Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Biomarkers of COVID-19 and technologies to combat SARS-CoV-2

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ABSTRACT

Due to the unprecedented public health crisis caused by COVID-19, our first contribution to the newly launching journal, *Advances in Biomarker Sciences and Technology*, has abruptly diverted to focus on the current pandemic. As the number of new COVID-19 cases and deaths continue to rise steadily around the world, the common goal of healthcare providers, scientists, and government officials worldwide has been to identify the best way to detect the novel coronavirus, named SARS-CoV-2, and to treat the viral infection – COVID-19. Accurate detection, timely diagnosis, effective treatment, and future prevention are the vital keys to management of COVID-19, and can help curb the viral spread. Traditionally, biomarkers play a pivotal role in the early detection of disease etiology, diagnosis, treatment and prognosis. To assist myriad ongoing investigations and innovations, we developed this current article to overview known and emerging biomarkers for SARS-CoV-2 detection, COVID-19 diagnostics, treatment and prognosis, and ongoing work to identify and develop more biomarkers for new drugs and vaccines. Moreover, biomarkers of socio-psychological stress, the high-technology quest for new virtual drug screening, and digital applications are described.

Abbreviations: **ACE2**, Angiotensin-converting enzyme 2; **ACEI**, Angiotensin-converting enzyme inhibitor; **AI**, Artificial intelligence; **AIOD-CRISPR**, All-In-One Dual CRISPR-Cas12a; **ARB**, Angiotensin receptor blocker; **ARDS**, Acute respiratory distress syndrome; **cDNA**, Complementary DNA; **COVID-19**, Coronavirus disease 2019; **CQ**, Chloroquine; **CT**, Computed tomography; **DC**, Dendritic cell; **ELISA**, Enzyme-linked immunosorbent assay; **EUA**, Emergency use authorization; **FDA**, U.S. Food and Drug Administration; **GenOMICC**, Genetics of Mortality in Critical Care; **HCQ**, Hydroxychloroquine; **LFAs**, Lateral flow assays; **LSPR**, Localized surface plasmon resonance; **mAb**, Monoclonal antibody; **MERS**, Middle East respiratory syndrome; **ML**, Machine learning; **NIAID**, U.S. National Institute of Allergy and Infectious Diseases; **NIH**, National Institutes of Health; **PAC-MAN**, Prophylactic Antiviral CRISPR in huMAN cells; **PCR**, Polymerase chain reaction; **PCT**, Procalcitonin; **RT-PCR**, Reverse transcription polymerase chain reaction; **SaaS**, Software as a Service; **SARS**, Severe acute respiratory syndrome; **SARS-CoV-2**, SARS coronavirus type 2; **TCM**, Traditional Chinese medicine; **UCSF**, University of California San Francisco; **UCB**, University of California Berkeley.

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1. Introduction: An unprecedented pandemic crisis

1.1. Responses to the novel coronavirus

On December 31, 2019, a cluster of unusual pneumonia-like cases was reported in Wuhan, China. This unknown disease was later identified as a novel coronavirus (SARS-CoV-2) infection and named COVID-19. The pandemic has since spread to a total of 213 countries and territories with more than **738,600** deaths among > **20.2 million** infected people (by August 10, 2020 − Worldometers).[1] The spread has been so swift that our general population, public health workers, medical professionals, scientists, and government officers have found it difficult to respond in a coordinated and quick manner. Many countries have taken vastly different but effective approaches to fight the virus,[2] ranging from the complete lock-down observed in Wuhan, China, widespread testing in South Korea, diligent contact tracing from Singapore, Taiwan, and Hong Kong; and a luckily averted initial plan by the UK government to build non-vaccinated natural herd immunity.[3]

The United States has taken its own unique approach to combat COVID-19, leaving it largely up to the individual states to implement shelter-in-place and social distancing policies as the coronavirus crisis began to consume the country. The delayed response by the federal government has been critiqued both within the U.S. and around the world, and has called into question the ability of the U.S. government to exert global leadership during the pandemic. The U.S. currently holds the record for the highest total numbers of COVID-19 cases **(> 5.2 million**) and deaths (**~166,000**) in the world (by 08−10-2020).[1]

The delayed response to the global crisis and other key missteps in early action have highlighted the fact that the U.S. government needs to: 1) invest more in public health infrastructure; 2) coordinate fiscal and health policy implementation; and, 3) slash the bureaucratic red tape. Although we could not have completely prevented the advent of this virus and its consequences, we can utilize biomarkers (for detection, diagnosis, treatment, and prevention) as an important tool to address the needs of the ongoing COVID-19 crisis.

1.2. Importance of biomarkers

Use of biomarkers are particularly important during our current pandemic state since they can enhance the development and approval of new, innovative drugs and biological products, particularly in the field of vaccines. Clinical biomarkers are generally defined as the *measurable biological indicators* of the presence, severity or type of disease in medical settings.[4] Biomarkers can be applied to describe observable characteristics of a certain disease and to determine optimal treatments based on these phenotypes, as well as genotypes, thence, they have received substantial attention.[5] In particular, respiratory disease biomarkers, such as those associated with acute respiratory distress syndrome (ARDS), have been associated with increased mortality (IL-8, ICAM-1) and improved survival (nitric oxide).[6] These biomarkers play a pivotal role in predicting future complications or severity of disease, and could be useful as an indicator for COVID-19 prognosis.

As the number of COVID-19 cases has rapidly increased, the amount of scientific publications on COVID-19 research has also grown exponentially. Among the myriad reports of coronavirus investigations and innovations, however, there remains a limited number of publications regarding COVID-19 biomarkers. In this current article, we aim to provide an overview of biomarker applications throughout this pandemic crisis and to review several known and emerging biomarkers for SARS-CoV-2 detection, COVID-19 diagnostics, treatment and prognosis, as well as ongoing biomarker development for new drugs and vaccines.

2. Basics and pathogenesis of SARS-CoV-2

SARS-CoV-2, the causative pathogen of COVID-19, is named for its close resemblance to the original SARS (severe acute respiratory syndrome) virus. The virus's entire genome has been sequenced and scientists have characterized the shape and structure of proteins on the viral surface down to the position of individual atoms. This information is vital in order to be able to identify novel biomarkers that can be used for detection, diagnosis, and prognosis in the pandemic response.

2.1. SARS-CoV-2 structure, RNA genome and proteins

Similar to known coronaviruses (*e.g.* SARS-CoV and MERS-CoV), SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus from the family *Coronaviridae*, genus *Betacoronavirus*. Its RNA sequence is approximately 30,000 bases in length.[7,8] The four main structural proteins of coronavirus particles are crown-like spike (S-) glycoprotein, membrane (M-) glycoprotein, envelope (E-) protein on the viral surface, and nucleocapsid (N-) protein (Fig. 1a).[7,9]

Among the known viral proteins, M- and E-proteins form a ball protecting the RNA genetic core, which is wrapped by the N-protein in a "beads-on-a-string" type conformation. The S-protein is responsible for facilitating entry of SARS-CoV-2 into the target cell, forming protrusions that can bind to receptors on target cells for infection and giving the virus a crown-like shape, hence the name "coronavirus". A detailed observation of the S-protein structure and sequence of the SARS-CoV-2 genome provide indications of the origin of this mysterious new coronavirus: *the bat*.[10]

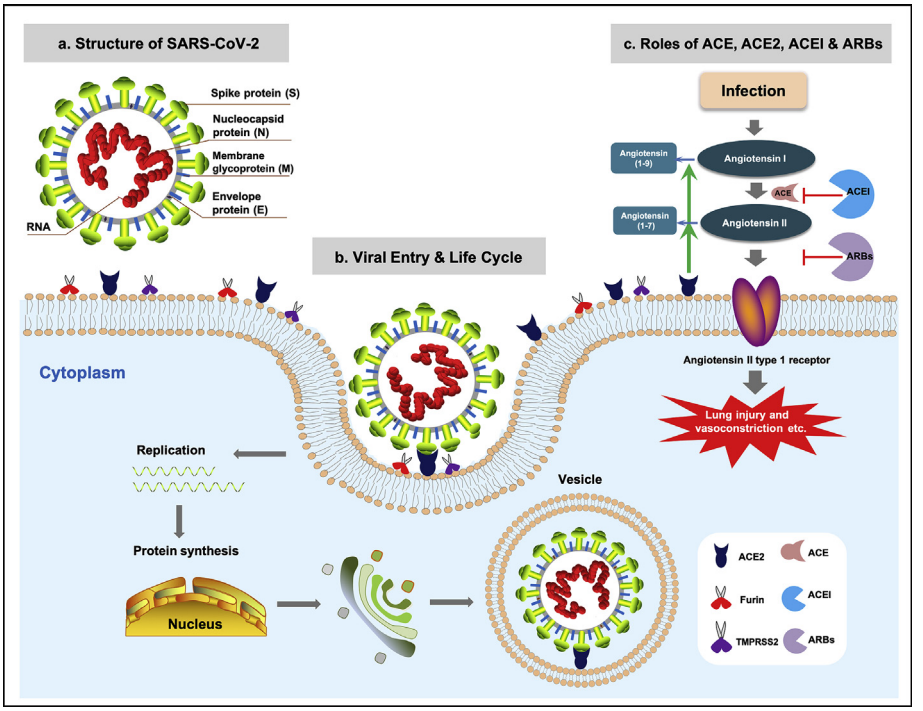

Fig. 1. Basics of the novel coronavirus SARS-CoV-2. a) Structure of SARS-CoV-2: Viral RNA and structural proteins (S, E, M, N); b) Viral entry and life cycle: Host-pathogen interactions; and c) Roles of ACE, ACE2, ACEIs and ARBs (ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers).

2.2. The origin of SARS-CoV-2

There have been a host of different theories surrounding the original source of SARS-CoV-2. One persistent myth of how the new coronavirus entered the human world is that it originated from a research laboratory. Scientists, however, remain firm that the virus was transmitted from natural selection in the environment. Evidence indicates that the virus originated in bats, then expanded to an unknown intermediate carrier before finally jumping to the human population.[11] Multiple analyses of the viral S-protein structure and genome sequence suggest that the intermediate host responsible for passing the SARS-CoV-2 from bats to human is *pangolins*.[10,12] Additionally, a new RmYN02 virus, recently discovered in bats, contains insertions of amino acids in its spike protein that are similar to SARS-CoV-2, providing further evidence to support the natural evolution of SARS-CoV-2.[13] Thus, many scientists agree the proximal origin of SARS-CoV-2 is not a laboratory construct or a purposefully manipulated virus, but a result of natural selection.[11]

2.3. SARS-CoV-2 entry required factors

Like all other viruses, SARS-CoV-2 invades host cells through an interaction between the spike protein (**S-protein**) on the *viral particle* surface and a *receptor protein* on the membrane of the host cell. Once inside, the virus hijacks the cell's reproductive machinery to produce more viral copies to eventually infect more cells. Structural analysis has suggested that the receptor for the virus is a protein called the angiotensin-converting enzyme 2 receptor (**ACE2**).[11,14,15] SARS-CoV-2 often requires cofactors, **furin** and **TMPRSS2**, two protein-cleaving enzymes that enable cellular infection by cleaving the viral S-protein and activating it for virus-cell fusion (Fig. 1b).[16,17] Additionally, furin plays an important role in the life cycle of SARS-CoV-2, which is distinctly different than SARS-CoV.[18] Several other protein-protein interactions have been reported between SARS-CoV-2 and human host cells that could potentially be targets for COVID-19 treatment.[19]

2.4. ACE2 and TMPRSS2 as potential therapeutic targets

SARS-CoV-2 entry, which is heavily dependent on the human ACE2 receptor and serine protease TMPRSS2, has been shown to be blocked *in vitro* by a serine protease inhibitor, camostat mesylate.[14] This finding suggests that the viral S-protein and cellular TMPRSS2 could be potential targets for therapeutic intervention. Examples of possible therapies include antibodies (convalescent or recombinant) against the spike protein and camostat-like protease inhibitors.

