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COVID-19: Discovery, diagnostics and drug development

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Summary

Coronavirus disease 2019 (COVID-19) started as an epidemic in Wuhan in 2019, and has since become a pandemic. Groups from China identified and sequenced the virus responsible for COVID-19, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and determined that it was a novel coronavirus sharing high sequence identity with bat- and pangolin-derived SARS-like coronaviruses, suggesting a zoonotic origin. SARS-CoV-2 is a member of the *Coronaviridae* family of enveloped, positive-sense, single-stranded RNA viruses that infect a broad range of vertebrates. The rapid release of the sequence of the virus has enabled the development of diagnostic tools. Additionally, serological tests can now identify individuals who have been infected. SARS-CoV-2 infection is associated with a fatality rate of around 1–3%, which is commonly linked to the development of acute respiratory distress syndrome (ARDS), likely resulting from uncontrolled immune activation, the so called “cytokine storm”. Risk factors for mortality include advanced age, obesity, diabetes, and hypertension. Drug repurposing has been used to rapidly identify potential treatments for COVID-19, which could move quickly to phase III. Better knowledge of the virus and its enzymes will aid the development of more potent and specific direct-acting antivirals. In the long term, a vaccine to prevent infection is crucial; however, even if successful, it might not be available before 2021–22. To date, except for intravenous remdesivir and dexamethasone, which have modest effects in moderate to severe COVID-19, no strong clinical evidence supports the efficacy of any other drugs against SARS-CoV-2. The aim of this review is to provide insights on the discovery of SARS-CoV-2, its virology, diagnostic tools, and the ongoing drug discovery effort.

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Introduction

The World Health Organization (WHO) announced on March 11th 2020, that the outbreak of “**CO**ro**na****V**irus **D**isease **2019**” (COVID-19), which initially started in Asia, had become a pandemic. As of September 4th 2020 the aetiologic agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread all over the world, leading to around 26 million confirmed cases and around 865,000 deaths.¹ The rapid availability of the genomic sequence of the viral RNA has been instrumental in the development of diagnostic tools and for the identification of experimental treatments. In this review, we will focus on the discovery of SARS-CoV-2, its virological features and pathogenesis, as well as diagnostic tools and, of course, drug development.

Overview of SARS-CoV-2 virology

The causative agent of COVID-19 is a novel coronavirus officially named SARS-CoV-2. It was named after SARS-CoV, because of their genomic homology.² Coronaviruses are enveloped, large, positive-sense single-stranded RNA viruses (+ssRNA) of the *Coronaviridae* family. Coronaviruses can infect a broad range of vertebrates, including bats, birds, pangolins, snakes, mice, and humans. Due to sequence similarities with RaTG13 bat and pangolin coronavirus strains, it is currently thought

that SARS-CoV-2 has a zoonotic origin and has secondarily acquired human-to-human spreading capacity.³ In particular, the acquisition of i) mutations in the receptor-binding area, ii) a polybasic furin cleavage site (RRRAR) at the junction of subdomain 1 and 2 of the spike protein and iii) a site of O-linked glycosylation in the same area, have enabled the virus to efficiently interact with high affinity (via its spike protein) with its *bona fide* cellular receptor (angiotensin-converting enzyme 2 [ACE-2]),⁴ to become more virulent and pathogenic, while potentially evading immune responses through O-glycan epitope masking.³

Fig. 1 provides general information on SARS-CoV-2 and its replication cycle, as well as a schematic representation of targets for drug development.

Where does the virus replicate?

Following replication and subgenomic RNA synthesis, the viral structural proteins are translated and inserted into the endoplasmic reticulum (ER). These proteins move along the secretory pathway into the ER–Golgi intermediate compartment. In infected cells, the CoV RNA-synthesising machinery associates with modified ER membranes that are transformed into the viral replication organelle; double-membrane vesicles appear to be the central hub for viral RNA synthesis.⁵ Notably, SARS-CoV-2

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Key point

An epidemic of acute respiratory syndrome (COVID-19) started in humans in Wuhan in 2019, and has since become a pandemic.



is present for significantly longer in stool samples than in respiratory samples.⁶

Mechanisms of virus-induced toxicity

The virus may be cytotoxic during the first days of infection. In biopsy or autopsy studies of patients infected with COVID-19, pulmonary pathology showed diffuse alveolar damage with the formation of hyaline membranes, infiltration of air spaces by mononuclear cells/macrophages, and a diffuse thickening of the alveolar wall.^{7,8} The lungs from patients with COVID-19 also showed severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes.⁹ Viral particles were observed in the epithelial cells by electron microscopy, suggesting that these lesions might be partially caused by direct cytotoxicity.

Future directions for basic research and target identification

A better understanding of the functions/roles of viral proteins, as well as of the viral replication cycle, with a particular attention on host-cell/virus interactions, will enable the identification of novel, or a better characterisation of existing, targets for antiviral development. The success of drug development for HCV has inspired scientists to achieve similar results for other viruses.¹⁰

Entry process

Many cell types express ACE2 and transmembrane serine protease 2 (TMPRSS2), the 2 cellular factors important for viral entry,¹¹ including nasal and lower airway epithelial cells (pneumocytes), lung resident immune cells, endothelial cells, as well as neurons, enterocytes, cardiomyocytes, hepatocytes and kidney cells.^{12–14} But the presence of mRNA in these cell types is not sufficient; further studies are needed to analyse the protein expression of these entry factors and to demonstrate *bona fide* viral entry and active replication in all these cell types. Interestingly, it was very recently shown that ACE2 is an interferon-stimulated gene (ISG),¹⁵ meaning that the presence of interferons in the microenvironment of the virus replication site could further enhance the spreading of the virus. The molecular details of the entry process, involving the spike protein and host receptor/co-receptor, have already been studied.¹⁶ The polybasic furin cleavage site at the junction of subdomain 1 and 2 of the spike protein may explain the large number of cell types that can be infected by the virus and the consequent diverse organ manifestations, possibly including thrombotic complications resulting from endothelial cell infection. This research will facilitate the identification of neutralising antibodies or small molecules, which could target this step of the life cycle.

Viral enzymes

Coronaviruses encode several enzymes that are crucial for the replication of the virus and are ideal

targets for antiviral development, including 2 proteases/proteinases (papain-like cysteine protease [PLpro] and 3-chymotrypsin-like protease [3CLpro]), the RNA-dependent RNA polymerase (RdRp), a helicase, an mRNA-cap-methyltransferase, and an exoribonuclease. These enzymes have been well studied for SARS-CoV, and thanks to the high homology between the 2 SARS-CoVs, we could expect functional similarities allowing for the possible repurposing of drugs.^{3,4,11}

The RdRp (also identified as Nsp12) bears the main enzymatic activity of the replicase complex. Recent advances in antiviral research against HCV^{10,17} have confirmed that RdRps are major targets for very specific antiviral discovery. Like HCV, the SARS-CoV-2 genome is characterised by a positive-sense single-strand RNA and both viruses share a similar replication cycle requiring an RdRp. This polymerase displays similar catalytic mechanisms and key conserved amino acids in the active site. The 3D structure of the SARS-CoV-2 RdRp was recently characterised.^{18,19} Interestingly it has a large N-terminal extension containing a kinase-like fold. The polymerase domain, like in HCV, is composed of 3 subdomains; a fingers subdomain, a palm subdomain, and a thumb subdomain. Moreover, 3CLpro is vital to viral replication and the 3CLpro cleavage sites are highly conserved, so it could be a promising drug target.¹⁸

PLpro and 3CLpro/Mpro are essential enzymes for the proteolytic processing of the CoV replicase polyprotein; their activities are needed very early in the infection process for the step-by-step release of other viral enzymatic activities. They are also attractive targets for specific antiviral discovery. The 3D structures of the SARS-CoV and SARS-CoV-2 proteases are available. Moreover biochemical assays are also available for functional testing, at least for the SARS-CoV proteins.²⁰ PLpro is a cysteine protease, encoded by Nsp3, and involved in the release of Nsp1 to 3, as well as in the regulation of host innate immunity, enabling viral escape.²⁰ Although the similarity is not very high between the PLpro of SARS-CoV-2 and that of SARS-CoV, the catalytic domain, around the triad Cys-His-Asp, is well conserved; therefore, drugs already in the pipeline for SARS-CoV might be repurposed.

