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Original Article

Current state of vaccine development and targeted therapies for COVID-19: impact of basic science discoveries ☆

Ali J. Marian*

Center for Cardiovascular Genetics, Institute of Molecular Medicine and Department of Medicine, University of Texas Health Sciences Center at Houston, Texas, USA

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ABSTRACT

Coronavirus disease-19 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 is closely related to two other coronaviruses that caused disease epidemic breakouts in humans in the last 2 decades, namely, severe acute respiratory distress syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). The similarities have enabled the scientists to apply the basic scientific discoveries garnered from studying the structure and modus operandi of SARS-CoV and MERS-CoV to develop therapies that specifically target SARS-CoV-2 and to develop vaccines to prevent COVID-19. Targeted therapies including the use of antibodies to prevent virus entry, nucleotide analogues to prevent viral replication, and inhibitors of proteases to prevent virion formation, among others, are being tested for their clinical efficacy. Likewise, complete sequencing of the SARS-CoV-2 and identification of its structural and nonstructural proteins have enabled development of RNA-, DNA-, and peptide-based vaccines as well attenuated viral vaccines to instigate the host-immune responses. The clinical impacts of the basic science discoveries are amply evident on the rapid pace of progress in developing specific antiviral therapies and vaccines against SARS-CoV-2. The progress emphasizes the merit of discovering the fundamental scientific elements, regardless of whether or not they have apparent or immediate clinical applications.

1. Introduction

Since first diagnosed in early December 2019 in the city of Wuhan in China, the coronavirus disease-19 (COVID-19) has become a pandemic with a colossal global impact. The disease is caused by a coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. COVID-19 along with severe acute respiratory distress syndrome (SARS) and Middle East respiratory syndrome (MERS) are the third epidemic outbreaks caused by coronaviruses in humans. The genome of SARS-CoV-2 has 80% sequence identity to that of SARS-CoV, which caused the first outbreak in humans more than a decade ago, and about 97% sequence identity to a bat coronavirus (CoV RaTG13) genome, the latter indicating its origin from the bat virus [2]. The genomic similarities have afforded scientists the opportunity to apply the knowledge gained from studying SARS-CoV and bat coronaviruses in deciphering the molecular underpinning of SARS-CoV-2 structure and function and therefore, the pathogenesis of COVID-19. Today, not only the genomic sequence of SARA-CoV-2 is fully known but also various protein constituents of the virus and its modus operandi for replication and infection of the host cells have been reasonably well-characterized. This is not to be interpreted that all is known and nothing is left to discover. To the contrary, there are plenty of unknowns about this virus and about this infection. Nevertheless, the fundamental discoveries about genetics and molecular biology of coronaviruses have paved the way in an unprecedented way for the rapid development of virus-specific therapeutics and vaccines, which are essential for the ultimate elimination of COVID-19.

2. A primer on structure and function of SARS-CoV-2

SARS-CoV-2 is a positive sense, single-stranded RNA virus whose genome is comprised of 29,903 nucleotides (NCBI Reference Sequence: NC_045512) [2]. The genome codes for the viral proteome, comprised of four major structural surface glycoproteins, namely, spike (S), matrix (M) and envelope (E) and the nucleo-

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* Corresponding author: AJ Marian, MD Center for Cardiovascular Genetics, Institute of Molecular Medicine and Department of Medicine, University of Texas Health Sciences Center at Houston, 6770 Bertner Street, Suite C900A, Houston, TX 77030.
E-mail address: Ali.J.Marian@uth.tmc.edu

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protein (N), as well as 16 nonstructural proteins (NSPs), the latter proteins are generated from cleavage of two large polypeptides [3]. The viral genome also contains several additional ORFs, which encode the accessory proteins involved in virus-host interactions [3].

The S glycoprotein (~141 kDa and ~1,270 amino acids) forms a homotrimer that protrudes from the cell membrane and gives the virus the appearance of a crown under electron microscopy, and hence the name coronavirus (Fig. 1) [4]. The S protein is responsible for attachment of the virus to the host cell receptors, namely the angiotensin converting enzyme-2 (ACE2) [5]. The M glycoprotein (~25 kDa, ~220 amino acids) is the most abundant structural protein, which through interactions with other structural proteins gives the virus its physical structure. The M protein also interacts with the S protein to retain the virus at the endoplasmic reticulum (ER)-Golgi complex, where the new virions are assembled and subsequently excreted via secretory vesicles. The excess load of the viral proteins during infection with SARS-CoV-2 could overwhelm the ER leading to ER stress and consequent activation of the unfolded protein response (UPR). The nucleoprotein or the N protein (~46 kDa, ~420 amino acid) directly interacts with the viral RNA as well as the membrane proteins, such as the M protein, and forms nucleocapsid, which provides stability to the viral genome within the envelope. It also enables viral replication. The E protein (~ 10 kDa, ~75 amino acid) is the smallest of the structural proteins and contributes to viral production and maturation, as its absence significantly reduces viral titers and leads to production of incompetent viral particles [6]. These structural proteins not only interact with each other but also with a large number of host proteins, which are collectively responsible for part of the phenotypic effects of infection with SARS-CoV-2.