Additionally, soluble ACE2 has been effective in the past to block the binding of SARS-CoV S-protein, potentially slowing down viral replication.[20] In fact, ACE2 and angiotensin have been found to be protective in a number of different lung injury models.[21,22] Thus, a closer look at the underlying mechanism of SARS-CoV-2 viral entry has buoyed another idea for treatment – giving patients decoy ACE2 receptors to direct SARS-CoV-2 away from vulnerable host cells. This approach has been shown to be effective in reducing viral growth in cell cultures as well as blood vessel and kidney organoids.[23] Building upon these results, Aperion Biologics is conducting a clinical pilot study on COVID-19 patients with a new drug APN01, which contains recombinant human ACE2 as its active substance.[24]

2.5. Concerns of ACE, ACE2 and their inhibitors and blockers

Beyond functioning as the key SARS-CoV and SARS-CoV-2 receptor, the primary role of ACE2 is to act as a regulator of the renin-angiotensin-aldosterone system, a hormone system that regulates blood pressure, blood volume, and electrolyte balance in the body. Due to the role that ACE2 plays in SARS-CoV-2 viral entry, there has been a growing concern that anti-hypertensive medications such as ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) could affect the severity and mortality of COVID-19 (Fig. 1c).[21]This concern is two-fold: 1) ACEIs could potentially inhibit ACE2 due to the high degree of sequence similarity between ACE and ACE2; and, 2) the use of ACEIs and ARBs could increase expression of ACE2, rendering patients more susceptible to viral host cell entry and propagation. Although a prior study has shown that ACEIs in clinical use did not directly affect ACE2 activity,[25] the significance of ACE2 expression on COVID-19 pathogenesis and mortality is still unclear and warrants further investigation.[21,22]

2.6. ACE2 expression in susceptible cell types and at-risk human populations

SARS-CoV-2 viral tropism: Respiratory cells are vulnerable to coronavirus infection because they express ACE2, which engages the viral S-protein, and TMPRSS2, which helps merge virus and target cell membranes. ACE2 is known to be expressed in the human nose, lungs, heart, kidneys, intestines, brain, and testicles (Fig. 2).[26] Results from multiple studies have indicated that the higher the ACE2 (or its expression) level is, the higher the COVID-19 risk. Recent analyses from single-cell sequencing datasets support the idea that COVID-19 is not just a respiratory disease but can also affect the gastrointestinal tract, the nervous system, and other organs in the body (*e.g.,* strokes, blood clots, hypoxia, and cardiovascular complications), thus explaining the multifaceted nature of COVID-19 infection.[27]

Vulnerable cell types: SARS-CoV-2 is mainly transmitted between people through respiratory droplets from the nose and lungs where some specific cell types have been identified as likely initial infection sites. Using single-nuclei and single-cell RNA sequencing, one study reported that ACE2 and TMPRSS2 are most richly expressed in *bronchial transient secretory* cells among diverse cell types in respiratory tissues (Fig. 2).[28] Similarly, another study analyzed the expression of viral entry-associated ACE2 and TMPRSS2 genes and found high expressions in nasal epithelial cells, specifically *goblet* and *ciliated* cells in the nose (Fig. 2).[29] These results were further confirmed by a larger study.[30]

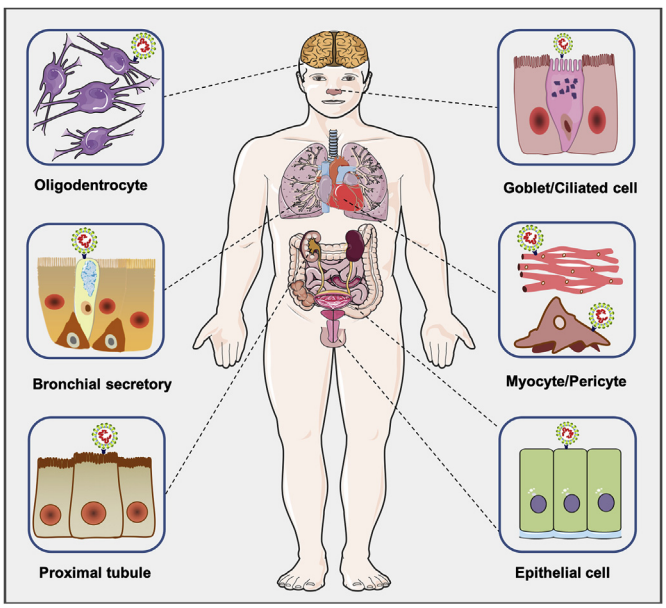

Fig. 2. ACE2 distribution in human tissues and specific cell types.

Besides cells in the respiratory system, cells in the eyes and some other organs also contain the critical viral-entry proteins. For instance, *gastrointestinal* cells are loaded with ACE2. A new study carried out in 3D models showed that SARS-CoV-2 could infect organoid cells not only from the airway, but also the gut.[31] Despite that, it remains unclear whether intestinal cells could actually get infected and produce viral particles. However, SARS-CoV-2 has been detected in human stool long after respiratory symptoms resolved and gastrointestinal symptoms in COVID-19 patients are prevalent, which suggests that the gut could be a likely reservoir, indicating the potential of transmission via the fecal-oral route.

Susceptible human populations: Interestingly, studies also indicated that ACE2 receptor density in bronchial cells is higher with age, and in men compared with women, which correlates to the higher infection rates in seniors and males as reported from epidemiological studies.[28] Plasma ACE2 has also been reported at a much higher level in men than in women.[32] Notably, the gene encoding ACE2 protein is X-linked and is expressed at a particularly high level in the testes, which could explain the gender differential observed in infection and mortality.[33] Moreover, ACE2 gene expression in nasal epithelium, reported as *age*-dependent, is significantly lower in younger children than adults, which may explain why COVID-19 is less prevalent in children.[34]

The *small airway epithelial* lung cells of current cigarette smokers and individuals with chronic obstructive pulmonary disease also have increased levels of ACE2,[35] which suggests that abstaining from smoking could lessen the chance that an infection with SARS-CoV-2 coronavirus will lead to severe COVID-19 disease.

Genetic variants linked to severity: Other genetic variants in the *ACE2* gene, reportedly linked to cardiovascular and lung conditions, could predispose infected individuals to more severe COVID-19 disease.[36] Recent studies have revealed that genes ***SLC6A20*** (an encoded amino acid transporter that interacts with ACE2) and ***TLR7*** (toll-like receptor 7 on chromosome X) also link to COVID-19 severity.[37,38]

Blood type and *ABO* gene: Several studies have suggested that blood type could be implicated in susceptibility and severity for COVID-19, with a particular focus on the role of the *ABO* gene.[37,39,40] Recently, 23andMe, a personal genomics and biotechnology company in California, has corroborated this link.[41] Their preliminary results suggest that **blood type O** appears to be protective against SARS-CoV-2 when compared to all other blood types. From a different viewpoint, however, a group of hematologists have argued that the serum ***anti-A antibody*** (more specifically IgG anti-A in blood O group) is more significant as a biomarker for COVID-19 protection than the blood type itself.[42]

2.7. Epigenetics of ACE2

The COVID-19 symptoms for infected individuals range from no symptoms at all to mild, moderate or severe disease. A few recent studies have pointed to epigenetics to explain the difference. One study reports that epigenetic dysregulation of ACE2 and other interferon-regulated cytokine genes may increase COVID-19 susceptibility and severity in lupus patients.[43] A separate study has reported that DNA methylation patterns associated with the ACE2 gene differ among individuals: by gender in lung tissues, and by age in epithelial cells.[44]

In general, epigenetic mechanisms are an important part of the pathophysiology and illness severity of COVID-19. It is not only identified in SARS-CoV-2, but also in other viral infections.[45] When these mechanisms are confirmed, epigenetic interventions influencing DNA methylation could be indicated as primary and/or secondary preventive options besides the current classical epidemic controls.

3. SARS-CoV-2 detection and COVID-19 diagnostics

Detection refers to the act of identifying disease-specific molecules, molecular changes, or distinct physiological signatures. These detection biomarkers enable diagnosis of disease and are useful not only for identifying patients, but also for detecting infected carriers who display no symptoms, a distinct characteristic of SARS-CoV-2. It is worth noting that since asymptomatic carriers do not manifest any clinical symptoms, their nucleic acid testing is only a measure of *viral detection* not *disease diagnosis*. For symptomatic COVID-19 patients, the methods described below could serve for both SARS-CoV-2 detection and COVID-19 diagnosis. For readers' convenience, we have summarized these detection methods and their applications in Table 1.

3.1. Biomarkers of viral detection

The SARS-CoV-2 RNA genome currently serves as a major biomarker of direct viral detection and the primary COVID-19 diagnosis. Viral proteins encoded by SARS-CoV-2 could theoretically serve as alternative biomarkers of viral detection, but due to the complexity of protein detection and the significantly larger amount of biological samples required, are often impractical targets. Many of these viral proteins, however, could serve as potential targets for anti-viral drugs or biomarkers of drug development for COVID-19 treatment,[46] which will be discussed in Section 4.

3.2. PCR-based analysis

The primary method of SARS-CoV-2 detection relies on polymerase chain reaction (PCR), a technique widely used in molecular biology to amplify DNA samples. Since the novel coronavirus genome consists of single-stranded RNA, reverse

Table 1
Summary of biomarkers of SARS-CoV-2 detection and COVID-19 diagnosis.