3CLpro/Mpro is encoded by Nsp 5, forms a functional homodimer, utilises a catalytic dyad Cys-His, and is involved in the release of Nsp4 to 16 from polyprotein. Its activity is key in the CoV replication cycle and its inhibition is very efficient at stopping viral replication. Due to the dimeric nature of this protease, not only catalytic inhibitors, but also allosteric ones can be developed, increasing the possibility of success. Moreover the very high similarity of 3CLpro/Mpro between the SARS-CoVs may allow for drug repurposing.²⁰ Specific antiviral screening has been started and

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Key point

SARS-CoV-2 is a member of *Coronaviridae*, a family of enveloped, positive-sense, single-stranded RNA viruses that infect a broad range of vertebrates.

Key point

Groups from China identified and sequenced the virus responsible for COVID-19, named SARS-CoV-2, and determined that it was a novel coronavirus that shared high sequence identity with bat-derived SARS-like coronavirus, suggesting it had originated in bats.

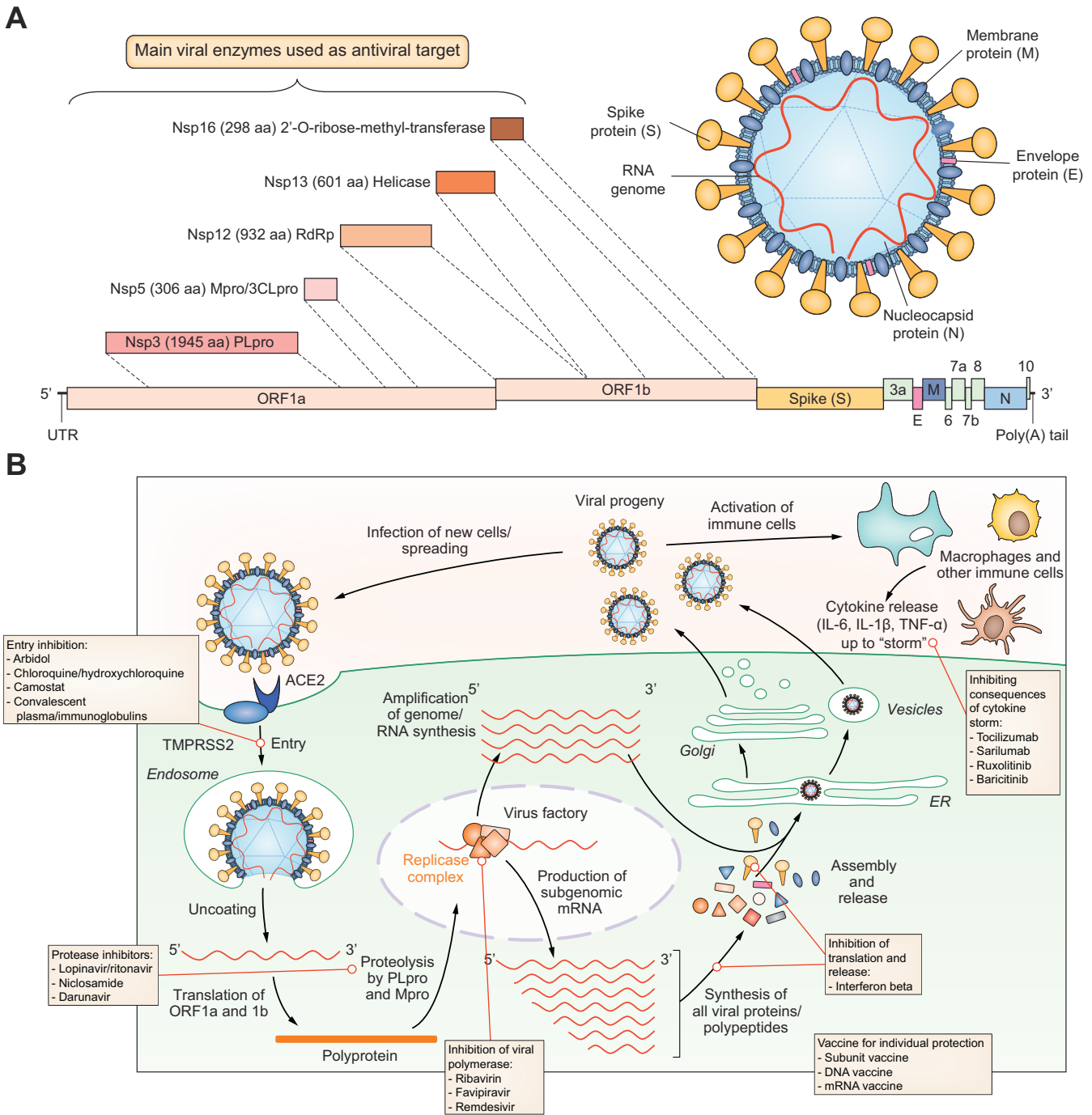


Fig. 1. Virology, replication cycle and targets for drug development. (A) Coronaviruses have a long, capped and poly-adenylated RNA genome, which contains between 8 to 10 ORFs, allowing structural, non-structural and accessory viral protein synthesis.⁸⁷ SARS-CoV-2 is 29,903 base-long and contains 6 major ORFs, as well as additional accessory genes; the reference sequence is registered in GenBank with ID: MN908947.3.¹ (A, B) Up to 28 different polypeptides are potentially produced *in fine* from the different ORFs and after polyprotein processing by viro-encoded proteases.⁸⁷ If the RNA genome contained in virions can already serve, after cell entry, as a template for the synthesis of non-structural proteins, which are involved in the early phase of virus replication (mainly by forming the replicase complex), subgenomic messenger RNAs are also produced in the late phase of the cycle to allow the synthesis of structural proteins (e.g. spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins), as well as other accessory polypeptides. Another main replication intermediate is the complementary minus-sense RNA, which is used by the viro-encoded RdRp, within the replicase complex, to amplify the full-length genome, which is then capped and polyadenylated by both viral and host enzymes before being incorporated into the viral progeny. (B) After entry into ACE2-positive (entry receptor) and TMPRSS2-positive (co-factor for entry) cells, and membrane fusion (*i.e.* uncoating process), a full-length genome is released into the cytoplasm of cells. This full-length polycistronic RNA is directly used to efficiently encode a polyprotein from the first ORFs present on the molecule, starting from 5' extremity, *i.e.* ORF1a and ORF1b; the latter is read after a frame-shift from ribosomal scanning of ORF1a. (A, B) The polyprotein is then processed by 2 viro-encoded proteases, PLpro/Nsp3 and 3CLpro/Nsp5 (also known as main protease [Mpro]), into 16 proteins/polypeptides (Nsp1 to 16). (B) These non-structural proteins/polypeptides are important for the early stages of infection, as they enable the formation of the replicase complex around the RdRp enzymatic activity, which is involved in the synthesis of negative-sense full-length RNA, as well as subgenomic messenger RNAs by a discontinuous transcription strategy.⁸⁷ The latter enables the efficient and stoichiometric synthesis of all other viral proteins/polypeptides, which are important for virus assembly and release of progeny virions. (B) Specific targets for drug development and current treatment options are indicated. ACE2, angiotensin-converting enzyme 2; 3CLpro/Nsp5, chemotrypsin-like protease; ORF, open reading frame; PLpro/Nsp3, papain-like cysteine protease; RdRp, RNA-dependent RNA polymerase; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2.

several drug candidates that target the 3CLpro of SARS-CoV-2 have already been identified.^{21,22}

Exacerbated innate immune functions

Besides the virological aspects of COVID-19, it is also important to better understand immunological factors, and how their mutual amplification is involved in the pathogenesis of the disease. While the virus can be studied in cell culture models, immunological factors can only be studied either in relevant animal models or during clinical studies, using patient samples. It is now rather well established that in patients with poor outcome there is an uncontrolled “cytokine storm”, featuring a local and systemic production of pro-inflammatory cytokines such as interleukin (IL)-6, tumour necrosis factor- α (TNF- α) and IL-1 β .^{23–27} Recently, it was reported that ACE2 is a human ISG; data suggest that SARS-CoV-2 could exploit species-specific interferon-driven upregulation of ACE2, a tissue-protective mediator during lung injury, to enhance infection.²⁸ More studies are needed to clarify the origin of this massive and uncontrolled cytokine production.

