput Screening Identifies Inhibitors of the SARS Coronavirus Main Proteinase. *Chemistry & Biology* **11**, 1445–1453 (2004).
282. Owa, A. B. & Owa, O. T. Lopinavir/ritonavir use in Covid-19 infection: is it completely non-beneficial? *Journal of Microbiology, Immunology and Infection* **53**, 674–675 (2020).
283. Griffin, S. Covid-19: Lopinavir-ritonavir does not benefit hospitalised patients, UK trial finds. *BMJ* **370**, m2650 (2020).
284. Zhang, L. *et al.* Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α -ketoamide inhibitors. *Science* **368**, 409–412 (2020).
285. Kouznetsova, V. L., Zhang, A., Tatineni, M., Miller, M. A. & Tsigelny, I. F. Potential COVID-19 papain-like protease PL^{pro} inhibitors: repurposing FDA-approved drugs. *PeerJ* **8**, e9965 (2020).
286. Kim, D. W. *et al.* Phenolic phytochemical displaying SARS-CoV papain-like protease inhibition from the seeds of *Psoralea corylifolia*. *Journal of Enzyme Inhibition and Medicinal Chemistry* **29**, 59–63 (2014).
287. Cho, J. K. *et al.* Geranylated flavonoids displaying SARS-CoV papain-like protease inhibition from the fruits of *Paulownia tomentosa*. *Bioorg Med Chem* **21**, 3051–3057 (2013).
288. Song, Y. H. *et al.* Papain-like protease (PLpro) inhibitory effects of cinnamic amides from *Tribulus terrestris* fruits. *Biol Pharm Bull* **37**, 1021–1028 (2014).
289. Pang, B., Zhang, J., Lee, M. S. & Zheng, W. Enlightenment from clinical trials on Chinese medicine for coronavirus disease 2019 (COVID-19). *Integr Med Res* **9**, 100481 (2020).
290. Leisman, D. E. *et al.* Cytokine elevation in severe and critical COVID-19: a rapid systematic review, meta-analysis, and comparison with other inflammatory syndromes. *The Lancet Respiratory Medicine* **8**, 1233–1244 (2020).

291. Smetana, K., Rosel, D. & Brábek, J. Raloxifene and Bazedoxifene Could Be Promising Candidates for Preventing the COVID-19 Related Cytokine Storm, ARDS and Mortality. *In Vivo* **34**, 3027–3028 (2020).
292. Yuan, S. *et al.* Mortality and pre-hospitalization use of low-dose aspirin in COVID-19 patients with coronary artery disease. *J Cell Mol Med* (2020) doi:10.1111/jcmm.16198.
293. Osborne, T. F. *et al.* Association of Mortality and Aspirin Prescription for COVID-19 Patients at the Veterans Health Administration. *medRxiv* 2020.12.13.20248147 (2020) doi:10.1101/2020.12.13.20248147.
294. Gokhale, Y., Mehta, R., Karnik, N., Kulkarni, U. & Gokhale, S. Tocilizumab improves survival in patients with persistent hypoxia in severe COVID-19 pneumonia. *EClinicalMedicine* **24**, 100467 (2020).
295. Lopes, M. I. *et al.* Beneficial effects of colchicine for moderate to severe COVID-19: an interim analysis of a randomized, double-blinded, placebo controlled clinical trial. *medRxiv* 2020.08.06.20169573 (2020) doi:10.1101/2020.08.06.20169573.
296. Olagnier, D. *et al.* SARS-CoV2-mediated suppression of NRF2-signaling reveals potent antiviral and anti-inflammatory activity of 4-octyl-itaconate and dimethyl fumarate. *Nature Communications* **11**, 4938 (2020).
297. Schulze-Topphoff, U. *et al.* Dimethyl fumarate treatment induces adaptive and innate immune modulation independent of Nrf2. *Proc Natl Acad Sci U S A* **113**, 4777–4782 (2016).
298. Chen, H. *et al.* Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. *J Clin Invest* **124**, 2188–2192 (2014).
299. Müller, S. *et al.* Dimethylfumarate Impairs Neutrophil Functions. *Journal of Investigative Dermatology* **136**, 117–126 (2016).
300. Brück, J., Dringen, R., Amasuno, A., Pau-Charles, I. & Ghoreschi, K. A review of the mechanisms of action of dimethylfumarate in the treatment of psoriasis. *Experimental Dermatology* **27**, 611–624 (2018).