Biomarker Target	Assay	Type	Product Name	Developer	FDA EUA	Description	Source
Viral RNA Genome	**PCR-based**	qSanger-based Laboratory testing	BillionToOne COVID-19 assay	BillionToOne	No	Can support higher testing capacity utilizing qSanger spike-in and ML algorithms.	https://billiontoone.com/covid-19/
		RT-PCR Point-of-care detection	Xpert Xpress	Cepheid	Yes	Automated molecular test for the qualitative detection of the virus; results available in ~45 min.	https://www.cepheid.com/coronavirus
		PCRPoint-of-care detection	Accula	Mesa Biotech	Yes	Qualitative, visually read test using throat and nasal swabs; results available in ~30 min.	https://www.mesabiotech.com/coronavirus
		RT-PCR Point-of-care detection	ID NOW	Abbott	Yes	Qualitative detection of nucleic acids via nasal, nasopharyngeal, and oropharyngeal swabs.	https://www.alere.com/en/home/product-details/id-now-covid-19.html
		RT-LAMP Point-of-care detection	RT-LAMP COVID-19 test	Beaumont Health System, Michigan	No	Technique that amplifies RNA rather than DNA; Test can be stored at room temperature, done in a single tube, and color-marked so that the mixture changes color if target RNA is present.	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0234682
	Digital PCR	Digital PCR Point-of-care detection	Naica™ System	Stilla Technologies	No	Digital PCR solution combined with a COVID-19 detection kit that can identify SARS-CoV-2 and measure its viral load.	https://www.stillatechnologies.com/wp-content/uploads/2020/05/PR_StillaTechnologies_COVID_19_04052020-1.pdf
		Droplet-digital PCR Point-of-care detection	Saliva-based COVID-19 test	University of Chicago	No	Saliva-based COVID-19 test utilizing droplet-digital PCR.	https://medicine.uchicago.edu/an-alternative-for-the-brain-tickler-uchicago-scientists-explore-saliva-testing-for-covid-19/
	CRISPR-based	CRISPR-Cas13 Point-of-care detection	Sherlock™ CRISPR SARS-CoV-2 test kit	Sherlock Biosciences	Yes	CRISPR-programmed detection of SARS-CoV-2 genetic signature via nasal, nasopharyngeal, oropharyngeal or BAL specimen; results available in ~1 h.	https://sherlock.bio/sherlock-biosciences-receives-fda-emergency-use-authorization-for-crispr-sars-cov-2-rapid-diagnostic/
		CRISPR-Cas12a Point-of-care detection	AIOD-CRISPR	University of Connecticut	No	Visual detection via CRISPR-based nucleic acid detection initiated by dual crRNAs.	https://www.biorxiv.org/content/10.1101/2020.03.19.998724v1
Spike Protein	**Serological**	ELISA Laboratory testing	COVID-19 ELISA IgG Antibody Test	MountSinaiLaboratory	Yes	Detects IgG antibodies as indicative of an immune response to SARS-CoV-2 in suspected patients.	https://www.mountsinai.org/about/newsroom/2020/mount-sinais-blood-test-to-detect-antibodies-to-covid19-receives-emergency-use-authorization-from-us-food-and-drug-administration-pr
	Recombinant antigens	LFA (IgM & IgG)Point-of-care detection	IgM & IgG Rapid Test Kit	Premier Biotech	No	Qualitative detection of SARS-CoV-2 antibodies in whole blood, serum, or plasma; results available in ~10 min.	https://premierbiotech.com/innovation/covid-19/
		LFA (IgM/IgG)Point-of-care detection	IgM/IgG Rapid Test Kit	RayBiotech	No	Detects IgG and IgM antibodies to the coronavirus N-protein in serum, plasma, and peripheral blood; results available in ~10 min.	https://www.raybiotech.com/coronavirus-research-products-covid-19/
Glycans	**Glycan biology**	LFA Point-of-care detection	Glycan recognition test	Iceni Diagnostics	No	Consists of gold nanoparticles coated with host carbohydrate structures that are recognized specifically by SARS-CoV-2.	https://www.genengnews.com/insights/iceni-diagnostics-hopes-for-home-use-coronavirus-test-this-autumn/

Abbreviations: AIOD-CRISPR, All-in-One Dual CRISPR-Cas12a; CRISPR, clustered regularly interspaced short palindromic repeats; BAL, bronchoalveolar lavage; COVID-19, coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; EUA, Emergency Use Authorization; FDA, U.S. Food and Drug Administration; hr, hour; Ig, immunoglobulin; LFA, lateral flow assay; min, minutes; ML, machine learning; N-protein, nucleocapsid protein; PCR, polymerase chain reaction; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT-PCR, reverse transcription polymerase chain reaction; and, SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

transcription PCR (RT-PCR) is commonly required to convert RNA transcripts into complementary DNA (cDNA) in a biological sample. Real-time PCR/RT-PCR enables early, sensitive, and specific viral detection because: 1) it *directly* detects nucleic acid (*e.g.*, viral RNA); and, 2) it *amplifies* the genomic material even when only a low level of the viral RNA is present in collected biological samples.47.

At the present, however, current PCR-based methods do not support the testing volume needed for a rapid COVID-19 response. In order to accommodate the higher testing demand, BillionToOne — a precision diagnostics developer — has recently developed a novel assay that utilizes high-throughput Sanger sequencing and machine learning (ML) algorithms to sequence the entire SARS-CoV-2 genome. However, high throughput detection methods such as BillionToOne's new assay, often require a centralized clinical laboratory with high-throughput equipment and well-trained personnel. Thus, point-of-care diagnostic applications are far more suitable for the simple, rapid, mobile testing that is critical to slow the spread of COVID-19.

3.3. Point-of-care detections

There are currently many small, rapid and readily useable devices for viral detection that are appealing to healthcare providers. One of the first to be approved by the U.S. Food and Drug Administration (FDA), was Cepheid's Xpert Xpress SARS-CoV-2 assay, an automated molecular test that targets multiple regions of the viral genome for *quantitative* detection of SARS-CoV-2 in around 45 min. Another assay, the Accula™ System, developed by Mesa Biotech, is a qualitative, visually read test for SARS- CoV-2 with results available in 30 min. The quickest point-of-care testing system currently on the market for qualitative detection of SARS-CoV-2, however, is Abbott's ID NOW™. This unique isothermal nucleic acid amplification technology provides molecular results in just 2—5 min, allowing medical workers to make effective clinical decisions much sooner than other testing methods. Recently, however, the accuracy of the Abbott ID NOW test has come under question.[48]

A new test, dependent on **RT-LAMP** (Reverse Transcription Loop-Mediated Isothermal Amplification), a technique similar to RT-PCR but amplifies RNA rather than DNA, has been developed by researchers from the Beaumont Health System in Michigan.[49] This method is simpler and cheaper than PCR, since it does not require the repeated heating cycles of PCR, and can detect infection in less than 45 min in urine, blood and saliva.

3.4. Advanced digital PCR detections

Recently, Stilla Technologies announced that its **digital PCR (dPCR)** test, the Naica system, is readily available to all research centers and hospitals involved in the fight against COVID-19. The Naica System reportedly offers a more accurate and sensitive method than RT-PCR, making it possible to cut down on the *false negative* results that currently hamper efforts to contain the re-emergence of the disease.

Additionally, an alternative analysis technique, **droplet-digital PCR** (**ddPCR**), with a greater sensitivity than the commonly used qPCR, is being trialed to detect SARS-CoV-2 in spit samples. A saliva-based digital testing method has two benefits over the nasal swab in that 1) it has been shown to be more sensitive for SARS-CoV-2 detection, and 2) is minimally invasive.[50]

3.5. CRISPR-based detections

Touted as "faster, better, and cheaper" than traditional PCR methods, CRISPR-based nucleic acid detection has received increased attention in recent years.[51] CRISPR tests are capable of diagnosing infections as accurately as conventional methods and can be easily converted to a point-of-care diagnostic. Sherlock Biosciences has designed the first CRISPR-based COVID-19 diagnostic to receive the FDA EUA (Emergency Use Authorization). This at-home assay depends upon the programming of a CRISPR molecule to pick up the genetic signature of SARS-CoV-2. If the signature is sensed, the CRISPR enzyme is activated and releases a detectable signal, providing results in an hour. Additional CRISPR-based efforts include the University of Connecticut's All-In-One Dual CRISPR-Cas12a (AIOD-CRISPR)[52] for detection and Stanford University's PAC-MAN (Prophylactic Antiviral CRISPR-Cas13 in huMAN cells) for treatment.[53] The latter has been shown to be effective *in vitro*, although an effective and safe *in vivo* delivery method into human cells must be developed before therapeutic use.[53]

3.6. Antibodies binding to S-protein against viral infection

In many infected patients, their *adaptive* immune response will naturally develop antibodies to the spike protein in a process called "*active immunity*". The antibodies bind to the S-protein and "*neutralize*" the virus by preventing it from interacting with ACE2, inhibiting its ability to infect more cells and therefore limiting disease progression. Similarly, this neutralization can also happen through "*passive immunity*" which involves giving patients antibodies against the spike protein as a treatment.

Thus, these antibodies are often used as a biomarker of viral detection, disease diagnosis *and* treatment. The antibodies to the spike protein can be measured by immunological assays like ELISA (enzyme-linked immunosorbent assay), which can detect antibodies, peptides, or proteins to verify the presence of virus in biological samples. ELISA kits are currently being used to detect the viral S-protein and human ACE2 to study the interaction of SARS-CoV-2 and its host receptor.[14,20] The CDC

has also developed a S-protein ELISA assay for use in performing contact investigations and conducting large-scale, cross-sectional studies to define disease burden in the population via sero-surveillance.[54]

For COVID-19, ELISA testing has been employed to: 1) diagnose patients who are more than 1-week post symptom onset, 2) determine potential immunity and risk of infection, 3) advance contact tracing, and 4) understand the extent of COVID-19 spread and immunity in communities through epidemiological studies.

3.7. Glycan as an emerging biomarker for viral detection

Both the viral S-protein and the host ACE2 receptor are known to be extensively glycosylated. When viruses invade the human body through the respiratory tract, they often utilize sugar chains on the surface of host cells, known as glycans. The viral S-protein has recently been shown to contain 66 glycosylation sites,[55] each of which can be occupied by up to 10 different glycans when tested with SARS-CoV-2 glycoproteins.[56] Despite this fact, glycans are often overlooked because of their chemical complexity and the limited throughput and sensitivity of existing analytical instruments. A new home-use coronavirus test that utilizes this glycan biology to identify the virus is currently under development by Iceni Diagnostics. Beyond viral detection, the SARS-CoV-2 glycans could be of benefit for COVID-19 drug design. For example, S309, a monoclonal antibody identified from a SARS-CoV-2 survivor, can recognize and interact with glycan epitopes on the spike protein and neutralize both SARS-CoV-2 and SARS-CoV.[57] This novel approach is advantageous since glycans remain relatively constant compared to the viral genetic code, which could be prone to mutation.

4. Biomarkers of COVID-19 treatment

Currently, there are no proven drug therapies for treating COVID-19. Under the FDA's EUA, however, several approved drugs, including small-molecule compounds and monoclonal antibodies (mAbs) have been in clinical trials. Other alternatives, such as convalescent plasma/serum treatment, allogeneic cell therapy, and traditional Chinese medicine (TCM) are also under consideration for COVID-19 treatment.

4.1. New therapies developed from existing drugs

To quickly seek a *new* treatment for COVID-19, a team of Calibr scientists at Scripps Research Institute is leveraging a unique resource — the ReFRAME drug collection of over 14,000 *old* compounds that have been previously approved by the FDA for other diseases or have been extensively tested for human safety. A medicine identified from the ReFRAME library could be rapidly repurposed and made available to treat COVID-19 patients on a much quicker timescale than new therapies since its safety, tolerated dose and manufacturing practices are already known.[58]

For instance, **dexamethasone** is a corticosteroid used in a broad range of conditions for its anti-inflammatory and immunosuppressant effects. Recent preliminary results from the large RECOVERY trial in U.K. suggest that the *low-cost* and *widely available* drug dexamethasone decreased the risk of death in severe COVID-19 patients by one-third in ventilated patients and 20% in patients receiving oxygen.[59] These findings suggest the drug could become standard care in COVID-19 severe patients.