Diagnostic tools for COVID-19

COVID-19 tests can be grouped as nucleic acid, serological, antigen, and ancillary tests, all of which play distinct roles in hospital, point-of-care, or large-scale population testing (Fig. 2).²⁹

Methods for the detection of viral nucleic acid

PCR tests for SARS-CoV-2 have been available since January 2020. Reverse transcription quantitative PCR (RT-qPCR)-based assays performed on respiratory specimens have emerged as the cornerstone of COVID-19 diagnostic testing. The USA Centers for Disease Control and prevention has developed a widely used SARS-CoV-2 RT-qPCR assay.³⁰ The kit contains PCR primer-probe sets for 2 regions of the viral nucleocapsid gene (N1 and N2), and for the human RNase-P gene to ensure that RNA extraction was successful. This assay differs from the WHO's assay, which targets SARS-CoV-2 RdRP and E genes.³¹ To avoid potential cross-reaction with other endemic coronaviruses, as well as potential genetic drift, at least 2 molecular targets should be included in the assay. Evolution and potential mutations in the SARS-CoV-2 genome support the need to continue optimising the oligonucleotides through global sharing of updated SARS-CoV-2 genomes.³² The theoretical specificity of most RT-qPCR assays is 100% because the primer design is specific to the SARS-CoV-2 genome. Occasional false positive results may occur due to technical errors or reagent contamination.³³ A cycle threshold (Ct) value of RT-qPCR less than 40 is generally interpreted as positive when results are interpreted as qualitative.^{34,35} Quantitative interpretation of Ct as an indicator of the copy number of SARS-CoV-2 RNA in specimens requires an

appropriate standard curve with an adequate limit of detection.³⁶ A rigorous assessment of the diagnostic accuracy of the many newly introduced SARS-CoV-2 assays has been hampered by availability.^{37,38} The sensitivity of viral RNA testing varies depending on the timing of testing relative to exposure. A false positive result erroneously labels a person as infected, with consequences including unnecessary quarantine and contact tracing.³⁹ False-negative results are more consequential, because infected people may not be isolated and can infect others. One modelling study estimated that the probability of a false-negative result in an infected patient decreases from 100% on day 1 to 67% on day 4.⁴⁰ On the day of symptom onset, the median false-negative rate estimation was 38%. A sample pooling strategy was suggested to offer a viable alternative to detect community transmission at a time when tests are in short supply globally.^{41–43} One potential limitation of pool testing is that the false-negative rate may increase, owing to dilution of positive samples. Point-of-care PCR kits can shorten the turnaround time for screening and diagnosing patients with suspected SARS-CoV-2. These rapid tests typically have lower throughput and are generally more expensive than other tests. Time efficient methods that do not require thermal cycling have been designed.⁴⁴ CRISPR-Cas12/Cas13-based assays are also currently in development for point-of-care use.^{45,46}

Nature of samples tested

The current diagnostic strategy to identify patients with COVID-19 is to test samples taken from the respiratory tract for the presence of SARS-CoV-2-specific nucleic acid targets.⁴⁷ A nasopharyngeal specimen is the preferred choice for testing, but oropharyngeal, mid-turbinate, or anterior nares samples are also acceptable.⁴⁸ Pharyngeal virus shedding was shown to be very high during the first week of symptoms.⁴⁹ Infectious virus was readily isolated from throat and lung samples, but not from stool samples. Serum and urine were usually negative for the presence of viral nucleic acid.^{50,51} The viral load in nasopharyngeal samples peaks within the first few days after symptom onset, before declining.^{48,51,52} For nasopharyngeal specimens, samples should be obtained using a flocked swab to enhance the collection and release of cellular material.^{53,54} Samples taken from sputum, endotracheal aspirates, and bronchoalveolar lavage may have greater sensitivity than upper respiratory tract specimens.⁵⁰ Inadequate sample collection may result in a false-negative test. The highest rates of SARS-CoV-2 positivity on RT-qPCR assays were obtained with bronchoalveolar lavage specimens.⁵⁰ A single nasopharyngeal swab has become the preferred swab, as it is well tolerated and safe. Saliva may also be an alternative specimen source that

Key point

The sequencing of the virus has allowed for the development of diagnostic tools (e.g., RT-PCR). Additionally, serological tests have enabled the identification of individuals who have previously been infected.

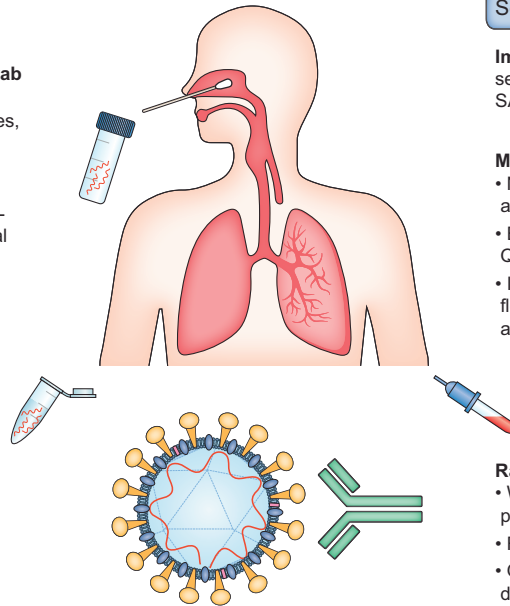
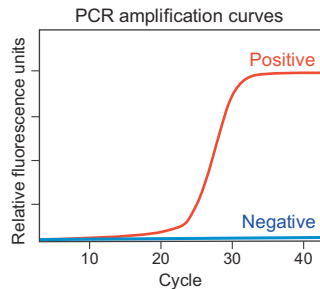
Molecular tests

Samples: respiratory tract:

- **Nasopharyngeal specimen using swab**
- Oropharyngeal and anterior nares specimen, sputum, endotracheal aspirates, bronchoalveolar lavage.

Detection of viral genome by real time RT-PCR-based assays with PCR primer-probe sets for regions of genes of the viral nucleocapsid, RNA pol, or envelope.

RT-PCR(+) from the first few days after symptom onset and prolonged up to 35 days (15 days on average)



Serological tests:

Immunoassays: Detection/quantification of seroconversion: Patient IgM and IgG specific to SARS-CoV-2 spike or nucleocapsid proteins.

Main types of immunoassays:

- **Neutralisation assay:** Quantitative information on antibodies able to inhibit virus growth *ex vivo*
- **Enzyme-linked immunosorbent assay (ELISA):** Quantification of antibodies specific to the virus
- **Immunochromatography assay:** qualitative lateral flow assay (rapid diagnostic test): detection of antibodies specific to the virus

Rapid diagnostic tests (RDT):

- Works with venous whole blood, serum, or plasma
- Rapid test (15 min) - No instrument required
- Only qualitative (aid screening and diagnosis in combination with RT-PCR)
- Aid in risk stratification and cohort study

Fig. 2. Diagnostic tools. ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription PCR.

requires less personal protective equipment and fewer swabs, but it requires further validation.^{55,56}

Serologic testing

While RT-qPCR-based molecular assays of respiratory specimens remain the current reference standard for diagnosis, point-of care technologies and serologic immunoassays have also rapidly emerged.^{57–59} Serologic tests that identify antibodies to SARS-CoV-2 from clinical specimens may be less complex than molecular tests.⁶⁰ As antibody responses to infection take days to weeks to be reliably detected,⁶⁰ their utility for diagnosing acute infections is limited.⁴⁸ Rapid antigen detection tests have recently entered the diagnostic market. Compared with RT-PCR, they are cheaper, and easy to use with faster turn-around times. The widespread and frequent use of such tests has recently been proposed but antigen rapid antigen detection tests's differ greatly in their ability to detect infectious cases, therefore requiring careful validation before routine application. Serologic assays might be more relevant in surveying for asymptomatic infection or in scenarios in which patients present with late complications of disease, when RT-qPCR may be falsely negative.^{55,61}

Seroconversion in most cases of COVID-19 occurs during the second week of symptoms.⁴⁹ For SARS-CoV-2 infection, the timing of seroconversion appears to be similar to or slightly earlier than in SARS-CoV infection.⁶² In a study of 285 patients with COVID-19, 100% of patients tested positive for antiviral IgG within 19 days after symptom onset, with seroconversion for IgG and IgM occurring

simultaneously or sequentially.⁶¹ Negative results would not exclude COVID-19 infection, particularly among those with recent exposure to the virus. The viral spike protein is perceived as the clear candidate for inclusion in an immunoassay that detects whether antibodies are present.^{58,63} The other protein that appears to be an important antigen for the development of serological assays is the N protein (structural component of the nucleocapsid). Indeed, antibodies to this protein are frequently detected in patients with COVID-19,^{64,65} suggesting that the N protein may be one of the immunodominant antigens for the early diagnosis of COVID-19.^{60,66,67} It is now established that pre-existing immune reactivity to SARS-CoV-2 can exist in the general population. Serum samples from patients with COVID-19 showed some cross-reactivity for the SARS-CoV nucleocapsid antigens.^{61,68,69} A recent study detected SARS-CoV-2-reactive CD4+ T cells in 50% of unexposed individuals, suggesting cross reactive T cell recognition between circulating "common cold" coronaviruses and SARS-CoV-2.⁶⁶ T cell reactivity was highest against proteins other than the coronavirus spike protein, but T cell reactivity was also detected against the spike protein. Several monoclonal antibodies have been described that target the spike glycoprotein of SARS-CoV-2 from memory B cells of an individual who was infected with SARS-CoV in 2003.⁶⁸ One antibody (S309) potentially neutralises SARS-CoV-2 by engaging the receptor-binding domain of the spike glycoprotein.