The viral genome also codes for 16 NSPs, which are involved not only in viral replication but also in suppression of the host defenses by various mechanisms, including degradation of host mRNAs and inactivation of protein translation. These NSPs along with the proteins coded by the ORFs and the structural proteins interact with over 300 human proteins to impact various biological effects [7]. Through meticulous cooperative interactions, the viral proteins confer the virus the ability to replicate effectively by copying the viral RNA, evading detection by the host-immune system, and destroying the host-defenses (reviewed in [8]). Several of these NSPs, because of their essential functions in viral survival and infectivity, are subject to specific therapeutic targeting.

3. A primer on the pathogenesis of COVID-19

The S glycoprotein, which gives the virus its crown, is critical for initiating the viral entry into the host cell (Fig. 1). The S protein is a homotrimeric protein comprised of S1 and S2 functional domains. The receptor-binding domain (RBD) of the S protein is located within the S1 domain. It recognizes the human ACE2 protein, which cleaves angiotensin 1 to angiotensin 1-9 and angiotensin II to angiotensin 1-7, the latter with potential beneficial effects on cardiovascular system [9]. The affinity of the S protein of SARS-CoV-2 for human ACE2 receptor is several-fold higher than that of the SARS-CoV [4]. The enhanced affinity might explains the rapid spread of COVID-19 (more contagious) as opposed to SARS.

In the open state, which is the predominant state of the S protein, the RBD of the S protein binds to the ACE2, initiating the viral entry into the host cell. The successful viral entry after attachment of the RBD of S protein to ACE2, however, requires proteolytic cleavage of the S protein by furin-like, trypsin-like, and cathepsin proteases, and the serine protease TMPRSS2 (Fig. 1) [10, 11]. Proteolytic modifications of the S1/ACE2 protein complex are considered a critical stage in viral entry, as inhibition of TMPRSS2 with serine protease inhibitor camostat mesylate blocks entry of SARS-CoV-2 into the epithelial cells [12]. TMPRSS2 also cleaves the ACE2 receptor, which might also facilitate entry of SARS-CoV-2 into the host cells [13]. In addition, FURIN is also a candidate to proteolytically modify the S protein and enhance viral entry into the host cell [10, 11]. Subsequent to the proteolytic cleavage of S1 by TMPRSS2, the S2 domain fuses with the host cell membrane, enables cellular entry of the virus by endocytosis, and subsequent release of the viral RNA into host cell cytoplasm. The canonical endocytic pathway is clathrin-dependent formation of membrane vesicles. However, viruses could enter the cell through noncanonical pathways, such as caveolae and flotillin-dependent endocytosis. The exact mode of endocytosis utilized by SARS-CoV-2 for entry into the host cell is not known and might vary according to the cell type.

Given the above mechanism of entry, coexpression of ACE2 and TMPRSS2 and possibly other host proteases on the same cell type is considered essential for infection with SARS-CoV-2. Data in the Human Protein Atlas (https://www.proteinatlas.org/) show that ACE2 is abundantly expressed in type II alveolar cells in the lung, nasal, and bronchial epithelial cells, as well as in the epithelial cells in the gastrointestinal tract (duodenum and small intestine), kidney, and testicular cells. Single cell RNA-sequencing data sets show predominant expression of ACE2 in nasal mucosa, lungs, and small intestine [14] [15]. An intriguing new finding is the role of interferons in regulating expression of *ACE2* gene, which raises questions about the potential beneficial or harmful effects of induction of interferons in response to infection with SARS-CoV-2 [14]. TMPRSS2 is expressed in the alveolar type II cells, gastrointestinal tract, kidney, epididymis, prostate, pancreas, and parathyroid glands [15,16]. Coexpression of ACE and TMPRRS2 in the lung provides a plausible explanation for the predominant involvement of the respiratory system in COVID-19.

Upon release into the host cell cytoplasm, the viral RNA recruits the host's translational ribosomes to synthesize various viral proteins necessary for its replication. Two large polypeptide chains are expressed from the viral RNA, which are then cleaved into NSPs, including RNA-dependent RNA polymerase. A combination of viral and host proteins, referred to as the replicase-transcriptase complex, is formed, which mediates synthesis of the new viral RNA and expression of the structural proteins, including the nucleocapsid. The newly synthesized viral RNA and the structural proteins are assembled in the endoplasmic reticulum (ER)-Golgi apparatus to form new virions. The ER-Golgi system also processes formation of the secretory viral vesicles and subsequent export of the newly formed viruses from the host cell through exocytosis.

4. A primer on host-immune response to SARS-CoV-2 infection

SARS viruses utilize a number of mechanisms to evade detection by the host-immune response, including suppression of host interferon response, mimicking host 5' cap on the mRNAs, and inhibition of signaling pathways that mediate host's cell gene expression [8]. Despite such evasive mechanisms, host's antigen presenting cells, such as the macrophages and the dendritic cells utilize their pattern recognition receptors, including the Toll-like receptors to recognize the pathogen-associated molecular pattern on viral proteins and instigate the host-immune responses. The recognition, through a series of signaling cascades, activate transcriptional factors and effectors of gene expression, including NFkB, IRF3, and MAPKs, and lead to expression of the proinflammatory cytokines and chemokines, such as IL6 and tumor necrosis factor a (TNFa) and MCP1 (reviewed in [17]). The host-immune cells express and release over a dozen cytokines and chemokines that induce a hyperinflammatory state, often referred to as the cytokine storm. The events predominantly occur in the lungs but largely because of systemic effects of the cytokines and chemokines, other organs, including the heart are also involved. In accord with the important role of proinflammatory cytokines, expression levels of several cy-