301. Gavazza, A. *et al.* Ozone Therapy as a Possible Option in COVID-19 Management. *Front. Public Health* **8**, (2020).
302. Hernández, A. *et al.* Ozone therapy for patients with COVID-19 pneumonia: Preliminary report of a prospective case-control study. *International Immunopharmacology* **90**, 107261 (2021).
303. Bojkova, D. *et al.* Proteomics of SARS-CoV-2-infected host cells reveals therapy targets. *Nature* **583**, 469–472 (2020).
304. Bausch Health Americas, Inc. *An Open-Label Study to Evaluate the Safety and Efficacy of VIRAZOLE® (RIBAVIRIN FOR INHALATION SOLUTION, USP) in Hospitalized Adult Participants With Respiratory Distress Due to COVID-19.* <https://clinicaltrials.gov/ct2/show/NCT04356677> (2020).
305. Tong, S. *et al.* Ribavirin therapy for severe COVID-19: a retrospective cohort study. *International Journal of Antimicrobial Agents* **56**, 106114 (2020).
306. Unal, M. A. *et al.* Ribavirin shows antiviral activity against SARS-CoV-2 and downregulates the activity of TMPRSS2 and the expression of ACE2 In Vitro. *bioRxiv* 2020.12.04.410092 (2020) doi:10.1101/2020.12.04.410092.
307. Bukreyeva, N. *et al.* The IMPDH inhibitor merimepodib suppresses SARS-CoV-2 replication in vitro. *bioRxiv* 2020.04.07.028589 (2020) doi:10.1101/2020.04.07.028589.
308. Hoffmann, M. *et al.* Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19. *Antimicrobial Agents and Chemotherapy* **64**, (2020).
309. Hofmann-Winkler, H. *et al.* Camostat Mesylate May Reduce Severity of Coronavirus Disease 2019 Sepsis: A First Observation. *Crit Care Explor* **2**, e0284 (2020).
310. KupferschmidtApr. 2, K., 2020 & Am, 11:10. These drugs don't target the coronavirus—they target us. *Science / AAAS* <https://www.sciencemag.org/news/2020/04/these-drugs-don-t-target-coronavirus-they-target-us> (2020).
311. Sun, Y. J. *et al.* TMPRSS2 structure-phylogeny repositions Avoralstat for SARS-CoV-2 prophylaxis in mice. *bioRxiv* 2021.01.04.425289 (2021) doi:10.1101/2021.01.04.425289.

312. Young, A. *et al.* Homologous protein domains in SARS-CoV-2 and measles, mumps and rubella viruses: preliminary evidence that MMR vaccine might provide protection against COVID-19. *medRxiv* 2020.04.10.20053207 (2020) doi:10.1101/2020.04.10.20053207.
313. Fromer, M. J. FDA Introduces New Phase 0 for Clinical Trials: Some Enthusiastic, Some Skeptical. *Oncology Times* **28**, 18–19 (2006).
314. Bjorn, G. Despite potential side effects, two drugs make a comeback. *Nature Medicine* **14**, 226–226 (2008).
315. Petsko, G. A. When failure should be the option. *BMC Biol* **8**, 61 (2010).
316. Long, M. J., Liu, X. & Aye, Y. Genie in a bottle: controlled release helps tame natural polypharmacology? *Current Opinion in Chemical Biology* **51**, 48–56 (2019).
317. Reddy, A. S. & Zhang, S. Polypharmacology: drug discovery for the future. *Expert Rev Clin Pharmacol* **6**, 41–47 (2013).
318. Van Norman, G. A. Drugs, Devices, and the FDA: Part 1: An Overview of Approval Processes for Drugs. *JACC: Basic to Translational Science* **1**, 170–179 (2016).
319. Chitalia, V. C. & Munawar, A. H. A painful lesson from the COVID-19 pandemic: the need for broad-spectrum, host-directed antivirals. *Journal of Translational Medicine* **18**, 390 (2020).
320. Ogando, N. S. *et al.* The Enzymatic Activity of the nsp14 Exoribonuclease Is Critical for Replication of MERS-CoV and SARS-CoV-2. *Journal of Virology* **94**, (2020).
321. Baddock, H. T. *et al.* Characterisation of the SARS-CoV-2 ExoN (nsp14ExoN-nsp10) complex: implications for its role in viral genome stability and inhibitor identification. *bioRxiv* 2020.08.13.248211 (2020) doi:10.1101/2020.08.13.248211.
322. Selvaraj, C. *et al.* Structure-based virtual screening and molecular dynamics simulation of SARS-CoV-2 Guanine-N7 methyltransferase (nsp14) for identifying antiviral inhibitors against COVID-19. *Journal of Biomolecular Structure and Dynamics* **0**, 1–12 (2020).