Enzyme-linked immunosorbent assays (ELISA) and chemiluminescent immunoassay (CLIA) are

common laboratory platforms that can measure antibody titres (IgG and IgM). A variation of these tests can use magnetic, protein-coated microparticles, known as a chemiluminescent microparticle immunoassay. Being able to quantify antibodies will be important to identify convalescent plasma donors with abundant titres and to study how the immune system responds to the virus. Neutralising antibodies play important roles in viral clearance and have been considered as a key immune product for protection or treatment against viral diseases. In COVID-19, transfusion of convalescent plasma or serum from recovered patients was also considered a promising therapy.^{70,71} The neutralisation assay is a laboratory-based test that uses live virus and cell culture methods to determine if patient antibodies can prevent viral infection *in vitro*.⁷²

Because immunofluorescence assays are labour intensive, a substantial number of the new commercial COVID-19 antibody tests – developed as screening tests – are not ELISA-based. They are lateral flow immunoassays (LFIAs), which provide no quantitative information. These qualitative LFIAs represent typically small, portable rapid diagnostic tests that can be used at point-of-care.

Conclusions on serologic testing

Antibody testing is ramping up quickly, with a growing list of commercial kits and test protocols from academic researchers,⁵⁷ although many questions remain to be answered. The first and most urgent is the validation of serologic tests. A recent meta-analysis showed wide ranging sensitivities, from 66% with LFIAs to 98% with CLIAs⁷³; sensitivities were higher with increased time after symptom onset. The specificities are excellent (99%). Assays must be optimised further, independently validated, and used as part of an algorithm to achieve the highest possible accuracy for decision making.^{74,75} Second, there is insufficient data on the magnitude and duration of antibody responses after infections. Although data suggest that neutralising titres correlate with severity of infection,⁶¹ it remains unclear whether this effect is caused by ongoing somatic hypermutation or ongoing production of highly potent antibodies that were initially generated. Moreover, any documentation that limits individual freedoms on the basis of biology risks becoming a platform for restricting human rights.⁷⁶

Pathophysiology and clinical characteristics of COVID-19

Pathophysiology

Several potential pathogenic mechanisms may be involved in COVID-19, including coagulopathy, endothelial dysfunction, and excessive release of pro-inflammatory cytokines. The endothelial dysfunction caused by infection activates excessive

thrombin generation and inhibits fibrinolysis, leading to hypercoagulability.⁷⁷ Lymphopenia is frequent in patients with COVID-19.⁷⁸ The cytokine release syndrome could have a major role in patients with severe COVID-19 as in acute respiratory distress syndrome (ARDS).⁷⁹ The pathological features of COVID-19-related ARDS are diffuse alveolar damage with hyaline membrane formation and fibrin deposition, as well as a few multinucleated enlarged cells.^{7,8} In patients who died from COVID-19-associated respiratory failure, the histologic pattern in the peripheral lung was diffuse alveolar damage with perivascular T cell infiltration.⁹ The lungs also showed distinctive vascular features, consisting of severe endothelial injury, but also widespread thrombosis with microangiopathy. Alveolar capillary microthrombi were frequent, with a high level of new vessel growth (intussusceptive angiogenesis).

Transmission by asymptomatic carriers

Several findings are consistent with person-to-person transmission of this novel coronavirus in hospital and family settings.^{47,80} There is also evidence of asymptomatic transmission, including a case of SARS-CoV-2 infection acquired outside of Asia in which transmission appears to have occurred during the incubation period.⁸¹ Additionally, in a previously reported family cluster, some family members had positive RT-qPCR results without any symptoms.⁴⁷

Clinical characteristics

Among 1,099 patients from China with laboratory-confirmed COVID-19, 5.0% were admitted to an intensive care unit (ICU), 2.3% underwent invasive mechanical ventilation, and 1.4% died.⁷⁸ The most common symptoms were fever and cough. The median incubation period was 4 days. In another study including 191 patients, of whom 54 died in hospital, half of these patients had a comorbidity, with hypertension being the most common, followed by diabetes and coronary heart disease.⁵² In-hospital death was associated with older age, higher sequential organ failure assessment score, and D-dimer greater than 1 µg/ml on admission. In another study of the 1,591 patients infected with SARS-CoV-2 admitted to ICUs in Italy, the median age was 63 years and 82% were male.⁸² Among 1,300 patients with available data on respiratory support, 99% needed respiratory support, including 88% who received mechanical ventilation and 11% who received non-invasive ventilation. Finally, in this case series of critically ill patients admitted to ICUs, the majority were older men and ICU mortality was 26%.

Moreover, data from previous coronavirus infections such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome, as well as emerging data from the COVID-19 pandemic, suggest that there could be substantial

Key point

Testing and tracing programmes will be essential. Later, testing, tracing and treating (T3) programmes will become mandatory, once effective and safe therapies are developed.

Key point

Early strong social distancing efforts are needed to stop transmission of the virus and are important measures to reduce case incidence. In addition, the use of masks, soaps, and disinfectants are critical to reduce or eliminate viral spread. Case isolation and contact tracing has also proven effective.

fibrotic consequences following SARS-CoV-2 infection.⁸³

Pulmonary imaging findings

The hallmarks of COVID-19 were bilateral and peripheral ground-glass and consolidative pulmonary opacities.⁸⁴ Notably, 56% of patients with early disease had a normal CT. A longer time after the onset of symptoms, abnormal CT findings were more frequent, including consolidation, bilateral and peripheral disease, greater total lung involvement, linear opacities, “crazy-paving” pattern and the “reverse halo” sign. Bilateral lung involvement was observed in 28% of cases in the early phase and 88% in late phase of the disease. CT scans at the time of symptoms may increase diagnosis rates, since RT-qPCR sensitivity may be as low as 60%.⁸⁵ Also, chest x-ray findings in patients with COVID-19 frequently showed bilateral lower zone consolidation.⁸⁶

Extrapulmonary manifestations

Coagulopathies

SARS-CoV-2-induced infection can be associated with a coagulopathy consistent with infection-induced inflammatory changes, as observed in patients with disseminated intravascular coagulopathy (DIC).⁸⁷ In patients with COVID-19, the initial coagulopathy is associated with elevated D-dimer and fibrin/fibrinogen-degradation products. COVID-19-associated coagulopathy should be managed as it would be for any critically ill patient, using thromboembolic prophylaxis and standard supportive care measures for those with sepsis-induced coagulopathy or DIC. Current data do not support the use of high-dose anticoagulants.⁸⁷

Among all the numerous clinical manifestations associated with COVID-19 infection, there have been reports of cardiological lesions with acute myocardial injury⁸⁸; neurological lesions with encephalitis and myalgia,^{89,90} cutaneous manifestations with rash and urticaria,⁹¹ and acute kidney injury⁹² (Fig. 3).