4.2. Examples of small-molecule therapies

Several of these already existing "*front-runner*" drug candidates have been approved by the FDA for clinical trials (discussed below). Other drugs that have not yet been tested, but are under consideration include ivermectin,[60] Calquence, and colchicine.[61] To help navigate through these potential therapeutic options for COVID-19, we have compiled a list of selected candidates in Table 2.

Remdesivir, developed by Gilead, has shown *in vitro* activity against SARS-CoV-2[62] as well as prophylactic and therapeutic efficacy in nonclinical models of other types of viruses, such as SARS-CoV, MERS-CoV and Ebola.[63,64] Since remdesivir is intracellularly metabolized to an analog of adenosine triphosphate, its primary mechanism of inhibition is its incorporation into the nascent RNA chain by viral RNA polymerase, thus halting viral replication.[63] Based on promising results from a randomized, double-blinded, placebo-controlled trial conducted by the National Institute on Aging[65] and the Gilead-sponsored open-label trial,[66] remdesivir has been granted EUA by the FDA as a treatment for COVID-19 patients with severe disease.

EIDD-2801 is a new antiviral drug, modified from EIDD-1931, that has been tested in cultured human lung cells infected with SARS-CoV-2 and mice infected with related coronaviruses SARS-CoV and MERS-CoV.[67] Results show that EIDD-2801 can prevent severe lung injury in infected animals when used prophylactically or administered early following infection.[67] The drug has been granted Investigational New Drug status by the FDA. If ongoing clinical trials are successful, the EIDD-2801 drug could be used to limit the spread of SARS-CoV-2 in addition to controlling future outbreaks of other emerging coronaviruses.

HCQ and CQ: Hydroxychloroquine (HCQ) and chloroquine (CQ), a decades-old anti-malaria drug touted by some political leaders, have received intense worldwide attention for its potential activity against SARS-CoV-2 *in vitro*.[68,69] Both CQ and HCQ share the same mechanisms of action, although HCQ is typically more tolerable and less toxic than CQ.[68,70] It has been suggested that CQ/HCQ may inhibit coronaviruses through changing the pH in the cell membrane surface to decrease viral

fusion to the host cell membrane, interfering with lysosomal activity and autophagy, suppressing viral nucleic acid replication, protein glycosylation and particle transport; and, blocking viral release.[68,70] Although these drugs are currently in use to treat medical conditions such as lupus and rheumatoid arthritis, they have not yet been proven to be effective for COVID-19.

Despite its early promise *in vitro*, results from trials worldwide have been disappointing. A clinical trial in Brazil was terminated early due to several patients developing fatal heart damage and/or arrhythmia[71] and HCQ has shown *no* evidence of clinical efficacy in *severely ill* COVID-19 patients in France[72] or the U.S.[73] Later studies additionally show that HCQ has no effect on alleviating mortality rates in hospitalized COVID-19 patients.[74,75] Thus, recruitment for the PRINCIPLE trial in the UK has been halted temporarily due to concerns about the safety of HCQ. Furthermore, the US FDA recently (June 15, 2020) repealed the EUA granted for HCQ/CQ because of a lack of consistent replication of earlier promising results and results from a randomized controlled clinical trial that showed no clinical benefit for HCQ.

4.3. Therapeutic antibodies

Monoclonal antibodies (mAbs) are produced by B-cells to target antigens. Since the first therapeutic mAb's approval by the FDA in 1986, therapeutic antibodies have become the predominant class of new drug development due to their high specificity.[76] During the current pandemic, many researchers have been trying to develop antibody treatments for COVID-19. Here, we discuss a few examples.

Kevzara® (also named **sarilumab**) is a mAb and an IL-6 receptor antagonist approved by the FDA in 2017 to treat rheumatoid arthritis. In light of the pandemic, it is thought that it can inhibit the inflammatory response in COVID-19 patients, who often exhibit elevated levels of pro-inflammatory molecules such as IL-6. Kevzara is currently undergoing Phase II and III clinical trials. Additionally, **leronlimab**, a mAb and CCR5 antagonist recently developed by CytoDyn, has been approved by the FDA as an Emergency Investigational New Drug. One of the multiple ongoing clinical studies of leronlimab has shown promising responses in *mild-to-moderate* patients.[77] Recently, the neutralizing IgG1 mAb **LY-CoV555** developed by Eli Lilly targets directly on the S-protein of SARS-CoV-2 and is in a small ongoing first trial.

In addition to the traditional mAb treatments described above, Active Motif Shanghai, in collaboration with Fudan University and its affiliated Public Health Clinical Center, has successfully used its single-cell **AbEpic** screening technology to produce *recombinant* human COVID-19 antibodies. It has been demonstrated that these recombinant antibodies bind to the viral S-protein and interfere with its interaction with the ACE2 receptor to neutralize SARS-CoV-2's activity in *in vitro* infection assays with pseudotyped viruses bearing the S-protein of SARS-CoV-2.[78] **47D11**, another *recombinant* mAb drug, has also shown early promise, potently inhibiting infection of kidney cells *in vitro*.[79] It acts by binding to cells expressing the full-length spike protein of SARS-CoV-2 in cell culture.

Ultimately, a cure for COVID-19 might come from a combination of drugs targeting multiple pathways. Examples of drug combinations currently under consideration are Actemra® (an arthritis mAb also called tocilizumad) with remdesivir[80] or leronlimab plus colchicine.[81] A pair of human neutralizing mAbs, B38 and H4, has also been shown to simultaneously block the binding between viral S-protein and cellular receptor ACE2.[82] Another cocktail pair, now named *REGN-COV2*, consists of two antibodies (REGN10933 and REGN10987) that are designed to bind non-competitively to the receptor binding domain of the S-protein, and was launched in a Phase II/III clinical trial by Regeneron Pharmaceuticals.

4.4. Allogeneic cell therapy

Cell-based therapies have recently come to the fore as a new paradigm in therapeutics. One allogeneic cell therapy is currently under consideration for COVID-19 treatment. Known as CAP-1002, the procedure comprises of infusing laboratory-grown, cardiosphere-derived cells, into COVID-19 patients. In a study of 6 critically ill COVID-19 patients, following intravenous infusion of **CAP-1002**, all six patients survived, and clinical biomarkers such as ferritin levels and absolute lymphocyte counts were observed to improve in comparison with a control group that experienced 18% mortality.[83]

4.5. Traditional Chinese medicine for COVID-19

Largely unheard of in the Western world, traditional Chinese medicine (TCM) was used to treat COVID-19 patients when there were no other effective remedies available in China. The China NIH publicly announced the three best formulated herb remedies (金花清感颗粒，莲花清瘟胶囊，血必净注射液) and three TCM recipes (清肺排毒汤,化湿败毒方，宣肺败毒方), which have been officially used to treat over 90% COVID-19 patients in China.[84] An evidence-based guideline with diagnosis and treatment protocols has been published for the use of healthcare professionals and researchers.[85] One clinical study of ***Lianhuaqingwen***, a Chinese herb product, has reported that TCM improved symptoms, reduced hospitalization days (average < 10 days) and minimized the chance of mild/moderate patients progressing to severe disease.[86] A protocol of a prospective systematic review and meta-analysis to evaluate TCM treatment in China has since been published as well.[87]

5. Biomarkers of COVID-19 prognosis

A prognostic biomarker is a clinical or biological characteristic that provides information on the likely patient health outcome (i.e. disease recurrence) irrespective of treatment.[88] Prognostic biomarkers frequently serve as a useful prediction

Table 2
Summary of biomarkers of COVID-19 treatment.

Product Name	Target	Basic Mechanism	Developer	FDA EUA	Source
Small-molecule therapy					
Remdesivir	Viral RNA polymerase	ATP analog that inhibits replication	Gilead	Yes	https://www.gilead.com/purpose/advancing-global-health/covid-19/remdesivir-clinical-trials
EIDD-2801	Viral RNA polymerase	Ribonucleoside analog that inhibits replication	Emory Institute for Drug Development	No - IND	https://stm.sciencemag.org/content/12/541/eabb5883
HCQ and CQ	Unclear	Hypothesized to change cell membrane pH to decrease viral fusion, interfere with lysosomal activity and autophagy, suppress viral replication, protein glycosylation, and particle transport, and block viral release	Various and marketed by Sanofi	No – Withdrawn by the FDA on 6-15-20	https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-revokes-emergency-use-authorization-chloroquine-and
Ivermectin	Unclear	Inhibits replication	Various	No	https://www.sciencedirect.com/science/article/pii/S0166354220302011
Calquence	BTK	Inhibits BTK to ameliorate effects of cytokine storm in severe patients	AstraZeneca	No	https://www.astrazeneca.com/media-centre/press-releases/2020/astrazeneca-initiates-calavi-clinical-trial-with-calquence-against-covid-19.html
Colchicine	Granulocytes and monocytes	Anti-inflammatory treatment	Various	No	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4656054/
Dexamethasone	Glucocorticoid receptor	Corticosteroid that binds to the glucocorticoid receptor to inhibit pro-inflammatory signals and promote anti-inflammatory signals	Various	No	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7343652/
CRISPR therapy					
PAC-MAN	Viral RNA genome	CRISPR-Cas13, a virus-killing enzyme and gRNA, commands Cas13 to destroy specific sequences in SARS-CoV-2	Stanford University	No	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7189862/
Monoclonal antibody					
Kevzara	IL-6 receptor	IL- 6 receptor antagonist	Regeneron Pharmaceuticals & Sanofi	No	https://investor.regeneron.com/news-releases/news-release-details/regeneron-and-sanofi-provide-update-us-phase-23-adaptive
Leronlimab	CCR5	Inhibits the migration of Tregs into areas of inflammation to prevent migration of macrophages and release of pro-inflammatory cytokines in lungs	CytoDyn	No - EIND	https://www.cytodyn.com/newsroom/press-releases/detail/424/cytodyn-reports-strong-results-from-eind-covid-19-patients
LY-CoV555 (IgG1 mAb)	S-protein	Directly block viral binding to host cells then neutralizing SARS-CoV-2	Eli Lilly	No	https://investor.lilly.com/news-releases/news-release-details/lilly-begins-worlds-first-study-potential-covid-19-antibody
S309	Glycan epitopes on S-protein	Interacting with S-protein at glycan sites and neutralizing SARS-CoV-2	Identified from a SARS-CoV infected individual in 2003	No	https://www.nature.com/articles/s41586-020-2349-y_reference.pdf
REGN-COV2	S-protein	Double antibody cocktail consisting of REGN10933 and REGN10987 to bind to RBD of S-protein to diminish ability of mutant viruses to escape treatment and protect against new spike variants	Regeneron Pharmaceuticals	No	https://investor.regeneron.com/news-releases/news-release-details/regeneron-announces-start-regn-cov2-phase-3-covid-19-prevention
CERC-002	LIGHT cytokine	Has the potential to block the actions of LIGHT cytokine to treat cytokine storm-induced COVID-19 ARDS	Cerecor	No - IND	https://www.globenewswire.com/news-release/2020/05/28/2040087/0/en/Cerecor-Announces-FDA-Clearance-of-IND-for-CERC-002-in-COVID-19-Induced-ARDS.html
Recombinant antibody					
47D11 (Recombinant mAb)	S-protein	Binds to cells expressing full-length S-proteins and neutralizes virus	Utrecht University, Erasmus Medical Center & Harbour BioMed	No	https://www.nature.com/articles/s41467-020-16256-y
AbEpic	S-protein	Binds to the S-protein and prevents it from interacting with ACE2	Active Motif	No	https://www.activemotif.com/blog-covid19-abs
APN01	ACE2	rhACE2 blocks viral entry and decreases viral replication	Aperion Biologics	No	https://clinicaltrials.gov/ct2/show/NCT04335136
Convalescent serum/plasma					
	S-protein	Passive immunity		Yes	