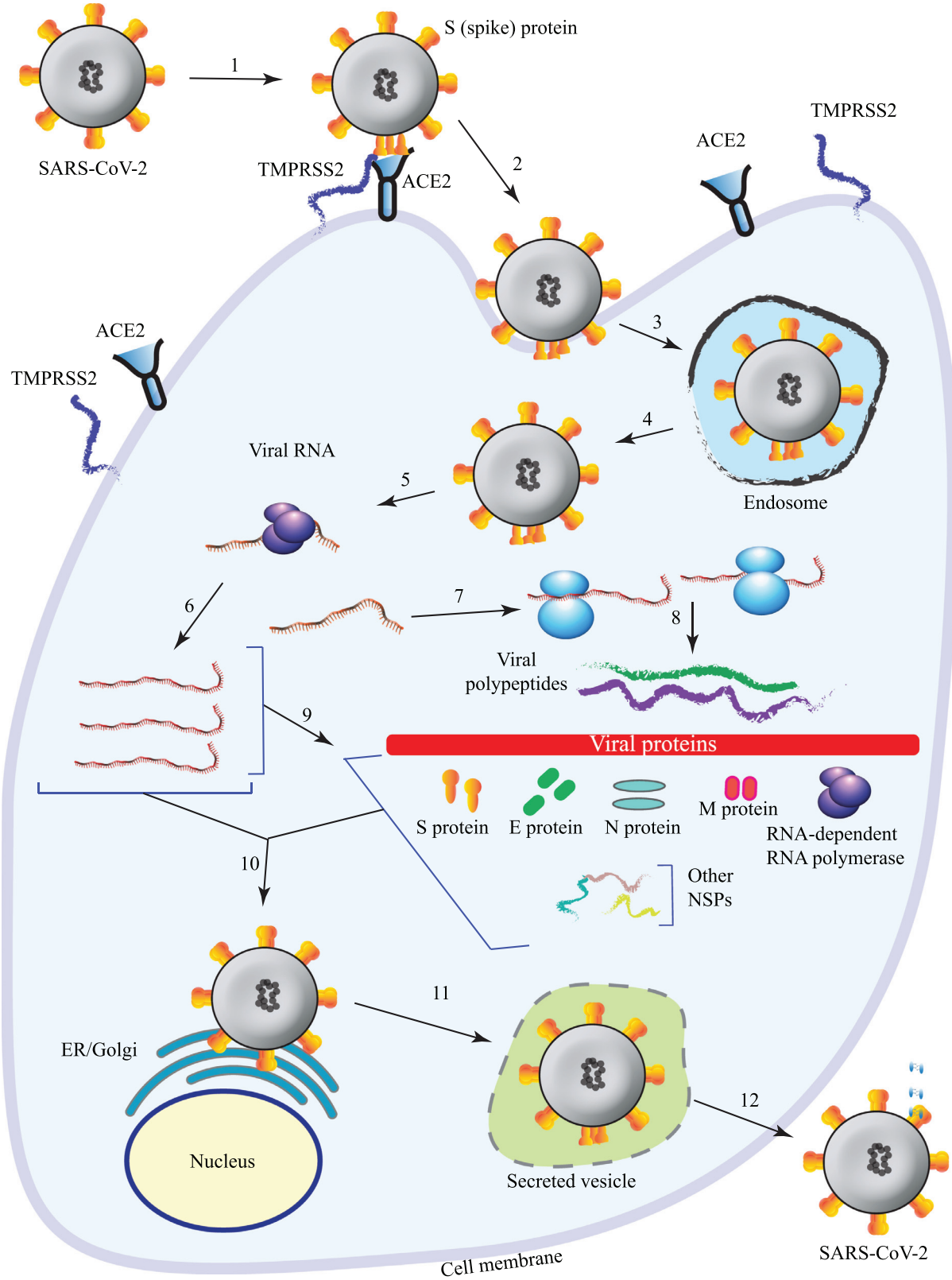

Fig. 1. Various steps (numbered) involved in the pathogenesis of COVID-19, which might serve as targets for specific therapy and vaccine development.
1. Attachment of SARS-CoV-2 to ACE2 receptors on host cell membrane
2. Integration of viral S protein with host cell membrane
3. Viral entry through endocytosis
4. Release of the virus from endosome to host cell cytoplasm
5. Formation of replicase-transcriptase complex
6. Synthesis of viral RNA
7. Translation of viral RNA into large polypeptides
8. Proteolytic cleavage of viral proteins into non-structural proteins
9. Translation of viral mRNAs into structural proteins
10. Assembly of viral RNA and proteins into virions at endoplasmic reticulum and Golgi apparatus
11. Formation of secreted vesicles
12. Exit of the newly formed virion from the host cell by exocytosis.

tokines and chemokines have been associated with the severity of the disease and the clinical outcomes in patients with COVID-19 [18].

At the cellular level, the immune response of the host, including expression of cytokines leads to activation and rapid expansion of the cytotoxic CD8+ T cells, which target and kills the host cells infected with the virus. Likewise, the CD4+ T cells are activated, which in turn signal a subset of the antibody producing B cells (plasma cells), prompting generation of antibodies. These antibodies are specific to the viral proteins and are comprised of IgM and IgG. Whereas the former rises within a few days and declines within several weeks, the latter rises within 2-3 weeks but stays much longer. It is the latter antibody along with the memory T cells that confer host immunity to reinfection with SARS-CoV-2.