COVID-19 and the liver

Elevation of liver enzymes occurs in 5 to 50% of patients. The pattern of liver injury is mainly hepatocellular rather than cholestatic,^{93,94} with hepatocyte degeneration, focal necrosis, capillary bile duct cholestasis and inflammation in the portal area, but interestingly SARS-CoV-2 cannot be detected in liver samples.⁹⁵ Frequently, the severity of liver injury has been correlated with the severity of COVID-19. The presence of underlying chronic liver diseases could render patients with COVID-19 at higher risk of severe liver injury, such as acute-on-chronic liver failure,⁹⁶ with data suggesting that non-alcoholic/metabolic fatty liver disease (NAFLD/MAFLD) could be an independent risk factor for severe COVID-19.^{97,98}

The virus was found in stool samples in around 50% of patients with COVID-19, with around 18% of them complaining of abdominal pain and diarrhoea.⁹⁹ It was demonstrated that SARS-CoV-2 is capable of productively replicating in ACE2-positive enterocytes.¹² Due to the abundance of the virus in the small intestine, liver cell exposure through the hepatic reticular system is expected. The default immune status of the liver might play a critical role in COVID-19 infection. Indeed, it has been shown that in patients with MAFLD, the polarisation status of macrophages might be skewed due to metabolic stimuli such as fatty acids, thus affecting host-inflammatory responses to signals generated from the gut-liver axis.⁹⁷ In COVID-19, the “cytokine storm” bears resemblance to that observed in patients with SARS.^{100–102}

However, SARS-CoV-2 could also have a direct cytotoxic effect, as its entry receptor ACE-2 is expressed on cholangiocytes.¹⁰³ Also, learning from the SARS experience, the use of antibiotics and antivirals, as well as possible secondary bacterial infections, might lead to liver injury in patients with COVID-19.¹⁰⁴ Moreover, tocilizumab has been evaluated for the treatment of patients with COVID-19 and serious lung damage accompanied by elevated blood levels of IL-6.¹⁰⁵ Prophylactic nucleoside analogues against HBV have been recommended for HBsAg-positive patients with COVID-19 for whom immunosuppressive therapy is planned.¹⁰² Liver damage, leading to drug withdrawal, has been reported in patients treated with remdesivir. Accordingly, remdesivir is not recommended for patients with alanine aminotransferase >5x the upper limit of normal or with hepatic decompensation.¹⁰⁶ Lastly, hypoxia and shock induced by COVID-19-related complications may also cause hepatic ischaemia.¹⁰⁷ To manage liver injury related to COVID-19, several guidelines have been issued.^{100–102,108}

Gastrointestinal manifestations

Clinically, approximately 10% of the patients with COVID-19 suffer from gastrointestinal symptoms such as nausea or vomiting, diarrhoea and anorexia,¹⁰⁹ with similar incidence among adults and children.¹¹⁰ Patients with gastrointestinal symptoms may require longer hospitalisations.^{78,79,111} In some patients, gastrointestinal (not respiratory) symptoms might be the presenting clinical features.^{112,113} The underlying mechanism may be related to the abundant expression of ACE2 mRNA and receptor protein on enterocytes.^{112,113} Histological changes, including plasma cell and lymphocyte infiltration into the lamina propria of enterocytes, suggested an immune-mediated response.¹¹⁴ The capability of SARS-CoV-2 to infect enterocytes has also been demonstrated in human intestinal organoids.¹² One of the major concerns around enteric infection is

Key point

Drug repurposing is a strategy to identify new uses for approved or investigational drugs that are outside the scope of the original medical indication. This strategy has been used to rapidly identify treatments for the COVID-19 infection that could move quickly to phase III.

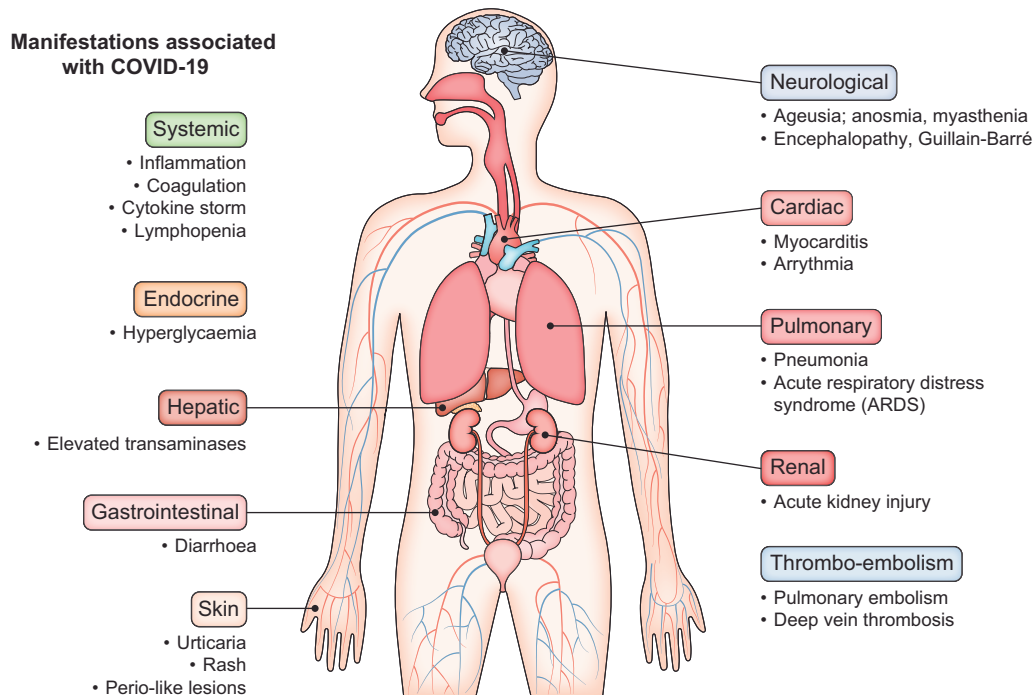


Fig. 3. Systemic manifestations of COVID-19. COVID-19, coronavirus disease 2019.

whether the faecal source can lead to fomite transmission, especially when infective aerosols are generated from the toilet plume. Indeed, a cluster of COVID-19 cases potentially linked to faecal transmission, analogous to “Amoy Garden” during the SARS outbreak in 2003, has recently been reported in Hong Kong.¹¹⁵ In accordance with a surface stability study on plastic and different materials, SARS-CoV-2 could remain viable for up to 72 hours.¹¹⁶ In 1 study, faecal samples remained SARS-CoV-2 positive despite respiratory clearance in 20% of patients.¹¹⁴ Taken together, determining the presence of SARS-CoV-2 in the stool is of great importance for the epidemiological control of COVID-19.

Co-infections

There is major concern regarding the potential for concomitant infection of SARS-CoV-2 with influenza or other respiratory diseases, such as respiratory syncytial virus, tuberculosis or even bacterial infections or mycoplasma. Co-infection with SARS-CoV-2 and influenza A virus in a patient with pneumonia has been reported in China.¹¹⁷ COVID-19 might be underdiagnosed because of false-negative tests for upper respiratory specimens or co-infection with other respiratory viruses.

Treatment strategies

Prevention and transmission control measures

Washing hands frequently, using masks and social distancing are important. China banned travel to

and from Wuhan city on 23 January 2020, delaying the arrival of COVID-19 in other cities by approximately 3 days.¹¹⁸ Suspending intra-city public transport, closing entertainment venues and banning public gatherings were associated with reductions in case incidence. Early on, the spatial distribution of COVID-19 cases in China was explained well by human mobility data.¹¹⁹ Following the implementation of control measures, this correlation dropped and growth rates became negative in most locations. A contact tracing application, which builds a memory of proximity contacts and immediately notifies contacts of positive cases could achieve epidemic control if used by enough people.¹²⁰

Timing of treatment

Much like with influenza, antiviral drugs likely need to be started early after infection to be effective. In turn, this makes it difficult to identify drugs that are indeed effective against the virus in clinical trials. Patients with early disease may benefit from antiviral agents to reduce viral load, patients with severe and late disease may benefit from anti-inflammatory drugs. Furthermore, in the early disease course, anti-inflammatory drugs might be harmful by increasing viral load.

Drug repurposing

Drug repurposing (also called drug repositioning or repurposing) involves identifying new uses for

Key point

To date, with the exception of intravenous remdesivir or dexamethasone which have a modest effect, no strong clinical evidence supports the efficacy of any drug against SARS-CoV-2.

Table 1. Drugs evaluated in clinical trials for the treatment of COVID-19 (not exhaustive).