Table 2 (*continued*)

Product Name	Target	Basic Mechanism	Developer	FDA EUA	Source
Recovered patient's serum/ plasma			Recovered COVID-19 patients		https://www.pnas.org/content/117/17/9490
Allogeneic cell therapy					
CAP-1002	N/A	Laboratory-grown, cardiosphere-derived cells for cell infusion	Capricor	No - IND	https://link.springer.com/article/10.1007/s00395-020-0795-1
Traditional Chinese medicine					
Lianhuaqingwen (莲花清瘟胶囊)	Whole body	Holistic approach	China	No	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7229744/

Abbreviations: ACE2, angiotensin converting enzyme 2; ATP, adenosine triphosphate; BTK, Bruton tyrosine kinase; CQ, chloroquine; EIND, Emergency Investigational New Drug; EUA, Emergency Use Authorization; FDA, U.S. Food and Drug Administration; gRNA, guide RNA; HCQ, hydroxychloroquine; IL-6, interleukin 6; IND, Investigational New Drug; mAb, monoclonal antibody; PAC-MAN, Prophylactic Antiviral CRISPR in human cells; RBD, receptor-binding domain; rhACE2, recombinant human angiotensin-converting enzyme 2; RNA, ribonucleic acid; S-protein, spike protein; and, Tregs, regulatory T cells.

for the development of *severe* disease. Although the majority of infected individuals report mild symptoms, many COVID-19 patients, particularly seniors and those with underlying medical conditions, are at risk of severe disease or even death. Therefore, early prediction of severe cases using relevant biomarkers is critical for COVID-19 patient management.

5.1. Biomarkers of COVID-19 severity

Clinical biomarkers routinely applied in hospital settings have been recently proposed, tested and analyzed to identify severe cases among COVID-19 patients. Levels of these serum biomarkers: serum urea, creatinine, cystatin C were reported to be elevated in severe COVID-19 patients when compared to mild cases.[89] Additionally, significant differences in serum direct bilirubin, cholinesterase and lactate dehydrogenase concentrations between severe and mild COVID-19 patients ($p < 0.05$) have also been reported.[89] Thus, monitoring the COVID-19 severity using these serum indicators is imperative to reduce mortality.

Inflammatory biomarkers have also been correlated with severity of disease. In a systematic review of inflammatory markers, levels of all markers tested (C-reactive protein, procalcitonin (PCT), IL-6, erythrocyte sedimentation rate, serum amyloid A and serum ferritin) were significantly *higher* in severe patients compared with non-severe cases.[90] Moreover, it corroborates that increased IL-6 levels are correlated with higher mortality rates. A meta-analysis has also shown that **PCT** values are associated with a nearly 5-fold higher severe COVID-19 risk, which might result from enhanced levels of other inflammatory markers, including **IL-6**.[91] Finally, the SARS-CoV-2 ORF3b protein, a potent interferon antagonist, suppresses production of type I interferon and this antagonism is reportedly elevated in severe COVID-19 cases.[92]

A novel cytokine as an inflammatory biomarker: LIGHT, encoded by the *TNFSF14* gene, is an important cytokine with inflammatory actions, playing a key role in regulating immune responses in the lung, gut and skin and in responding to viral pneumonia. **LIGHT** levels were significantly elevated in the serum of hospitalized patients with COVID-19 versus healthy controls and highly correlated with the disease severity and mortality in a COVID-19 ARDS biomarker study.[93] The cytokine stimulates T-cell and B-cell response, induces the release of other cytokines such as IL-1, IL-6, IL-8, IL-10, TNF and GM-CSF, and as data suggested, is a potential key driver of cytokine storm leading to ARDS and death. Anti-LIGHT monoclonal antibody, **CERC-002**, has been granted Investigational New Drug status by the FDA to undergo a clinical trial in patients with COVID-19 cytokine storm-induced ARDS to assess the efficacy and safety of the drug.[94]

Immune defensing cells or T-lymphocytes (particularly $CD4^+$ and $CD8^+$ cells) are among the first human cells to respond to the threat of SARS-CoV-2. In a recent study, all twenty of the COVID-19 patient participants produced $CD4^+$ T-cells and antibodies (IgG and IgA) targeting the viral S-protein, and 70% of cases produced measurable $CD8^+$ T-cells.[95] These results confirm that the human immune system can mount a substantial and lasting response to the novel coronavirus. On the other hand, lymphopenia has also been observed in COVID-19 patients, in which, levels of $CD4^+$ and $CD8^+$ T-cells were decreased in *severe* patients in comparison with *mild* cases, but restored when the viral infection was cleared.[96]

Biomarkers of biological age: One important predictor of severity of COVID-19 is age. However, pre-existing conditions (such as: diabetes, cardiovascular disease, chronic kidney disease, hypertension, obesity, lifestyle *etc*) are also associated with increased mortality. This phenomenon seems to indicate that for COVID-19, *biological* age (how old one's body *seems*) is just as relevant as *chronological* age (the number of living years). A good biomarker of *biological* age takes into account genetics, lifestyle factors, health conditions, and toxic exposures (pathogens or chemicals). The most prominent biomarkers of biological age are the *epigenetic clock* and the *glycan clock*.[97] Epigenetic clocks, partially originated from chronological age, include DNA methylation (*e.g.* GrimAge methylation clock), telomere length and several other models, and have recently been shown to predict prevalence and incidence of leading causes of death and disease.[98] Glycan clocks, associated with unhealthy lifestyle indicators, are also potentially effective tools for biomarker discovery since IgG glycosylation appears to be closely linked with both chronological and biological ages.[99]

5.2. COVID-19 recurrence

There are concerns that patients who recover from COVID-19 may be at risk of reinfection. A growing number of recovered patients in Wuhan, China and South Korea appeared to be relapsing.[100,101] Reports that the coronavirus can "*reactivate*" in recovered patients could be inaccurate, however, since diagnostic tests for COVID-19 have the potential of producing false negatives or positives. Additionally, when patients are sequentially tested, some will toggle between negative and positive results, creating the appearance of reinfection. While human data is limited, studies in rhesus macaques reported the lack of reinfection,[102,103] suggesting that immunity acquired following primary infection may protect against subsequent exposure to the virus.[104]

5.3. Convalescent serum or plasma

Convalescent sera from recovered COVID-19 patients, approved to be safe for treatment, is currently in use for treating the critically ill or to boost immediate immunity for at-risk individuals (Fig. 3a).[105,106] A recent Belgium study, using single-cell sequencing, is the first to show that virus-specific antibodies in the plasma can boost a newly identified class of dendritic cells (DCs) – called inflammatory type 2 conventional DC or inf-cDC 2 – to enhance host immunity.[107] Another study conducted in Texas, recently asserted the safety of convalescent plasma as a treatment option, reporting that 80% (20/25) of severely ill COVID-19 patients (without controls) were discharged after treatment combined with other drugs (*e.g.* HCQ etc.).[108] To verify these results, the FDA has approved a clinical trial to be carried out by Johns Hopkins University researchers. One barrier for clinical trials, however, is amassing enough biospecimens from donors to test.

5.4. Serological antibody detection of IgM & IgG

The presence of antibodies in the blood against viral proteins (*e.g.* S-protein and N-protein) could be used as an indicator of a prior infection regardless of whether symptoms are present. A whole set of serological assay-based products, such as ELISAs and lateral flow assays (LFAs), have been developed to detect SARS-CoV-2 S- or N-proteins or IgM/IgG antibodies in human serum, plasma, whole blood, or finger prick samples (Fig. 3b).

Primary SARS-COV-2 infection is characterized by the presence of detectable IgM antibodies 3–7 days after the onset of infection. *Secondary* viral infection is characterized by the elevation of SARS-COV-2-specific IgG, which is often accompanied by elevated levels of IgM. In combination with direct viral RNA detection, these serological antibody (IgM and IgG) tests provide a spectrum of phenotypic outcomes of a person's current, historical and future status of COVID-19 (summarized in Table 3). Please note, positive IgG (or IgM/IgG) indicates a person's active immunity, but it is unclear how long the immunity lasts.

Seroprevalence surveys, based on detection of *anti*-SARS-CoV-2 antibodies, have been conducted in many geographical areas in an attempt to reveal the extent of the spread of SARS-CoV-2. A recent example is a country-wide survey from Spain, which estimated 5% of the country's population had been exposed to the virus.[109] Unfortunately, several of these surveys, particularly one conducted in Santa Clara County, California,[110] have generated criticism regarding the accuracy of their results. The University of California at San Francisco (UCSF) and Berkeley (UCB) recently tested 12 antibody assays (10 lateral flow assays, LFAs, and 2 ELISAs) to evaluate their accuracy. They reported that IgM detection was more variable than IgG, and detection accuracy was highest when IgM and IgG results were combined. The authors concluded that informed use of serology will require evaluations covering the full spectrum of SARS-CoV-2 infections, from asymptomatic or mild infection to severe disease, and later convalescence.[111]

5.5. Undetected asymptomatic SARS-CoV-2 carriers

Another point-of-care test, developed by researchers at Stanford University, can detect the IgG and IgM antibodies that indicate prior infection with SARS-CoV-2, in the form of a *simple finger prick* sample. A study in Major League Baseball has been launched to recruit and test as many as 10,000 people to help determine how the virus has spread in metropolitan areas and to develop a better understanding of the true infection rate in the general population.[112] Similar efforts are being launched by the U.S. NIH,[113] as well as the Chinese government,[114] to yield a clearer picture of the true magnitude of the COVID-19 pandemic in their respective countries.

A study conducted in China with 37 asymptomatic individuals who were infected with SARS-CoV-2, reported that the virus carriers who fail to develop COVID-19 symptoms may have a weaker immune response to the virus.[115] The authors also observed that the asymptomatic patients' IgG levels began to diminish rapidly within 2–3 months after infection, which may have implications for future immunity strategy and serological surveys.