323. Sun, Y., Abriola, L., Surovtseva, Y. V., Lindenbach, B. D. & Guo, J. U. Restriction of SARS-CoV-2 Replication by Targeting Programmed –1 Ribosomal Frameshifting In Vitro. *bioRxiv* 2020.10.21.349225 (2020) doi:10.1101/2020.10.21.349225.
324. Han, Y. *et al.* Identification of SARS-CoV-2 inhibitors using lung and colonic organoids. *Nature* **589**, 270–275 (2021).
325. Gordon, D. E. *et al.* A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* **583**, 459–468 (2020).
326. Aye, Y., Li, M., Long, M. J. C. & Weiss, R. S. Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies. *Oncogene* **34**, 2011–2021 (2015).
327. Mortimer, S. E. *et al.* IMP dehydrogenase type 1 associates with polyribosomes translating rhodopsin mRNA. *J Biol Chem* **283**, 36354–36360 (2008).
328. Chaigne-Delalande, B. *et al.* The immunosuppressor mycophenolic acid kills activated lymphocytes by inducing a nonclassical actin-dependent necrotic signal. *J Immunol* **181**, 7630–7638 (2008).
329. Tang, L., Yin, Z., Hu, Y. & Mei, H. Controlling Cytokine Storm Is Vital in COVID-19. *Front. Immunol.* **11**, (2020).
330. Xiang, Y. *et al.* Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2. *Science* **370**, 1479–1484 (2020).
331. Schoof, M. *et al.* An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. *Science* **370**, 1473–1479 (2020).
332. Thomford, N. E. *et al.* Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. *International Journal of Molecular Sciences* **19**, 1578 (2018).
333. Siddiqui, A. A., Iram, F., Siddiqui, S. & Sahu, K. Role of Natural Products in Drug Discovery Process. *International Journal of Drug Development and Research* **6**, (2014).
334. Zhang, Z.-R. *et al.* A cell-based large-scale screening of natural compounds for inhibitors of SARS-CoV-2. *Signal Transduction and Targeted Therapy* **5**, 1–3 (2020).

335. Khatri, H. R. *et al.* Modular Total Synthesis and Cell-Based Anticancer Activity Evaluation of Ouabagenin and Other Cardiotonic Steroids with Varying Degrees of Oxygenation. *J. Am. Chem. Soc.* **141**, 4849–4860 (2019).
336. Orellana, A. M., Kinoshita, P. F., Leite, J. A., Kawamoto, E. M. & Scavone, C. Cardiotonic Steroids as Modulators of Neuroinflammation. *Front. Endocrinol.* **7**, (2016).
337. Dawadi, S. *et al.* Discovery of potent thrombin inhibitors from a protease-focused DNA-encoded chemical library. *PNAS* **117**, 16782–16789 (2020).
338. Norman, A. *et al.* Discovery of Cyclic Peptide Ligands to the SARS-CoV-2 Spike Protein using mRNA Display. *bioRxiv* 2020.12.22.424069 (2020) doi:10.1101/2020.12.22.424069.
339. Dai, W. *et al.* Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science* **368**, 1331–1335 (2020).
340. Douangamath, A. *et al.* Crystallographic and electrophilic fragment screening of the SARS-CoV-2 main protease. *Nature Communications* **11**, 5047 (2020).
341. Tan, X. *et al.* Semi-synthesis of Ubiquitin-propargylamide for identifying deubiquitinase targeting inhibitors. *Chinese Chemical Letters* **30**, 743–746 (2019).
342. Klemm, T. *et al.* Mechanism and inhibition of the papain-like protease, PLpro, of SARS-CoV-2. *The EMBO Journal* **39**, e106275 (2020).
343. Long, M. J. C. & Aye, Y. Privileged Electrophile Sensors: A Resource for Covalent Drug Development. *Cell Chemical Biology* **24**, 787–800 (2017).
344. Vogenberg, F. R., Isaacson Barash, C. & Pursel, M. Personalized medicine: part 1: evolution and development into theranostics. *P T* **35**, 560–576 (2010).
345. Zhang, Q. *et al.* Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. *Science* **370**, (2020).
346. Docherty, A. B. *et al.* Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study. *BMJ* **369**, m1985 (2020).