5. A brief overview of clinical manifestations of COVID-19

The virus is transmitted from person to person via airborne respiratory droplets and aerosols. SARS-CoV-2 is more contagious but less lethal than SARS. Every infected person is estimated to transmit the virus to 2.1 individuals. Given the above basic reproductive number, herd immunity is expected to be achieved when about 2/3rd of the population develop immunity [19]. It is estimated that between 10% and 30% of the infected individuals remain asymptomatic, albeit the true number of infected asymptomatic individuals might be even higher. The asymptomatic individuals by shedding the virus might be an important source of spread of the virus in the population. Given the relatively high number of asymptomatic infected individuals, one might posit that that exposure to the virus is inevitable.

Several factors have been associated with the clinical presentation of COVID-19, including age, biological sex, pre-existing medical conditions, such as cardiovascular disease, diabetes, and obesity. Otherwise, the reasons for: inter-individual variability in susceptibility to SARS-CoV-2, which ranges from an asymptomatic course to that of severe disease requiring intubation, are largely unknown. The variability likely reflects complex stochastic interactions among multiple factors, including possible exposure load, viral shedding, pre-existing neutralizing antibodies that cross-react with SARS-CoV-2 proteins, differences in the host cellular immune responses, composition of the immune cells, including T cells, blood groups, and genetic composition of the individuals [20,21,22], for example, genetic variability in the human leukocyte antigens (HLA) is implicated in affecting susceptibility to SARS-CoV-2 and severity of COVID-19 [23].

The majority of the infected individuals (>80%) who are diagnosed with COVID-19 are minimally or mildly symptomatic. Viral shedding may occur about 3 days before development of symptoms and continues for a week after the onset of symptoms. In those who become symptomatic, the incubation period varies from 2 days to 14 days (average ~ 5 days). The most common symptoms are fever or chills, cough, dyspnea, fatigue, myalgia, headache, new loss of taste (dysgusia) or smell (anosmia), sore throat, rhinorrhea, nausea, vomiting, and diarrhea. The disease is self-limiting in the majority of patients but about ~15% of the patients require hospitalization primarily because of hypoxia, particularly in those with comorbid conditions. About 5% of the infected symptomatic patients develop acute respiratory distress syndrome and require oxygen supplementation through intubation and other invasive procedures. Lungs are the primary organ involved but involvement of other organs, mostly indirectly because of cytokine storms is not uncommon. The heart is involved in about 20% of the cases, as evidenced by elevated B-type natriuretic peptide (BNP) and cardiac troponin I (TNNI3) as well as regional and global wall motion abnormalities with reduced ejection fraction on echocardiography. Direct cardiac involvement is less certain. Cardiac involvement is a major determinant of clinical outcomes in patients with COVID-19. In advanced cases multi-organ failure, including coagulopathy and lymphopenia, develops and progresses to death. The overall case-fatality of symptomatic patients with COVID-19 varies with age and is highest in the elderly [24]. It is less (about 3.5%) in those diagnosed by testing alone (reverse-transcriptase polymerase chain testing alone), which includes symptomatic and asymptomatic individuals. The overall mortality varies from less than 3% in the young to ~30% in the elderly hospitalized patients, and to more than 70% in those requiring endotracheal intubation [24,25].

A number of factors are implicated in increased susceptibility to COVID-19 and its clinical outcomes, including aging and comorbid conditions. Elderly with diabetes and cardiovascular diseases are particularly susceptible. Several factors are implicated in increased susceptibility of the elderly to COVID-19, in addition to the high prevalence of co-morbid conditions, such as the presence of underlying inflammatory state, referred to as inflammaging, reduced innate, and adaptive immunity, altered airway function, and altered expression of ACE2 and other molecules involved in the pathogenesis of COVID-19 (reviewed in [26]). Likewise, mortality of COVID-19 is the highest in the elderly in part because of the changes discussed above and partly because of the presence of concomitant cardiovascular diseases, diabetes mellitus and others.

Although males and females are equally susceptible to infection with SARS-CoV-2, there is a sex-dependent difference in the clinical outcomes of COVID-19 with male individuals exhibiting the worse clinical outcomes. Mechanistically, there is not compelling evidence to attribute the sex-dependent differences in the clinical outcomes to differential expression of ACE2, TMPRSS2, or furin proteases.

The sexual dimorphism in the clinical outcomes, which is more pronounced in the older individuals, likely, reflects sexual dimorphism of the immune system. In general, there is a progressive decline in the adaptive immunity in men in response to pathogen as opposed to women [27,28,29]. The molecular basis of differential adaptive immunity between the two sexes has been attributed to the effects of the sex hormones on expression of genes involved in immunity, Y- and X-linked genes involved in immunity, nonhomogenous X-chromosome inactivation, and cell type-specific differential gene expression [27,28,29]. In addition, it is known that TMPRSS2 is an androgen-responsive gene, which in part might account the worse clinical outcomes in male patients [30].

Cardiovascular involvement in COVID-19: SARS-CoV-2 enters the cell through the ACE2 receptors, upon modifications with TMPRSS2 and possibly by furin-like and CTSL (cathepsin L) proteases. Therefore, SARS-CoV-2 tropism is largely determined by the expression of protein constituents involved in viral entry. Single cell sequencing data show that ACE2 is expressed at high levels in cardiac pericytes but relatively low to moderate levels in other cardiac cell types, including myocytes, fibroblasts, smooth muscle cells, and endothelial cells [31,32]. In contrast, TMPRSS2 and CTSL are expressed at relatively low levels in the cardiovascular cells [31, 32]. Because tropism of the SARS-CoV-2 depends on coexpression of ACE2 and host proteases, viral myocarditis is not a common feature of COVID-19. However, histological and magnetic resonance imaging evidence of myocarditis, such as interstitial fibrosis, inflammatory cellular infiltrates, and necrosis, has been reported in patients with COVID-19. Whether these changes are the direct consequence of viral infection or are secondary to increased levels of cytokines, catecholamines, or hypoxia, and other factors remains uncertain.