6. COVID-19 prevention: development of new vaccines

To ultimately defeat SARS-CoV-2, public health workers need to protect the general population from further viral infection. The key question is whether *herd immunity* is a more realistic goal in the near future than originally thought. Historically, the herd immunity for some infectious diseases can go into effect when 40% of the people in a population become immune, but in

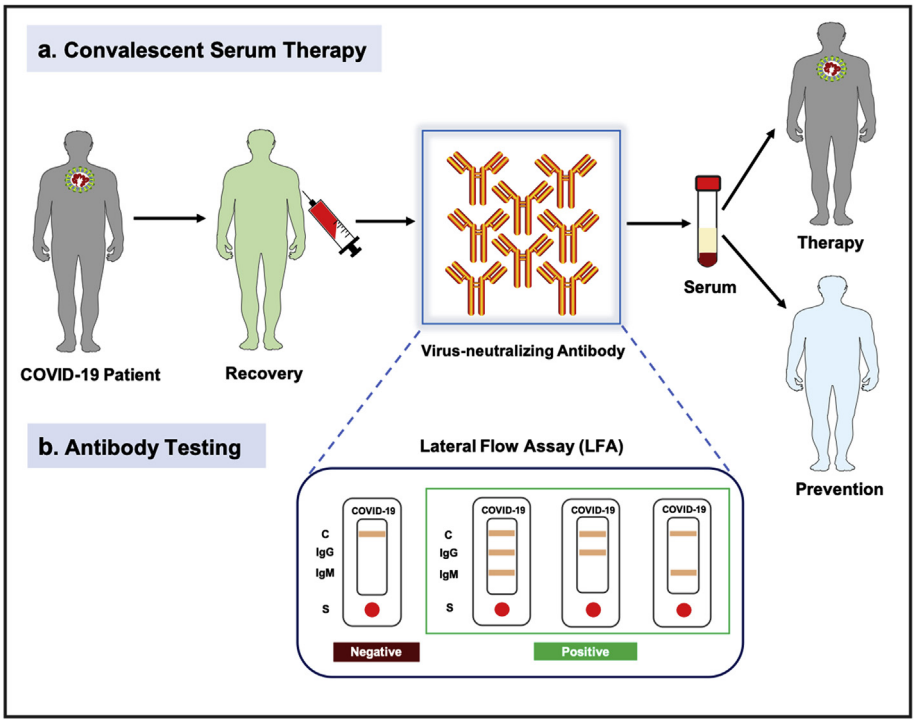

Fig. 3. Schematic of serological antibody treatment and prevention for COVID-19. a) Convalescent serum therapy; and, b) Antibody testing by a lateral flow assay.

Table 3
Outcomes identified from detections of viral RNA and serological antibodies (IgM & IgG).

Viral Detection	IgM	IgG	COVID-19 Phenotypes	Immunity	Notes
Positive	Positive	Positive	Confirmed COVID-19 patient	Developing	
Positive	Positive	Negative	Early-stage diagnosed patient	Not yet	Primary response
Positive	Negative	Positive	Asymptomatic but healthy viral carrier	Yes	
Positive	Negative	Negative	Very early-stage of viral infection	No	Not enough antibodies
Negative	Positive	Positive	Convalescent COVID-19 patient	Yes	
Negative	Positive	Negative	Primary infections with other virus[a]	No	Retests needed
Negative	Negative	Positive	Recovered COVID-19 patient	Yes	
Negative	Negative	Negative	Uninfected health people	No	

[a] Need to retest to avoid false negative of the viral infection and false positive of IgM.

most cases, 80–95% of the population must be immune to the disease to stop its spread.[116] Thus, development of new vaccines will be a crucial step to combat COVID-19.

As an RNA virus, SARS-Cov-2 lacks error-repairing mechanisms during replication, and therefore, could have a relatively high mutation rate. Therefore, one challenge for new vaccine invention will be staying ahead of the viral mutations. Fortunately, a recent Italian study has reported that the novel coronavirus seems to act as a slow-mutating pathogen and is so far relatively stable with only 5 mutants so far reported in Italy.[117] There are many novel coronavirus vaccine candidates under development in the pipeline for COVID-19. A few key examples are described below and listed in a summary Table 4.

6.1. Basics and types of vaccines

Vaccination is a proven way to prevent many infectious diseases (*e.g.* polio, measles etc.). Recent advances in technology have led to the development of a few new types of vaccines. In addition to *traditional* whole **pathogen**-based vaccines, *modern* types of vaccine include **antigen**- and **nucleic acid**-based vaccines, which are perhaps the most applicable to COVID-19. In particular, the nucleic acid-based approach uses genetically engineered RNA or DNA to code for an antigen, such as the S-protein on SARS-CoV-2, to prompt an immune response to the virus.

6.2. Broader collaborations to race against SARS-CoV-2

Efforts to develop vaccines against SARS-CoV-2 are currently ongoing at various companies and research institutions. To increase the chances of success, however, the research world needs an intensive collaborative effort and a panel of expertise

Table 4
Summary of new vaccines developed for COVID-19 prevention.

Vaccine	Target	Basic Mechanism	Developer	US Warp Speed	Clinical Trial Phase	Source
Vector-based						
Ad5-nCOV	S-protein	Uses replication-defective adenovirus type 5 vector to express S-protein	China CanSino Biologics	No	III	https://www.precisionvaccinations.com/vaccines/ad5-ncov-covid-19-vaccine
AZD1222 (ChAdOx1)	S-protein	Uses attenuated adenovirus vector to express S-protein	AstraZeneca & Oxford University	Yes	II & III	https://www.astrazeneca.com/media-centre/press-releases/2020/astrazeneca-and-oxford-university-announce-landmark-agreement-for-covid-19-vaccine.html
Ad26.COV2−S	S-protein	Recombinant vaccine using a genetically modified adenovirus vector (Ad26) to produce S-protein antigen to induce an immune response	Janssen Pharmaceutical (a Johnson & Johnson Company)	Yes	I & II	https://www.janssen.com/infectious-diseases-and-vaccines/vaccine-technologies
V590	S-protein	Vaccine developed from rVSV to shuttle a SARS-CoV-2 virus surface protein into host cells	Merck & IAVI	Yes	Preclinical	https://investors.merck.com/news/press-release-details/2020/IAVI-and-Merck-Collaborate-to-Develop-Vaccine-Against-SARS-CoV-2/default.aspx
Merck vaccine candidate	S-protein	Uses measles virus vector to introduce S-protein gene into host cells	Merck	Yes	Preclinical	https://www.sciencemag.org/news/2020/05/merck-one-big-pharma-s-biggest-players-reveals-its-covid-19-vaccine-and-therapy-plans
DNA-based						
INO-4800	S-protein	INO-4800 DNA plasmid encodes for S-protein	Inovio Pharmaceuticals & Beijing Advanccine Biotechnology	No	I	http://ir.inovio.com/news-releases/news-releases-details/2020/INOVIOs-COVID-19-DNA-Vaccine-INO-4800-Demonstrates-Robust-Neutralizing-Antibody-and-T-Cell-Immune-Responses-in-Preclinical-Models/default.aspx
mRNA-based						
mRNA-1273	S-protein	Encodes for prefusion stabilized form of S-protein	Moderna	Yes	III	https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-positive-interim-phase-1-data-its-mrna-vaccine
BNT162	S-protein	Utilizes different mRNA formats combined with LNP formulation to either target larger spike sequence or RBD from S-protein	Pfizer & BioNTech SE	Yes	II & III	https://www.genengnews.com/covid-19-candidates/biontech-pfizer-and-fosun-pharma-bnt162/
Protein-based						
PittCoVacc	S-protein	Laboratory-manufactured pieces of S-protein delivered by microneedle array	University of Pittsburgh	No	Preclinical	https://www.upmc.com/coronavirus/vaccine
NVX-CoV2373	S-protein	Vaccine created using recombinant nanoparticle technology to generate stable, prefusion S-protein antigen along with saponin-based Matrix-M adjuvant	Novavax	Yes	I & II	https://ir.novavax.com/news-releases/news-release-details/novavax-announces-positive-phase-1-data-its-covid-19-vaccine
Oral Vaccines						
Vaxart COVID-19 Oral Vaccine	Mucosal immunity	Oral tablet vaccine consisting of an Ad5 vector, vaccine antigen, and a TLR3 adjuvant designed to release contents in the small bowel to activate mucosal immunity	Vaxart	Yes	Preclinical	https://investors.vaxart.com/news-releases/news-release-details/vaxarts-covid-19-vaccine-selected-us-governments-operation-warp
Thin-film oral vaccine	Antibody-mediated immunity	Novel thin, peelable film containing live adenovirus that is administered orally through sublingual and buccal routes	University of Texas, Austin	No	Preclinical	https://advances.sciencemag.org/content/6/10/eaau4819

Abbreviations: DNA, deoxyribonucleic acid; EUA, Emergency Use Authorization; FDA, U.S. Food and Drug Administration; LNP, lipid nanoparticle; mAb, monoclonal antibody; mRNA, messenger ribonucleic acid; RBD, receptor-binding domain; rVSV, recombinant vesicular stomatitis virus; and, S-protein, spike protein.

in diverse areas of research working together with a common goal. Recently, a ***public-private*** effort was launched to accelerate the development of COVID-19 vaccines and treatments. Coordinated by the NIH, the ACTIV (Accelerating COVID-19 Therapeutic Interventions and Vaccines) partnership will work with government agencies and members of the pharmaceutical industry, to provide infrastructure, subject matter expertise and/or funding to advance the most promising vaccines and therapeutic candidates into clinical trials. Even so, most experts agree it is likely to take at least a year until a vaccine is available for safe and widespread application to a general population.

Recently, two pharmaceutical giants, Sanofi and GSK, have announced a joint effort to develop an adjuvanted vaccine for COVID-19. Sanofi will contribute its S-protein COVID-19 antigen while GSK will contribute its adjuvant technology, which has been proven to reduce the amount of vaccine protein required per dose. Both companies plan to initiate Phase I clinical trials in the second half of 2020.

As an example of ***academia-industry*** collaboration, AstraZeneca, Oxford University, and its spinout company Vaccitech are working collaboratively to enable rapid production and distribution of their own vaccine candidate, **ChAdOx1**, based on a non-replicating **ch**impanzee **ad**enovirus vector. The ChAdOx1 construct is designed to trigger production of the viral S-protein which then primes the immune system to recognize SARS-CoV-2 infection. AstraZeneca will oversee the first human Phase II/III trial of ChAdOx1 nCoV-19, now renamed to **AZD1222**, in the U.K., which will be carried out in June 2020. Another adenovirus vector-based vaccine candidate, **Ad5-nCOV**, has been developed by China CanSino Biologics and is also undergoing clinical trials.

6.3. An example of DNA-based vaccine

DNA vaccines are composed of double-stranded plasmids meant to produce a specific immune response in the body. A candidate vaccine known as **INO-4800**, produced by *Inovio Pharmaceuticals* in collaboration with *Beijing Advanccine Biotechnology*, has launched a small clinical trial in the U.S. in April. More trials of INO-4800 are planned to launch in May in South Korea and China.