347. Pairo-Castineira, E. *et al.* Genetic mechanisms of critical illness in Covid-19. *medRxiv* 2020.09.24.20200048 (2020) doi:10.1101/2020.09.24.20200048.
348. Consortium, W. S. T. Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results. *New England Journal of Medicine* (2020) doi:10.1056/NEJMoa2023184.
349. Kingston, D. G. I. Taxol, a molecule for all seasons. *Chem. Commun.* 867–880 (2001) doi:10.1039/B100070P.
350. Honigsbaum, M. *Living with Enza: The Forgotten Story of Britain and the Great Flu Pandemic of 1918*. (Palgrave Macmillan US, 2009). doi:10.1007/978-0-230-23921-0.
351. Sattui, S. E. *et al.* Swinging the pendulum: lessons learned from public discourse concerning hydroxychloroquine and COVID-19. *Expert Review of Clinical Immunology* **16**, 659–666 (2020).
352. Shyr, D. & Shyr, Y. The design and analysis of non-randomized studies: a case study of off-label use of hydroxychloroquine in the COVID-19 pandemic. *Expert Opinion on Investigational Drugs* **0**, 1–7 (2020).
353. Anderson, S., Windscheffel, J. & Gunning, K. Covid Media Misguidance: A Case Report Highlighting the Influence of Media on Patient Medication Decision Making. *Innov Pharm* **12**, 1–1 (2021).
354. Erlanson, D. A. Many small steps towards a COVID-19 drug. *Nature Communications* **11**, 5048 (2020).
355. Anonymous. EU and South Korean research organisations sign an agreement to work together on the development of a COVID-19 treatment using Raloxifene. *Shaping Europe's digital future - European Commission* <https://ec.europa.eu/digital-single-market/en/news/eu-and-south-korean-research-organisations-sign-agreement-work-together-development-covid-19> (2020).
356. Goldstein, J. R. & Lee, R. D. Demographic perspectives on the mortality of COVID-19 and other epidemics. *PNAS* **117**, 22035–22041 (2020).

357. Omori, R., Matsuyama, R. & Nakata, Y. The age distribution of mortality from novel coronavirus disease (COVID-19) suggests no large difference of susceptibility by age. *Scientific Reports* **10**, 16642 (2020).
358. Wise, J. Covid-19: Experts divide into two camps of action—shielding versus blanket policies. *BMJ* **370**, m3702 (2020).
359. Iacobucci, G. Covid-19: Isolating older people would be “age based apartheid,” says NHS boss. *BMJ* **370**, m3788 (2020).
360. Armitage, R. & Nellums, L. B. COVID-19 and the consequences of isolating the elderly. *The Lancet Public Health* **5**, e256 (2020).
361. Smith, M. L., Steinman, L. E. & Casey, E. A. Combatting Social Isolation Among Older Adults in a Time of Physical Distancing: The COVID-19 Social Connectivity Paradox. *Front. Public Health* **8**, (2020).
362. Pearce, N. *et al.* Is death from Covid-19 a multistep process? *medRxiv* 2020.06.01.20116608 (2020) doi:10.1101/2020.06.01.20116608.
363. Shanks, G. D. COVID-19 versus the 1918 influenza pandemic: different virus, different age mortality patterns. *Journal of Travel Medicine* **27**, (2020).
364. Klennerman, P. & Zinkernagel, R. M. Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. *Nature* **394**, 482–485 (1998).
365. Gudbjartsson, D. F. *et al.* Spread of SARS-CoV-2 in the Icelandic Population. *New England Journal of Medicine* **382**, 2302–2315 (2020).
366. Tosif, S. *et al.* Immune responses to SARS-CoV-2 in three children of parents with symptomatic COVID-19. *Nature Communications* **11**, 5703 (2020).
367. Grant, M. C. *et al.* The prevalence of symptoms in 24,410 adults infected by the novel coronavirus (SARS-CoV-2; COVID-19): A systematic review and meta-analysis of 148 studies from 9 countries. *PLoS One* **15**, (2020).

368. Lu, Y. *et al.* Early Warning Indicators of Severe COVID-19: A Single-Center Study of Cases From Shanghai, China. *Front. Med.* **7**, (2020).
369. Aly, M. H. *et al.* <p>Indicators of Critical Illness and Predictors of Mortality in COVID-19 Patients</p>. *Infection and Drug Resistance* vol. 13 1995–2000
<https://www.dovepress.com/indicators-of-critical-illness-and-predictors-of-mortality-in-covid-19-peer-reviewed-article-IDR> (2020).
370. Helmy, Y. A. *et al.* The COVID-19 Pandemic: A Comprehensive Review of Taxonomy, Genetics, Epidemiology, Diagnosis, Treatment, and Control. *Journal of Clinical Medicine* **9**, 1225 (2020).
371. Fang, S. G., Shen, H., Wang, J., Tay, F. P. L. & Liu, D. X. Proteolytic processing of polyproteins 1a and 1ab between non-structural proteins 10 and 11/12 of Coronavirus infectious bronchitis virus is dispensable for viral replication in cultured cells. *Virology* **379**, 175–180 (2008).
372. Daly, J. L. *et al.* Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* **370**, 861–865 (2020).