Drug	Company	Current use and/or initial target	Mode of Action	Mode of Administration	Clinical trials for COVID-19 (examples) (NCT)(not exhaustive)
Antiviral					
Remdesivir	Gilead	Experimental, Ebola	Nucleotide analogue	intravenous	NCT04252664; NCT04280705; Solidarity (WHO); NCT04292899
Chloroquine/hydroxychloroquine (Aralen/Plaquenil)	Sanofi	Malaria	Heme polymerase inhibitor	Oral	NCT04333732; NCT04341727; NCT04358068; NCT04315948
Lopinavir + ritonavir (Kaletra)	AbbVie	HIV	Protease inhibitor	Oral	NCT04307693; NCT04372628; NCT04255017; NCT04276688
Favipiravir (Avigan)	Fujifilm	Experimental, Influenza	RNA polymerase inhibitor	Oral	NCT04333589; NCT04310228; NCT04346628
Umifenovir (Arbidol)	Pharmstandard	Experimental, Influenza	Inhibits membrane fusion (entry)	Oral	NCT04333589
Camostat	Ono Pharmaceutical	Experimental	Protease inhibitor	Oral	NCT04374019
Ribavirin	Bausch Health	Hepatitis C	Lower respiratory tract infection due to RSV	Inhalation	NCT04356677
Anti-inflammatory					
Interferon alfa-2b		Hepatitis C	Immune modulator	Sub-cutaneous	NCT04349410
Tocilizumab (Actemra)	Roche	Rheumatoid arthritis	IL-6R Ab	intravenous	NCT04310228; NCT04331795; NCT04320615; NCT04310228
Sarilumab (Kevzara)	Sanofi	Rheumatoid arthritis	IL-6R Ab	intravenous	NCT04315298; NCT04359901
Baricitinib (Olumiant)	Eli Lilly	Rheumatoid arthritis	Inhibition of JAK	Oral	NCT04340232; NCT04373044

Ab, antibody; COVID-19, coronavirus disease 2019; RSV, respiratory syncytial virus.

approved or investigational drugs that are outside the scope of the original medical indication.¹²¹ This strategy offers various advantages over developing an entirely new drug, with a reduced risk of failure because safety has already been evaluated. The timeframe and the cost can also be reduced, because most of the preclinical testing and safety assessments have already been done. There have been extensive efforts to repurpose approved drugs during the COVID-19 pandemic. A selection of drugs being tested for COVID-19 is presented in Table 1. As an example, the design of "Solidarity" – a large randomised trial that is currently ongoing – is provided in Fig. 4.

Existing antiviral medicines targeting the virus

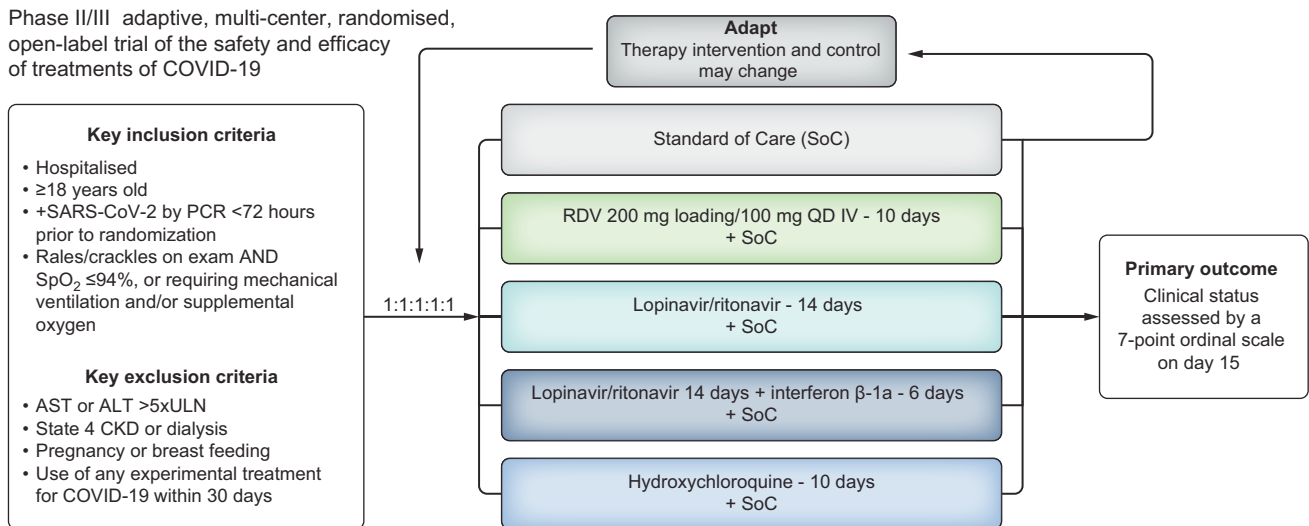
Hydroxychloroquine

Hydroxychloroquine is a medication used to prevent and treat lupus and malaria. Hydroxychloroquine has also been combined with azithromycin, an antibiotic. Hydroxychloroquine is hypothesised to inhibit SARS-CoV-2 entry into cells, although there is limited data, mostly coming from case reports and small studies.¹²² A systematic review on the efficacy and safety of hydroxychloroquine for the treatment of COVID-19 concluded that there is currently no evidence from RCTs for its efficacy.¹²³ In a multicentre, open-label, randomised controlled trial, 150 patients admitted to hospital with laboratory-confirmed COVID-19 were included in the intention-to-treat analysis (75 patients assigned to hydroxychloroquine plus standard of care [SOC], 75 to SOC).¹²⁴ There was no difference in terms of efficacy between the 2 arms. Adverse events were higher in patients treated with hydroxychloroquine.

Lopinavir

Lopinavir is an antiretroviral protease inhibitor used in combination with ritonavir in HIV therapy; it has shown some antiviral activity against SARS-CoV.¹²⁵ A randomised, controlled, open-label trial involving hospitalised adult patients with confirmed SARS-CoV-2 infection and COVID-19-related severe respiratory illness was performed.¹²⁶ Patients were randomly assigned to receive either lopinavir-ritonavir, in addition to SOC, or SOC alone. There were no differences between groups (virological factors, duration of disease, mortality), indicating that there is no benefit in hospitalised adult patients with severe COVID-19. Cell culture data suggest that this compound demonstrates activity with an EC₅₀ of 26.6 μM.¹²⁷ One wonders why a compound with such weak activity was selected for clinical trials. Human trials of repurposed drugs that are essentially ineffective against SARS-CoV-2 in culture are being repeated over and over again, wasting time and resources.

Phase II/III adaptive, multi-center, randomised, open-label trial of the safety and efficacy of treatments of COVID-19



Source: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments>

Fig. 4. WHO master protocol: Solidarity trial. ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; COVID-19, coronavirus disease 2019; RDV, remdesivir; SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2; SOC, standard of care; SpO₂, oxygen saturation; ULN, upper limit of normal.

Remdesivir

Remdesivir is a prodrug of a nucleotide analogue that is intracellularly metabolised to an analogue of adenosine triphosphate that inhibits viral RNA polymerases. Remdesivir has broad-spectrum activity against members of several virus families, including filoviruses (e.g., Ebola) and coronaviruses (e.g., SARS-CoV and MERS-CoV).¹²⁸ Six large studies are ongoing (Table 2). Unfortunately, remdesivir must be given intravenously for at least 5 days, although an aerosol formulation is being developed.

A report based on the compassionate use of remdesivir for patients hospitalised with severe COVID-10 has been published.¹²⁹ From the 53 patients whose data were analysed, clinical improvement was observed in 36/53 patients (68%). In addition, a randomised, double-blind, placebo-controlled, multicentre trial was performed in China.¹⁰⁶ Mortality at day 28 was similar between the 2 groups (14% died in the remdesivir group vs. 13% in the placebo group). There was no difference in the 2 groups regarding clinical improvement or decrease in viral load. This trial did not attain the predetermined sample size because the outbreak of COVID-19 was brought under control in China, therefore, it is difficult to reach a definitive conclusion.

Gilead is conducting 2 randomised, open-label, multicentre, phase III clinical studies to evaluate the safety and efficacy of 2 dosing durations – 5 days and 10 days – of remdesivir in adults diagnosed with COVID-19 (The Simple studies). The first SIMPLE study includes hospitalised patients with confirmed SARS-CoV-2 infection, oxygen saturation of 94% or less while breathing ambient

air, and radiologic evidence of pneumonia.¹³⁰ In the second Simple study, patients were randomised to receive open-label remdesivir for 5 or 10 days or SOC alone. At Day 11, a higher proportion of patients in the 5-day treatment group achieved improvement in clinical status vs. the SOC group, achieving statistical significance for a ≥1-point improvement in ordinal scale ($p = 0.026$) (Gilead press release). However, most clinicians would have preferred to see a decrease in mortality on treatment. Clearly another controlled study will have to be performed soon.

Other antiviral drugs being tested for COVID-19 include arbidol,^{131,132} favipiravir,¹³³ famotidine,^{134,135} and camostat (TMPRSS2 inhibitor).¹¹

Existing antiviral medicines targeting inflammation

Dexamethasone

Glucocorticoids may modulate inflammation-mediated lung injury and thereby reduce progression to respiratory failure and death. In a controlled, open-label trial of patients hospitalised with COVID-19, patients were randomly assigned to receive oral or intravenous dexamethasone (6 mg once daily) for up to 10 days or to receive SOC alone.¹³⁶ In the dexamethasone group, the incidence of death was lower than that in the SOC group among patients receiving invasive mechanical ventilation (29.3% vs. 41.4%) and among those receiving oxygen without invasive mechanical ventilation (23.3% vs. 26.2%) but not among those who were receiving no respiratory support at randomisation (17.8% vs. 14.0%).