6.4. Vaccines developed with mRNA-based technology

There has been growing interest for the past two decades in mRNA-based technology as a new tool in prophylactic vaccine development against infectious diseases.[118] mRNA vaccination is an attractive alternative to conventional vaccine approaches because of its potent, long-lasting and safe immune responses observed in animal models and encouraging data from early human clinical trials. There are several mRNA-based vaccines that are currently undergoing development such as **mRNA-1273** by Moderna and **BNT162(b1)** by Pfizer Inc. and BioNTech SE. mRNA-1273 is a novel lipid *nanoparticle* (LNP)-encapsulated mRNA vaccine encoding for a prefusion stabilized form of the spike (S) protein.[119] A NIAID-led study of mRNA-1273 in a Phase I open-label, dose-ranging trial released positive response from a preliminary report[120] and Phase II/III "COVE" trials are being planned and conducted soon in the summer, while BNT162 (b1) is also undergoing a clinical Phase I/II study.

6.5. Innovative vaccine delivery techniques

A team from the University of Pittsburgh has recently used *engineered spike protein* to make a new vaccine named **Pitt-CoVacc**. When tested in mice, the vaccine demonstrates a response within 2 weeks and produces antibodies specific to SARS-CoV-2 at quantities thought to be enough to neutralize the virus. Another important advantage of PittCoVacc is its delivery by *microneedle array* in a fingertip-sized skin patch.[121] If tested successfully in humans, this simple and easy to use delivery method can be very beneficial for widespread vaccination in the general population.

As an alternative vaccine delivery method, Vaxart has developed an oral COVID-19 vaccine that is administered by tablet rather than injection, and is currently in preparation for a Phase I clinical trial.[122] Similarly, another oral vaccine has been developed with a novel preparation technique that takes the form of a thin, peelable film containing live virus, administered orally via sublingual and buccal routes.[123] This vaccine, if proved to be effective, is ground-breaking in that it can be stored at room temperature for up to 3 years and can maintain viability through repeated cycles of freezing and thawing.[123] This method would facilitate vaccine preservation, transportation, and administration without the need for vials, fridges, or needles.

6.6. Further vaccine insights

Recent insights that could aid COVID-19 vaccine development include critical findings regarding the importance of lung memory T cells and a DNA origami approach previously used for HIV vaccine development. Research from the Salk Institute has uncovered how memory T cells that are responsible for long-term immunity in the lungs can be reactivated more easily than previously thought.[124] The results reveal a novel tissue-specific paradigm for the reactivation of memory $CD8^+$ T cells which could aid in the development of universal vaccines for both influenza and SARS-CoV-2. In a separate study, *in vitro* tests have shown that DNA origami particles, which are folded intricately to mimic the size and shape of viruses, provoked a strong immune response from human immune cells.[125] The researchers are now working to adapt this approach to develop a potential vaccine for SARS-CoV-2.

6.7. Operation Warp Speed in the U.S

As a ***public-private*** partnership to facilitate and accelerate the development, manufacturing, and distribution of COVID-19 vaccines, therapeutics, and diagnostics, Operation Warp Speed has been initiated by the U.S. federal government. Among the companies chosen for funding to expedite development and preparation for manufacturing their respective vaccine candidates are: Johnson & Johnson (Janssen Pharmaceutical), AstraZeneca-University of Oxford, Pfizer-BioNTech, Moderna, Merck, Vaxart, Inovio, and Novavax.[126] All of the vaccine candidates from these selected companies are summarized in Table 4. Operation Warp Speed has not gone without criticism, however, as its proposed haste to have one or more vaccines sufficiently studied for safety and efficacy, approved internationally, and manufactured in hundreds of millions of doses by the end of 2020 has provoked fear of error and could impact public confidence in a new vaccine.

7. Emerging biomarkers & newly developed technologies

In the efforts to vanquish COVID-19, numerous modern technologies have been developed, opening up novel avenues for COVID-19 research and biomarker discovery, which are summarized in Table 5.

7.1. Innovative approaches to drug screening

Interactome protein map: A research team at UCSF, in collaboration with scientists at Mount Sinai, Institute Pasteur and others, have identified 66 druggable human proteins that can be targeted by 69 compounds, either approved or in development. The process entailed cloning, tagging, and expression of viral proteins in human cells to identify viral-host protein interactions via affinity-purification mass-spectrometry (AP-MS).[19] These elegant antiviral tests revealed that **Sigma 1** and **Sigma 2 receptors** are the key host targets that SARS-CoV-2 binds to, and one promising experimental molecule **PB28** has strong efficacy and high selectivity against the off-targets – suggesting PB28 may be optimized towards therapeutics.[19]

Ultra-high-throughput proteomics: Severe COVID-19 patient proteomes in blood plasma were analyzed and compared to controls by utilizing a high-throughput mass spectrometry platform. The study identified 27 potential protein biomarkers that are differentially expressed, including complement factors, the coagulation system, inflammation modulators, and pro-inflammatory factors upstream and downstream of **IL-6**.[127]

Hunting for new biomarkers: Genomics England and the GenOMICC (Genetics of Mortality in Critical Care) in the UK are spearheading a human genome initiative to sequence 35,000 COVID-19 patients' genomes to look for genetic links or markers of the disease susceptibility and severity in order to understand how a person's genes may affect how they react to the virus. Additional metabolomics approachesaim to measure all the metabolites and other small molecules in a sample; and is being used to survey blood, urine, feces and saliva samples to identify chemical biomarkers of COVID-19. These data-rich **omic-based** approaches will generate immense sets of data for new biomarker discovery and provide useful information for virtual new drug screening.

AI & ML platform: Artificial Intelligence (AI) with machine learning (ML) is quickly becoming a vital component of everyday medical testing and has now been applied for COVID-19 drug screening due to the fact that AI can make higher-order correlations and link all kinds of datasets beyond human capabilities. AI/ML algorithms have been used to screen 90,000 candidate compounds, identifying a shortlist of 31 that are already in Phase II or III, or approved; *five* of which on the top-ranked drugs are already in trials for COVID-19. BenevolentAI (London, UK) is one of several groups leveraging AI to find existing drugs that have already been approved by regulators and could therefore be repurposed to fight SARS-CoV-2. Their top selection is **baricitinib** (a JAK inhibitor), a drug to treat rheumatoid arthritis, which is now in clinical trials in the U.S. with a planned expansion to Europe and Asia.[128,129]

Efforts to diagnose COVID-19 without a test have also relied on AI. The researchers behind the COVID Symptom Study (>3.9 million people joined) tracking app have developed an AI-based mathematical model that can predict whether an individual has COVID-19 based on their age, sex and four key symptoms: anosmia (the loss of sense of smell or taste), fatigue, persistent cough, and loss of appetite.[130] Additionally, AI/ML has now been applied to chest computed tomography (CT) scans for rapid and accurate diagnosis and prognosis of patients with COVID-19.[131,132]

Structure-based approach: One antiviral drug target is the SARS-CoV-2 main protease (M^{pro}), which plays an essential role in mediating viral replication and transcription. The molecular structure of the viral M^{pro}, identified by X-ray,[133] provides a basis for design of new drugs from structure-based virtual screens. One team has tested more than 10,000 compounds and identified six drug candidates targeting the viral M^{pro} through a combination of structure-based virtual *in silico* and high-throughput *in vitro* screenings.[134] Additional studies using similar approaches have been conducted to screen SARS-CoV-2 M^{pro} inhibitors among food, plants, and marine natural products.[135,136]

Moreover, a computational tool developed to identify cancer immunotherapy targets has been used as a SARS-CoV-2 vaccine design concept.[137] Focused on identification of highly conserved regions of the viral genome and newly acquired adaptations, this computational *in silico* analysis, though yet to be experimentally validated, prioritizes viral targets based on their ability to stimulate a lasting immune response.

Table 5
Emerging biomarkers and new technologies for COVID-19.

Technology	Example	Developer	Source
Omic-based			
Protein Interactomics	SARS-CoV-2 protein interaction map	QBI Coronavirus Research Group	https://www.nature.com/articles/s41586-020-2286-9
High-Throughput Proteomics	Patient proteome analysis via high-throughput mass spectrometry platform	European collaborative team	https://www.sciencedirect.com/science/article/pii/S2405471220301976
Human Genomics	Human genome sequencing	Genomic England & GenOMICC	https://www.genomicsengland.co.uk/covid-19/
Metabolomics	COVID-19 MS Coalition	Mass Spectrometry Coalition	https://covid19-msc.org/
AI/ML-based			
	AI drug discovery and development platform	BenevolentAI	https://www.benevolent.com/covid-19
	COVID Symptom Study tracking app	ZOE, King's College of London, and Massachusetts General Hospital	https://covid.joinzoe.com/us
Structure-based			
	M(pro) structure-based virtual *in silico*	Various	https://www.nature.com/articles/s41586-020-2223-y
	High-throughput *in vitro* screenings	China collaborative team	https://www.nature.com/articles/s41586-020-2223-y
	Computational *in silico* analysis of SARS-CoV-2 viral targets	University of Pennsylvania & Children's Hospital of Philadelphia	https://www.cell.com/cell-reports-medicine/pdfExtended/S2666-3791(20)30,048-3
Nanotechnology			
Nanoparticles	LSPR sensing	Empa, ETH Zurich & Zurich University Hospital	https://pubs.acs.org/doi/10.1021/acsnano.0c02439
	Nanosponges	University of California, San Diego	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7301960/
Nanopore Sequencing	LamPORE	Oxford Nanopore Technologies	https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30,251_8/fulltext
Single-Molecule Imaging	Nanoimager	ONI	https://oni.bio/covid19
Digital Technology			
	COVID-19 Voice Detector	Carnegie Mellon University; and Cambridge University	https://voca.ai/corona-virus/https://covid-19-sounds.org/en/
	COVID-19 Self-Diagnosis Tool	Apple	https://www.apple.com/newsroom/2020/03/apple-releases-new-covid-19-app-and-website-based-on-CDC-guidance/
	COVID-19 Compass	Seqster	https://seqster.com/press/press-releases/seqster-launches-covid-19-compass-based-on-cdc-guidelines-for-healthcare-enterprises
	Smartphone-based Multiplex 30-min Viral RNA Test	Universityof Illinois,Urbana-Champaign	https://pubmed.ncbi.nlm.nih.gov/32334422/
Biobanking			
Biospecimens	COVID-19 Biobanking Accelerator	LabVantage Solutions	https://www.labvantage.com/your-lab-type/biobanking/
Patient data	CentraXX Database	KAIROS	https://www.kairos.de/en/kairos/ongoing-project/covid-19-projekt-coronaboxx-field-test/
Protein data	Protein Data Bank	Collaborative team from UVA, UAM, Poznan University of Technology, ICHB PAN, and NIH	https://covid-19.bioreproducibility.org/
Imaging	COVID Digital Pathology Repository	Indica Labs and Octo	https://www.biospace.com/article/releases/indica-labs-octo-and-axle-work-with-nih-to-launch-a-global-covid-19-digital-pathology-repository/

Abbreviations: AI, artificial intelligence; COVID-19; coronavirus disease 2019; ICHB PAN, Institute of Bioorganic Chemistry of the Polish Academy of Sciences; LSPR, localized surface plasmon resonance; min, minute; M(pro), NIH, National Institutes of Health; SARS-CoV-2 main protease; MS, mass spectrometry; QBI, Queensland Brain Institute; RNA, ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UAM, Adam Mickiewicz University; and UVA, University of Virginia.