Despite the paucity of firm data on direct myocardial involvement in COVID-19 patients, there are ample data on secondary involvement of the cardiovascular system, manifesting as elevated blood levels of cardiac enzymes; such as cardiac troponin I and B-type natriuretic peptide, regional and global wall motion abnor-

malities, cardiac arrhythmias, intravascular thrombosis, and heart failure (reviewed in [33,34,35]). More importantly, concomitant cardiovascular disease and secondary cardiac involvement are major determinants of clinical outcomes, including mortality in patients with COVID-19 [36,37].

Coagulopathy, represented by increased D-Dimer levels, disseminated intravascular coagulation (DIC), and thrombocytopenia, and thrombotic events, is a common manifestation of COVID-19 [38,39]. Increased coagulopathy is in part because of the so-called "cytokine storm" manifesting with increased levels of proinflammatory cytokines; such as IL6 and TNF-a, and in part because of endothelial dysfunction, and increased levels of prothrombotic factors [39]. Approximately, 5% of the hospitalized patients experienced venous thromboembolism, and the overall thrombotic complication rate is about 10% [40]. Because of multiplicity of the prothrombotic factors in COVID-19 patients, the conventional preventive anticoagulation is often insufficient, and an intense anticoagulation regiment might be needed. However, there is no consensus on the proper anticoagulation approach, and the risk of bleeding is relatively high [40]. Nevertheless, the patients are commonly treated with the low molecular weight heparin, adjusted according to body weight and renal function (reviewed in [41]).

6. Targeted drug therapies

The knowledge gained through basic science discoveries about the structure and function of the SARS-CoV-2 has enabled the researchers to test the existing compounds and to perform large-scale screening to identify new compounds that effectively target the specific components of the virus. Likewise, sequencing of the viral genome and identification of its proteome have enabled the investigators to develop vaccines by using the viral RNAs and specific epitopes of the viral proteins as antigens. Over 3,000 clinical trials, including ~ 500, phase 3 clinical trials, have been registered worldwide that are designed to test effectiveness of over four dozen different compounds, ranging from the epigenetic modulator JQ1 to medicinal herbs, in prevention and treatment of COVID-19 (https://clinicaltrials.gov/). In the following sections, a selected number of interventions that directly target specific components pertaining to viral entry and replication are discussed along with various approaches to vaccine development.

6.1. Therapies targeting viral entry

Entry of the SARS-CoV-2 into the host cells could be simplified into a multistep process comprised of proteolytic cleavage of the S1 subunit and ACE2, binding of the viral S proteins through the RBD in the S1 subunit to the ACE2 receptors on the host cells, fusion of the viral S protein with the host cell membrane, and endocytosis (Fig. 1). This is then followed by release of the viral RNA into host cell cytoplasm and synthesis of viral proteins. The newly synthesized viral proteins and viral RNA are assembled into virions in the ER/Golgi apparatus followed by excretion from the host cells by exocytosis. A number of interventions are pursued to target specific steps involved in viral entry, which are discussed briefly.

6.1.1. Recombinant ACE2

The rationale for the use of recombinant ACE2 is to occupy the ACE receptors and hence, attenuate its availability for binding to SARS-CoV-2. Therefore, administration of the recombinant ACE2 by increasing circulating levels of ACE2 is expected to competitively bind and neutralize the ability of viral S protein to bind to the cellular ACE2 receptors. Consequently, the approach is expected to reduce viral entry into the host cell and attenuate the phenotypic consequences. Studies in cultured cells and organoids have shown efficacy of the recombinant human ACE in inhibiting cellular entry of SARS-CoV-2 [42]. A phase 2 clinical trial is designed to test effects of intravenous injection of recombinant ACE2 on a composite endpoint of all-cause mortality, invasive mechanical ventilation and hospital discharge (NCT04335136). Two other studies are also registered at ClinicalTrials.gov.

6.1.2. Neutralizing antibodies from convalescent plasma

The rationale for the approach is self-evident, as convalescent plasma, containing neutralizing antibodies, has been used for treatment of infectious diseases for decades. A neutralizing antibody functions by binding to a specific domain of the viral protein or host receptor for the virus, blocking viral entry into the cell. The main target of neutralizing antibodies is the S protein of SARS-CoV-2, which is responsible, after conformational changes and proteolytic modifications, for viral entry into the host cell through binding to the ACE2 receptors. The sera of patients who have recovered from COVID-19 contains several potent neutralizing monoclonal antibodies that effectively block binding of the S protein to the ACE2 receptors [43]. In the COVID-19 patients, the host B cells produce multiple neutralizing antibodies that target multiple epitopes on the viral proteins. These antibodies have been extracted, purified, and used for therapeutic purposes. The availability of multiple antibodies enables using a cocktail of antibodies to prevent possible loss of efficacy of a single antibody. The loss of efficacy is typically due to loss of antigenicity of the target due to replication mutagenesis, which by affecting protein structure and function, leads to a loss of antigenicity of the targeted epitope. Overall, the main function of the neutralizing antibodies is prevention of viral entry. This is in contrast to nucleoside analogues and inhibitors of viral proteases that target viral replication and virion formation within the host cell, respectively.