In a recent trial involving patients with ARDS who were undergoing mechanical ventilation,

Key point

Better knowledge of the virus, its enzymes, and immune response is essential for the development of direct-acting antivirals and effective vaccines.

mortality at 60 days was 15 percentage points lower among those receiving dexamethasone than among those receiving SOC.¹³⁷ In the early phase of the infection, anti-inflammatory drugs may not be efficient and may even be harmful (by increasing viral load). Viral shedding in SARS-CoV-2 appears to be higher early in the illness before declining thereafter.^{49,54,138} The fact that dexamethasone confers a greater survival benefit in patients with COVID-19 who are receiving respiratory support, or are recruited after the first week of their illness, suggests that by this stage the disease is dominated by inflammation, with active viral replication playing a secondary role. Clearly a trial of the combination of remdesivir and dexamethasone may yield interesting results.

Interferon beta-1b

The early triple combination of interferon beta-1b, lopinavir-ritonavir, and ribavirin was safe and superior to lopinavir-ritonavir alone in alleviating symptoms and shortening the duration of viral shedding and hospital stay in patients with mild to moderate COVID-19. Future clinical studies using interferon beta-1b as a backbone are warranted.¹³⁹

Tocilizumab & Sarilumab

Tocilizumab (Actemra), also known as atiluzumab, and sarilumab (Kevzara) are both immunosuppressive drugs, mainly used for the treatment of rheumatoid arthritis. They are both humanised monoclonal antibodies against the IL-6R and are given by injection. Clinical trials are ongoing. Moreover, other monoclonal antibodies or agents targeting other inflammatory cytokines (TNF- α , IL-1 β ...) should be tested.

Kinase inhibitors

The Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway has been implicated as a key driver in many inflammatory diseases. With the development of small molecule inhibitors that can selectively and specifically target key JAKs involved in controlling downstream inflammation, exploration of their utility across a broad range of diseases has become a rapidly expanding field,^{140,141} including for other viral infections (e.g. HIV¹⁴²⁻¹⁴⁴). Baricitinib and ruxolitinib are 2 known JAK inhibitors. Recently, artificial intelligence enabled the identification of a group of approved drugs that could inhibit clathrin-mediated endocytosis and thereby inhibit viral infection of cells.^{145,146} The drug targets are members of the numb-associated kinase (NAK) family. Baricitinib was identified as a NAK inhibitor, with a particularly high affinity for

Key point

A vaccine to prevent infection is crucial; however, even if 50% effective or more, the immunological protection might not persist.

Table 2. Clinical trials of remdesivir for treatment of COVID-19.

Study ID	Study Design	Location	Sponsor	Study size (randomisation)	Primary endpoint/outcome
Terminated studies					
NCT04257656 (terminated)	Double-blind, placebo-controlled (severe)	Beijing, China	Capital Medical University, China	N = 453 (2:1) 10 day RDV:Placebo	Time to clinical improvement by Day 28
NCT04252664 (suspended)	Double-blind, placebo-controlled (mild/moderate)	Wuhan, China	Capital Medical University, China	N = 308 (1:1) 10 day RDV:Placebo	Time to clinical recovery by Day 28
Ongoing studies					
NCT04292899	Open-label (severe)	Global	Gilead	Part A N = 400 (1:1) 10 day RDV:5 day RDV	Endpoint: Clinical status at Day 14 on 7-point ordinal scale Endpoint: Clinical status at Day 11 on 7-point ordinal scale Outcome: Time to recovery [Timeframe: Day 1 through Day 29] Outcome: Percentage of individuals reporting each severity rating on a 7-point ordinal scale [Timeframe: Day 15]
NCT04292730	Open-label (moderate)	Global	Gilead	Part A N = 600 (1:1:1) 10 day RDV:5 day RDV:SoC	Endpoint: Clinical status at Day 11 on 7-point ordinal scale Outcome: Time to recovery [Timeframe: Day 1 through Day 29]
NCT04280705	Adaptive, double-blind, placebo-controlled	Global	NIAID	N = 572 (1:1) 10 day RDV:Placebo	Endpoint: Clinical status at Day 14 on 7-point ordinal scale Endpoint: Clinical status at Day 11 on 7-point ordinal scale Outcome: Time to recovery [Timeframe: Day 1 through Day 29] Outcome: Percentage of individuals reporting each severity rating on a 7-point ordinal scale [Timeframe: Day 15]
NCT04315948	Adaptive, open-label	Europe	WHO/Institut National de la Santé Et de la Recherche Médicale, France	N = 3,100 (1:1:1:1:1) 10 day RDV:LPV/r: LPV/r+IFN: Hydroxychloroquine:SoC (1:1:1:1:1)	Endpoint: Clinical status at Day 14 on 7-point ordinal scale Endpoint: Clinical status at Day 11 on 7-point ordinal scale Outcome: Time to recovery [Timeframe: Day 1 through Day 29] Outcome: Percentage of individuals reporting each severity rating on a 7-point ordinal scale [Timeframe: Day 15]
Solidarity master protocol	Adaptive, double-blind, placebo-controlled	Global	WHO	10 day RDV:LPV/r: LPV/r+IFN: Hydroxychloroquine:SoC	Endpoint: Clinical status at Day 14 on 7-point ordinal scale Endpoint: Clinical status at Day 11 on 7-point ordinal scale Outcome: Time to recovery [Timeframe: Day 1 through Day 29] Outcome: Percentage of individuals reporting each severity rating on a 7-point ordinal scale [Timeframe: Day 15]

COVID-19, coronavirus disease 2019; IFN, interferon; LPV/r, lopinavir + ritonavir; RDV, remdesivir; SoC, standard of care.

AAK1, a pivotal regulator of clathrin-mediated endocytosis. This drug is also known to target JAK and could have a dual action against the virus and inflammation.¹⁴⁷ The NIH/NIAID sponsored ACTT-2 study is still ongoing and compares remdesivir to remdesivir plus baricitinib in patients with moderate to severe COVID-19. In a small uncontrolled cohort of Veterans Affairs patients with moderate-severe COVID-19, treatment with baricitinib plus hydroxychloroquine was associated with recovery in 11 of 15 patients.¹⁴⁸

Two other kinase inhibitors, namely imatinib mesylate and dasatinib, could also be envisaged to treat COVID-19.¹⁴⁹ Furthermore, ruxolitinib (another JAK inhibitor-Incyte) is being evaluated in a multicentre phase II clinical trial.¹⁵⁰

Therapeutic antibodies

Therapeutic antibodies are becoming increasingly attractive for the treatment of SARS-CoV-2, as they can be designed to specifically target viral antigens. REGN-COV-2 is a dual-antibody cocktail that contains 2 potent, non-competing and virus-neutralising antibodies (Regeneron, press release). The 2 antibodies of REGN-COV-2 bind non-competitively to critical portions of the receptor-binding domain of the virus' spike protein. The treatment could also help prevent infection by blocking the ability of the spike protein to bind to target host cells and facilitate viral entry. In addition to Regeneron, Eli Lilly, AbCellera and other companies also began testing their antibody treatment in humans.

Convalescent plasma

The immediate use of convalescent plasma provides a promising treatment. In a preliminary uncontrolled case series of 5 critically ill patients with COVID-19 and ARDS, administration of convalescent plasma containing neutralising antibody was followed by improvement in their clinical status.⁷¹ The limited sample size of this study precludes a definitive statement about the efficacy of this treatment.

Vaccines

Vaccines are the most effective strategy for preventing infectious disease as they reduce morbidity and mortality, and they are more cost-effective than treatment. Despite previous coronavirus epidemics, there is still no approved vaccine for human coronaviruses.

We will have to improve our understanding and knowledge regarding immune responses to SARS-CoV-2. Interestingly, in rhesus macaques, comparing the humoral and cellular immunity between primary infection and re-challenge revealed notably enhanced neutralising antibody and immune responses.¹⁵¹ These results suggest

that primary SARS-CoV-2 exposure protects against subsequent reinfection in rhesus macaques. In humans, a large study of the Icelandic population reported that the humoral response did not decline within 4 months after infection, that 44% of persons who had been infected had not been diagnosed with PCR, and that the fatality rate was 0.3%.¹⁵² We must also recall that cases of SARS-CoV-2 reinfection have been reported. Epidemiological, clinical, serological and genomic analyses confirmed that the patient had reinfection instead of persistent viral shedding from first infection.¹⁵³ This case leads to several open questions: How frequent is reinfection? Are reinfections less severe than the first infection? Will a vaccine protect against reinfections? These results suggest SARS-CoV-2 may continue to circulate among the human population despite herd immunity (whether due to natural infection or vaccination). Further studies of patients with reinfection will shed light on protective correlates important for vaccine design.