7.2. Nanotechnology for the viral detection

Nanotechnology is increasingly being used to detect, prevent, and treat SARS-CoV-2 infection, mostly by targeting the spike protein that presents on the surface of the virus. While the bulk of COVID-19 testing relies on RT-PCR technology, significant evidence shows that tests based on this method are far from the golden standard that one would typically expect

from a clinical diagnostic as both the *sensitivity* and *specificity* of the RT-PCR tests have come under question. Shortages of reagents and lack of trained personnel to run the RT-PCR tests also poses a barrier to generating timely testing results. *Nano*technology could eliminate some of these problems.

Scientists from the University of Maryland School of Medicine have recently developed an experimental diagnostic test for COVID-19 using **nanoparticle-based** technology called localized surface plasmon resonance (LSPR) sensing. The detection of SARS-CoV-2 RNA by LSPR has been demonstrated to be highly accurate, sensitive and rapid, albeit expensive (~$50—300K).[138] Another experimental diagnostic in development is a genome sequencing platform, called LamPORE, based on **Oxford nanopore** technologies.[139] Other novel new nanotechnologies in development include:

Nanosponges, developed by University of California, San Diego researchers, uses nanoparticles labelled with human ACE2 and CD147 to attract and neutralize the SARS-CoV-2 virus in *cell cultures*, causing the virus to lose its ability to hijack host cells and reproduce.[140] **Nanoimager**, utilizes nanotechnology to provide advanced microscopy and imaging. As a small black box with a footprint the size of an iPad, its imaging can be done practically anywhere. The use of single-molecule imaging technology in virus research could open a new window into SARS-CoV-2.[141]

7.3. Emerging digital biomarkers of COVID-19

There is no shortage of ways that technologists are harnessing modern technology to track the spread of the coronavirus pandemic, from fever-identifying drones (Australian Dragonfly) to 3D-printed ventilator components (Isinnova in Italy), and, from the tracking of mobile location data (MIT's app) to a COVID voice detector. As demand for preventive and precision healthcare continues to grow, so will the need for developing a new class of **digital biomarkers** that can generate *more* data and provide information in *real time.* Instead of blood tests and medical imaging, digital biomarkers use *sensors* and *algorithms* across a plethora of available connected hardware and software tools, such as personal *smartphones* or other home-based products.[142] The molecular software-hardware combination of these products has created new opportunities for public health and biomedical research.

COVID voice detector: At Carnegie Mellon University in the US and Cambridge University in the UK, researchers are developing free experimental, voice-activated online tools using AI, to analyze the sound of coughs and other noises to provide a diagnosis of infection. Both teams emphasize that the software does not replace medical tests that would be used to produce a more accurate diagnosis. Concerns of this technology include ethics of tentative diagnoses and the potential for false positives/negatives.

Smartphone-based self-diagnosis: Smartphone and data-rich companies like Apple and Google have been developing digital health programs for years, using AI and machine learning (ML) technologies to usher in a new class of medical devices such as *Apple Watch*, *Samsung Galaxy Watch*, *Fitbit* etc.[142] In coordination with the CDC, White House and FEMA, Apple has recently developed and released a new at-home COVID-19 self-diagnosis tool based on CDC guidance. Another study has developed a point-of-care system that integrates with a smartphone for detecting live virus from nasal swab samples.[143] This platform comprises a cartridge-housed microfluidic chip that carries out isothermal amplification of viral nucleic acids from the nasal samples, which are then detected using the smartphone camera.

SaaS-based platform: COVID-19 Compass is a Software as a Service (SaaS) online app with a new module built into the Seqster interoperability platform. It will allow: 1) healthcare enterprises to remotely and seamlessly track the health of research subjects who may have been exposed to the disease; and, 2) patients to share their key health metrics related to COVID-19. It is based on CDC guidelines, best clinical practices, illness severity, and risk factors such as age, gender, location, potential exposure, and pre-existing health conditions. This comprehensive **SaaS** platform has recently been deployed by LabVantage Solutions to build a COVID-19 Biobanking Accelerator (discussed below).

Advantages overcome challenges: As most viral tests rely on labor- and time-intensive laboratory preparation and analysis techniques, testing for SARS-CoV-2 from nose swabs can take days. Additionally, since available technologies remain expensive, technically challenging, and labor intensive, there is an urgent need for *low-cost portable platforms* that can provide fast, accurate, and multiplex diagnosis of infectious disease at the point of care. Hopefully, these above *digital* biomarkers and *smartphone*-based devices can overcome many of the challenges associated with rapid pathogen testing and contribute to current COVID-19 responses.

7.4. Biobanking plays a role

Widespread laboratory testing is a critical component in the battle to control the coronavirus pandemic. For biomarker application, the first important step is biobanking, a term that references *biospecimen* collection, storage and database. Biobanking has been critical in research and development during prior pandemics,[144] and continues to be a major tool in the arsenal of public health agencies and healthcare providers scrambling to fight COVID-19.

Biobanking in times of COVID-19: A COVID-19 Biobanking Accelerator, built by LabVantage Solutions, is designed to enable laboratories to enter biospecimens into a biobank much more rapidly, with the potential to greatly accelerate COVID-19 research and testing. Biobanks will be an essential part in researching long-term consequences for those affected by COVID-19. However, large amounts of data from COVID-19 positive patients, who don't require hospitalization, is being lost. As a result of its patient-centric platform, KAIROS is able to offer clients the ability to collect data from these patients in their CentraXX database. Another web resource contains validated SARS-CoV-2-related structural models from the Protein Data

Bank for potential drug targets. Its creation was due to concern over the elevated risk of error from the current rush to perform and publish research regarding COVID-19.

In addition to biobanking samples and data collection, other types of biological information (*e.g.* chest CT scan) could be digitally or virtually converged. IT systems providers Indica Labs and Octo have launched the COVID Digital Pathology Repository, an online collection of high-resolution microscopic images of COVID-related human tissues hosted at the NIH, which enables international collaboration by providing a centralized and cloud-based repository. The platform allows for the sharing and annotating digital whole slide images of lung, liver, kidney and heart tissues from patients infected with SARS-CoV-2. The repository creation will help speed research of the pathology, treatment, and prevention of COVID-19.

8. Important biomarkers of socio-psychological stress

Although most of the current research regarding COVID-19 and SARS-CoV-2 focuses directly on COVID-19 patients and issues of detection, diagnosis, treatment and prognosis, the pandemic has also caused the general population unprecedented social, economic, and psychological distress. In particular, COVID-19 has had disproportionate impacts on socioeconomically disadvantaged individuals and communities of color. Thus, it is important that these emotional outcomes are also addressed in research with careful monitoring of psychosocial needs, and integration of basic mental health services into general pandemic health care.

8.1. Questions we are facing

The coronavirus outbreak has made us completely change our modern behaviors. We are reinventing socialization practices, work routines, exercise regimens, and methods of communication. We have to adapt our life to the new normal: spending more time with family, altered personal freedom, and potential changes in our jobs. In this dramatically changed world, we find ourselves putting our fears and stress into context. Some final questions we are facing now:

1. How can we become more mentally resilient?
2. What will things look like on the other side of the infection peak, biologically and psychologically?
3. How can we measure our mental health and social stress scientifically?
4. Are there available *psychosocial stress biomarkers* that can be applied in near future studies?

8.2. Measurable biomarkers of psychosocial stress

Scientists have long been investigating psychosocial stressors or various internal and external factors that negatively affect the *homeostatic equilibrium* of individuals at the molecular to the whole-body level, inducing the so-called '*state of stress*'. Stress affects people's welfare status and induces energy-consuming mechanisms to combat the subsequent ill effects; thus, these individuals may be immunocompromised, making them vulnerable to pathogens (*e.g.* coronaviruses). These physiological effects of psychological stress can be manifested and monitored by the *quantitative* and *qualitative* measurement in a number of biological markers.

For example, salivary **cortisol** in excess levels could adversely affect various physiological systems, including the hypothalamic-pituitary-adrenal axis, sympathetic-adrenal-medullary, central nerve, and immune systems. Stress hormones, like **glucocorticoids** along with cytokines, act as master homeostatic regulators in circulation, which mediate several conditions like post-traumatic stress disorder.[145] Other potential biomarkers of stress include **allostatic load** (a summary measure of the cumulative biological burden from daily stress),[146] **thermal stress** markers (heat shock proteins), **innate immune** markers (acute phase proteins), **oxidative stress** markers (reactive oxygen species and 8-OH-dG etc), and **chemical secretions** (secretory IgA, chromgranin A, and cortisol) in the saliva and/or urine.[145,146]

8.3. Managing the stress related to COVID-19

To minimize the long-term mental health impact of COVID-19, a multifaceted and concerted effort from the entire healthcare system at large is needed and a list of suggested strategies includes:

1) monitoring sources of misinformation,
2) enhancing social support networks,
3) reducing the stigma associated with disease,
4) increasing available psychosocial services, particularly online services.[147,148]

Additionally, adequate training of healthcare personnel and the optimal use of technological advances to deliver mental healthcare are also required to manage the emotional response or stress to the outbreak.[149] The mental health interventions

and effective therapeutic development can be implemented at multiple levels – the general population, among healthcare workers, and in vulnerable populations.

9. Conclusion: key messages

Coronaviruses have plagued humanity for a long time (1965-2020). Several versions are known to trigger common colds or flus and more recently, two types have set off outbreaks of deadly illnesses: severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). The impact of these prior outbreaks, however, has been mild compared with the global havoc unleashed by the new coronavirus SARS-CoV-2. In the span of only a few months, it has triggered lockdowns in dozens of nations and claimed more than 738,600 lives. And the disease continues to spread.

While the world faces this urgent crisis, biomarkers will increasingly be indispensable tools for viral detection, disease diagnosis and treatment, and COVID-19 prevention and screening. Various new therapeutic drugs and preventive vaccines in the pipeline are being developed with cutting-edge technologies. In this review article, unique from many others, we have specially included the importance of ***socio-psychological biomarkers***, the emerging influence of ***digital biomarkers***, and ***TCM*** approach that is being widely used in China, in addition to new drugs being developed in the Western countries. We believe that the application and combination of all sorts of biomarkers in COVID-19 diagnostics, detection, treatment, and prevention will be the ultimate weapon to win the war of fighting SARS-CoV-2.

It is an extraordinary achievement that scientists and researchers currently know so much about the virus, from its genomic sequences (wild type and mutants) to its protein interactions with host factors, as just a few months ago, SARS-CoV-2 was a pathogen completely unknown to the scientific community. Today, SARS-CoV-2 is a subject of intensive scientific study on an unprecedented scale: vaccine projects proliferate, antiviral drug trials have been launched, new diagnostic tests are appearing, and more novel biomarkers are emerging. The question for tomorrow and the future is quite straightforward: how might the knowledge we have learned over the past seven months help put an end to this pandemic and better prepare for the next ones? Our answer is: early action, preparedness and prevention. The importance of taking a preventive approach has been confirmed in a recent analysis of ecology and economics for pandemic prevention.[150] Proverbially, as Benjamin Franklin pointed out in the 18th century, "*an ounce of prevention is worth a pound of cure*".

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Declaration of competing interest

The authors declare that they have no potential conflict of interest.

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