Preliminary studies in a small number of patients have shown beneficial effects of transfusion of plasma from patients who have recovered from COVID-19 on viral load, radiological findings, and survival of patients with severe COVID-19 [44,45]. However, the beneficial effects have not been observed consistently [46]. Over 50 clinical trials, including two dozen phase 3 studies, are currently underway and two randomized studies have been concluded with inconclusive findings [46,47]. Based on the safety profile, preclinical data, nonrandomized studies, and considering subgroup data in a randomized clinical trial, the Food and Drug Administration (FDA) recently approved convalescent plasma from COVID-19 patients as a "may be effective" treatment in hospitalized patients with COVID-19.

6.1.3. TMPRSS2 inhibitors

The prevailing data suggest that proteolytic cleavage of the S protein and ACE2 receptors are an essential step for the entry of SARS-CoV-2 into the host cells [12]. In support of the critical role of this protease in viral entry, data in mice show that deletion of *Tmprss2* gene abrogates infection with viruses similar to SARS-CoV-2 [48]. Camostat mesylate and nafamostat mesylate are serine protease inhibitors, which are commercially available and are currently being used for treatment of chronic pancreatitis and cystic fibrosis, respectively. Preclinical studies show treatment with camostat mesylate blocks entry of SARS-CoV-2 into the epithelial cells [12]. Likewise, nafamostat has been reported to reduce TMPRSS2-dependent fusion of MERS-CoV with host cell membrane in an in vitro assay [49]. Clinical evidence in COVID-19 patients is limited to case reports suggesting clinical efficacy [50]. Potential efficacy of camostat and the more potent nafamostat in the treatment of patients with COVID-19 is being tested in several randomized clinical trials.

TMPRSS2 gene is an androgen responsive gene [30]. Given the key role of this protease in SARS-CoV-2 entry into the host cells,

Table 1
Results of randomized studies with specific therapeutic agents in hospitalized patients with COVID-19 (randomized evaluation of COVID-19 therapy: https://www.recoverytrial.net/results).

Therapeutic agent	Target	Mechanism of action	Study Design	Primary endpoint: 28-day mortality
Dexamethasone (Dexa)	General inflammation	A steroid	Dexa: 6 mg po qd PO or IV, N = 2,104 Usual care: N = 4,321	35% mortality reduction in ventilated patients (95% CI: 0.48-0.88) 20% mortality reduction in those requiring oxygen (95% CI: 0.67-0.96) No benefit in those not requiring respiratory support
Hydroxychloroquine (HCQ)	Viral entry by endocytosis	Impairs lysosome function	HCQ: N = 1,542 Usual care: N = 3,132	HCQ: 25.7% Usual care:23.5% Hazard ratio: 1.11 (95% CI: 0.98-1.26) No effect on hospital stay or other outcomes
Loinavir-Ritonavir (L-R)	Protease/cytochrome P450 inhibitors	Viral replication	L-R: N = 1,596 Usual care: N = 3,376	L-R: 22.1% Usual care: 21.3% Relative risk: 1.04 (95%CI: 0.91-1.18) No benefit on secondary endpoints

anti-androgenic agents, such as bicalutamide and enzalutamide are being tested in prospective phase 2 clinical trials in patients with COVID-19.

6.1.4. Targeting endocytosis

The precise membrane trafficking of SARS-CoV-2 into the host cell is unknown. Nevertheless, it involves endocytic pathways, which could be targeted to prevent viral entry. A number of currently available drugs have been shown to target the endocytic pathways, largely through acidification of the endosomes. The list includes chloroquine, hydroxychloroquine, sertraline, chlorpromazine, and amiloride, among others. There has been considerable interest in the use of chloroquine and its less toxic version hydroxychloroquine, because of the initial reports on favorable clinical outcomes and the reduction in the viral load (reviewed in [51]). However, the initial enthusiasm for the potential efficacy of hydroxychloroquine (alone or in combination with azithromycin) in the treatment of patients with COVID-19 has been dampened in the absence of compelling clinical evidence of efficacy in the observational data and small-scale randomized clinical trials [52]. There were no beneficial effects of treatment with hydroxychloroquine in the Randomised Evaluation of COVID-19 Therapy (RECOVERY) study in UK (Table 1), which randomized 1,542 hospitalized patients to the hydroxychloroquine and 3,132 patients to the usual care (https://www.recoverytrial.net/results/hydroxychloroquine-results). The 28-day mortality in the hydroxychloroquine group was 25.7% as compared to 23.5% in the usual care group (hazard ratio: 1.11, 95% confidence interval [CI]: 0.98-1.26). The lack of efficacy is further compounded by the QT prolongation and potentially pro-arrhythmic side effects, observed with these drugs [53]. While a few studies have been discontinued, several large-scale randomized clinical trials are still ongoing to test efficacy of hydroxychloroquine in the treatment of patients with COVID-19.

6.2. Therapies targeting viral replication

Replication of SARS-CoV-2 requires copying of the viral RNA to make new strands, synthesis of new viral protein, including proteolytic cleavage of the large polypeptides into multiple units, co-assembly of the viral proteins and RNA to form virions, and processing of the virions for release from the host cells (Fig. 1). These components involved in viral replication are potential therapeutic targets, as discussed below.