In the past two decades, the world has seen three coronaviruses emerge and cause outbreaks that have caused considerable global health consternation,¹⁵⁴ with no vaccine available up to now. Regarding vaccine development, among the different strategies, we can recall the use of recombinant subunit vaccines, DNA vaccines and mRNA vaccines. Subunit vaccines are believed to be highly safe because they are expected to induce the immune system without introducing infectious viruses.¹⁵⁵ A better knowledge of SARS-CoV-2 spike and/or N protein organisations will be required to develop such vaccines. The SARS-CoV-2 spike glycoprotein mediates host cell attachment and is required for viral entry; it is the primary vaccine target for many candidate SARS-CoV-2 vaccines.

DNA vaccines are based on direct injection of plasmids encoding the desired viral antigens, which induce a large range of immune responses. mRNA-based vaccines contain mRNAs encoding the antigens, which are translated at the host cellular machinery by vaccination.¹⁵⁶ mRNA vaccines have advantages over conventional vaccines, including the absence of genome integration, the improved immune responses, their rapid development, and the production of multimeric antigens.^{156,157}

A preliminary report on an mRNA vaccine against SARS-CoV-2 has been published.¹⁵⁸ The candidate vaccine mRNA-1273 (Moderna) is a lipid nanoparticle-encapsulated, nucleoside-modified mRNA-based vaccine that encodes the SARS-CoV-2 spike glycoprotein stabilised in its prefusion conformation. A phase I, dose-escalation, open-label trial was conducted including 45 healthy adults, who received 2 vaccinations, 28 days apart,

Box 1. COVID-19: future research goals.

1. Define mechanisms determining establishment of SARS-CoV-2 infection: characterize all steps of the virus replication cycle
2. Define structure and function of the SARS-CoV-2 enzymes and their interactions
3. Understand physiopathology and immune response
4. Improve methods for study of the replication cycle and virus-host interactions to reveal new targets for therapeutic approaches
5. Develop and validate diagnostic tools improving sensibility and specificity (serology, rapid diagnostic test)
6. Understanding modes of transmission of SARS-CoV-2 to improve prevention
7. Describe all the clinical manifestations of the disease
8. Understand if humoral and cell-mediated immune responses induce protection against infection
9. Conduct randomized clinical trials with repurposing drugs & new specific direct-acting antivirals & anti-inflammatory drugs
10. Develop vaccine with safety and efficacy

COVID-19, coronavirus disease 2019.

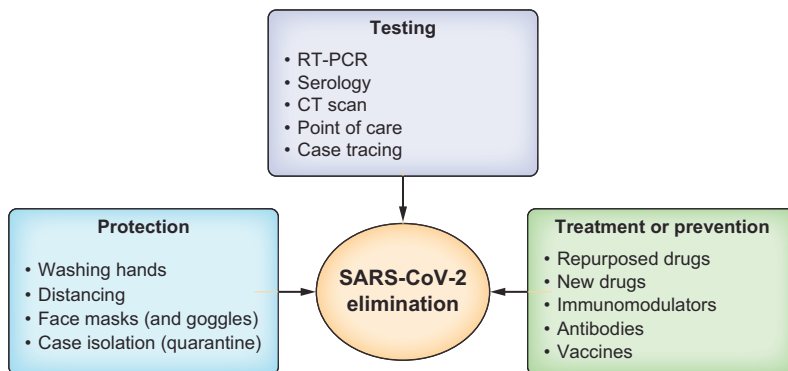


Fig. 5. Milestones for SARS-CoV-2 elimination. To achieve SARS-CoV-2 elimination there will be a need to improve protection, testing, treating and preventing strategies. Test and trace programmes will be essential. Later, test, trace and treat (T3) programmes will become mandatory once effective and safe therapies are developed. SARS-CoV-2, severe acute respiratory syndrome-coronavirus 2.

with mRNA-1273. After the second vaccination, serum-neutralising activity was detected in all participants evaluated. The pseudovirus neutralising activity was low before the second vaccination, which supports the need for a 2-dose vaccination schedule. Finally, the mRNA-1273 vaccine-induced anti-SARS-CoV-2 immune responses in all participants, with no limiting safety concerns. The significance of SARS-Cov-2 binding and neutralising antibody titres and their capacity to prevent infection will have to be determined. Humoral and cell-mediated immune responses have been associated with vaccine-induced protection against challenge or subsequent re-challenge after SARS-CoV-2 infection in a rhesus macaque model.¹⁵⁹ Long-term assessment will be relevant given that natural history studies suggest that SARS-CoV may not generate long-lived antibody responses.¹⁶⁰ Furthermore, safety evaluations are mandatory since there have been concerns about the potential for vaccine-associated enhanced respiratory disease. Of the 3 doses evaluated, the 100 µg dose

elicits high neutralisation responses and Th1-skewed CD4 T cell responses, coupled with a reactogenicity profile that is more favourable than that of the higher dose.

In addition, we want to mention the results of 2 early phase COVID-19 vaccine trials, one at Oxford University (UK), with support from AstraZeneca,¹⁶¹ and the second supported by CanSino Biologics in China.¹⁶² Both groups used an adenoviral vector, and both report the vaccine achieving humoral responses against the SARS-CoV-2 spike glycoprotein receptor-binding domain by day 28, as well as T cell responses. Both report local and systemic mild adverse events such as fever, fatigue, and injection site pain. Neither trial reported a severe adverse event.

Although these preliminary data are encouraging, SARS-CoV-2 is a novel pathogen in humans, and many of the technologies being used to build vaccines are relatively untested. There is still a long way to go and phase III trials of these vaccines will require thousands of participants in order to confirm efficacy and safety.

Conclusions

Box 1 summarises the future goals of COVID-19 research. The rapid sequencing of the virus has enabled the development of diagnostic tools. Test and trace programmes are essential and later, “test, trace and treat (T3)” programmes will become mandatory once effective drugs have been identified and safe therapies developed (Fig. 5). There remain several important issues that require clarification. It will be important to precisely determine how transmissible and pathogenic SARS-CoV-2 is in the ongoing and future epidemic. Furthermore, it is important to improve diagnostic tools. Ideally a single or combined test that provides virological and serological output would be ideal. In many countries, at the end of containment, strict recommended measures will be important to avoid new waves of contamination. However, few innovative treatment modalities have been discovered since the bulk of the effort to date has been focused on a vaccine. Vaccines might not be enough to quell this pandemic. Although many repurposed drug candidates are being evaluated, many are redundant and lack a strong rationale for clinical development. There is a small chance that some trials could grind to a halt, simply because the pandemic has been so well controlled by lockdowns and other measures. However, the risks of epidemics of coronavirus remain clear and present and it is imperative that work continues to develop vaccines and effective drugs for coronaviruses, to prevent future social and economic hardships around the world.

Abbreviations

ACE-2, angiotensin-converting enzyme 2; ARDS, acute respiratory distress syndrome; CLIA,

chemiluminescent immunoassay; 3CLpro/Nsp5, chemotrypsin-like protease; COVID-19, coronavirus disease 2019; Ct, cycle threshold; DIC, disseminated intravascular coagulopathy; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; ICU, intensive care unit; IL, interleukin; ISG, interferon-stimulated gene; JAK, Janus kinase; LFIA, lateral flow immunoassays; NAK, numb-associated kinase; PLpro/Nsp3, papain-like cysteine protease; RdRp, RNA-dependent RNA polymerase; RT-qPCR, reverse transcription quantitative PCR; SARS, severe acute respiratory syndrome; SARS-CoV, SARS-coronavirus; SARS-CoV-2, SARS-coronavirus 2; SOC, standard of care; TMPRSS2, transmembrane serine protease 2; TNF- α , tumour necrosis factor- α ; WHO, World Health Organization.

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Conflict of interest

Tarik Asselah has acted as a speaker and investigator for AbbVie, Janssen, Gilead, Roche, and Merck. David Durantel, Eric Pasmant and George Lau have nothing to declare. Raymond Schinazi was an unpaid consultant for Lilly and holds equity in Lilly and Gilead.

Please refer to the accompanying [ICMJE disclosure](#) forms for further details.

Authors' contributions

TA designed, supervised and prepared the manuscript. All the authors contributed to the drafting of the review, the critical revision of the manuscript and its final approval.

Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhep.2020.09.031>.

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