6.2.1. Targeting RNA synthesis by nucleoside analogues

The approach exploits the rare error of viral RNA-dependent RNA polymerase during RNA synthesis, resulting in incorporation of modified nucleotides into the viral RNA during synthesis, which leads to premature termination of RNA elongation, effectively abolishing viral replication [54]. A library of nucleoside analogues, which were initially developed and approved for treatment of other viral diseases, such as Ebola virus disease and influenza, are candidates to effectively terminate SARS-CoV-2 replication upon incorporation into its RNA chain during synthesis and terminating its elongation [55]. The list of potential candidates includes remdesivir, favipiravir, ribavirin, galidesivir, tenofovir, sofobuvir, and EIDD-2801 among others. Upon phosphorylation to the triphosphate these nucleosides forms convert to active drugs and terminate viral RNA synthesis upon incorporation.

Among the nucleoside analogues, remdesivir, an adenosine analogue, which was originally developed for treatment of Ebola virus disease, has been approved by FDA for clinical use in patients with COVID-19. Remdesivir incorporates into viral RNA during synthesis and inhibits replication of the virus. Because of its anti-viral effects against RNA viruses, including coronaviruses, FDA has approved its use in patients with COVID-19 on an emergency use basis. However, data on clinical efficacy of remdesivir, which has been tested in small to moderate sample size clinical studies, have been mixed and largely inconclusive [56-59].

Likewise, small scale preliminary studies with favipiravir, which targets RNA-dependent RNA polymerase, suggest partial beneficial effects [60,61]. Several randomized clinical trials (Phase 2 and 3) are testing efficacy of favipiravir in hospitalized patients with COVID-19. EIDD-2801 is a recently developed orally bioavailable ribonucleoside analogue, which inhibits SARS-CoV-2 replication *in vitro* in human airway epithelial cells and improved pulmonary pathology in a mouse model [62]. Safety, tolerability, and pharmacokinetic of EIDD-2801 has been tested in healthy individuals through a double-blind randomized placebo-controlled study (Phase 1). Phase 2 clinical trials are underway, however, the results are not yet available.

6.2.2. Protease inhibitors

Lopinavir/ritonavir, a combination of a protease inhibitor and P450 inhibitor, respectively, used for treatment of HIV, is also can-

didate to inhibit SARS-CoV-2 proteases and therefore, its replication. Small sample size studies, however, have not shown beneficial clinical effects in patients with COVID-19 [63, 64]. Likewise, data in the RECOVERY clinical trial in UK (https://www.recoverytrial.net/results/lopinavar-results), which included 1,596 hospitalized patients in the lopinavir/ritonavir treatment group and 3,376 patients in the usual care group, showed no difference in 28-day mortality (22.1% vs. 21.3% in the usual care group, relative risk: 1.04, 95% CI: 0.91-1.18) (Table 1). The difference in the effectiveness of this drug between patients with HIV and COVID-19 might reflect the differences in the structure and functions of proteases involved in processing of SARS-CoV-2 and HIV polypeptides

6.3. Targeting the downstream pathways activated by SARS-CoV-2

A diverse array of pharmacological and non-pharmacological interventions is applied to prevent, attenuate, or reverse, in part, the response of the host cells to infection with SARS-CoV-2. A notable phenotypic feature of COVID-19 is the cytokine storm, characterized by marked elevation of proinflammatory and cytotoxic cytokines, such as IL1, IL6, IL10, IFN-g and TNF-a, among others [65]. Consequently, specific inhibitors of cytokines, such as tocilizumab, a monoclonal antibody against IL6, have been used in treatment of patients with COVID-19. Preliminary observational data show that treatment with tocilizumab is associated with improved laboratory and clinical outcomes, including the risk of mechanical ventilation and hospital-related mortality [66,67,68]. However, the unpublished report of a phase 3 randomized double-blind clinical trial (COVACTA trial) showed no beneficial effects on mortality at 4 weeks between patients treated with tocilizumab or placebo (NCT04320615).

Notable among various interventions is the use of dexamethasone, which was found to reduce 28-day mortality modestly in those requiring respiratory support in the RECOVERY study [69]. Thus far, dexamethasone is the only drug shown to reduce COVID-19 mortality (Table 1). Details of empiric and general therapies are not discussed given the focus of this review on specific anti-viral therapy.

7. Vaccine

The clinical impact of major scientific advances is also amply evident in the current efforts to rapidly develop an effective vaccine against COVID-19. The advances have enabled shortening of the typical time required for developing an effective vaccine from more than a decade to anticipated less than a couple of years. This feat has been accomplished primarily because of the technological advances in molecular biology and genetics and the fact that almost all structural and functional components of SARS-CoV-2 have been delineated, even though their functions are not fully understood. The most challenging aspect of vaccine development is identification of the causal agent, which in the case of COVID-19, in contrast to AIDS, not only was identified soon after the start of the breakout but also its genome was fully sequenced within a month or two after its isolation [2]. In addition, building on the knowledge gained from studies of previous outbreaks of diseases caused by coronaviruses, namely SARS-CoV and MERS-CoV, the protein constituents of SARS-CoV-2 and their modus operandi were already partially understood. Moreover, technological advances have made it possible to generate RNA- and DNA-based vaccines, as was accomplished in the case of vaccine for Ebola virus disease. It is quite remarkable that the first RNA vaccine was injected to human volunteers within 3 months after sequencing of the SARS-CoV-2 genome. Given the rapid progress of scientific discoveries, it is a matter of time that an effective and safe vaccine for the prevention of COVID-19 will be developed. As is inherent to any advances, the success will be interrupted by intermittent setbacks, but the progress is inevitable. The characteristics of an ideal vaccine include ease of manufacturing in large quantity and in high purity, stable storage and transportation, convenient route of administration, absence of fortuitous adverse effects, and a low cost. Recognizing the impact of fundamental basic science discoveries on developing an effective vaccine, the US Department of Health and Human Services has launched the Operation Warp Speed, which is designed to streamline the regulatory process in vaccine development and commercialization. The bottleneck is no longer the lack of basic scientific data but rather in conducting large-scale clinical trials that are essential to demonstrate efficacy of the new vaccines. Multiple approaches to vaccine development are being pursued, which are summarized in Table 2.

Replication fidelity of SARS-coronaviruses is relatively high, because of their RNA proofreading functions through 3'- 5' exonuclease activities of NSP14 and NSP12, which results in a low rate of new mutations, estimated to be at $2x10^{-5}$ [70]. Nevertheless, the rare errors that occur during viral genome replication could results in generation of variants that could affect infectivity of the virus, lead to antigenic drift, and modify the host responses to the viral antigen, and hence, impede generation of an effective vaccine. This antigen drift could pose considerable challenges for developing a vaccine that maintains its effectiveness for a long period. A notable example is a single amino acid substitution, namely p.D614G, located in the S protein, which has become the dominant variant in the current pandemic in the Western world, as opposed to D614 variant during epidemics in Wuhan, China. Experimental and clinical data suggest that the G614 variant increases infectivity and is associated with a higher viral load [71]. Preliminary data suggest that the p.D614G variant does not affect antigenic properties of the S protein and therefore, is unlikely to have an effect on efficacy of the vaccines targeting the S protein [72].

7.1. Messenger RNA vaccines

The rationale is to deliver an mRNA that codes for a viral protein as an antigen presented to the host-immune system in order to elicit an immune response and produce neutralizing antibodies. The mRNA vaccine differs from the conventional vaccine, which typically utilizes an inactivated organism or its protein as an antigen to stimulate the host-immune system. An mRNA, containing an ORF, is first transcribed *in vitro* from a DNA template using an RNA polymerase. The ORF codes for the protein of interest that serves as the antigen. The translation is achieved using the host translational machinery. The mRNA-based vaccines have several advantages, including relative safety, reliance on host translational machinery, lack of integration in the genome, and the relative ease and scalable production in the laboratory. However, there are important challenges, such as effective delivery, stability of the mRNA in the host system, fortuitous immune response, and instability upon storage, unless frozen.

The mRNA vaccine is advocated to be as the most efficient and less time-consuming approach to develop a vaccine against COVID-19. Therefore, the RNA vaccines have emerged as the prime candidates for vaccination against COVID-19. The S protein of SARS-CoV-2 is the prime target of RNA vaccines as it closely resembles that of SARS-CoV, making it possible to apply the existing knowledge about the S protein to develop an effective vaccine against SARS-CoV-2. In support of the potential utility of the S protein as an antigen, data in a small number of patients with COVID-19 show neutralizing antibodies against the RBD and the N-terminal domain of the S proteins, rendering these domains as attractive targets for vaccine development [73].

An RNA vaccine that codes for the S protein is considered an efficient approach to produce neutralizing antibodies that prevent

Table 2
Vaccine platforms against SARS-CoV-2.

Vaccine technologies	Principle	Advantage	Disadvantage	Clinical trials stage
mRNA-based	Delivery of modified mRNA	Scalable production Cytoplasmic No vector or foreign DNA Two or more antigens Self-amplifying mRNAs provide sustained expression Cellular and humoral responses	Stringent preparation/storage Less stable Low efficiency of delivery Transient expression (except SAM) Fortuitous immune response Cost	Phase 3
DNA-based	Vector-based delivery of a viral gene	Easy to generate and stable Storage at room temperature Scalable Cellular and humoral responses Two or more antigens No adjuvant Ease of delivery Low cost	Issues associated with a vector DNA, such as immunogenicity and genomic integration and pre-existing immunity Purity Pathogenicity due to recombination with wild type virus	Phase 2
Peptide-based	A fragment or whole length viral peptide	Non-infectious Robust immune response Safe Ease of delivery	Challenging manufacturing Stability Need for adjuvant	Phase 2
Live attenuated virus	De-optimization of the genome (to reduce pathogenicity)	Multiple viral antigens Strong immune response	Safety concerns Labor intensive	Phase I
Inactivated virus	Chemically or UV inactivated virus	Relatively simple Strong immune response	Risk of partial inactivation Risk of becoming pathogenic	Phase I

binding of the virus to the ACE2 receptor and its entry into the host cells. The choice of the S protein as a target would have an inherent shortcoming, if SARS-CoV-2 enters the cell via a mechanism independent of ACE2, an issue that has not been totally resolved. There are a number of additional technical issues with the RNA-based vaccines, which pertain to stability of the mRNA, efficiency of its uptake by the host cell, and its release from the encapsulated nanoparticles into the cell cytoplasm to code for the intended protein. To increase stability of the mRNA and prevent rapid degradation by host ribonucleases, the mRNA is encapsulated in lipid nanoparticles or various compounds and injected intramuscularly into the host (reviewed in [74]). The first mRNA vaccine against COVID-19 was the mRNA-1273 vaccine, which is a modified viral RNA encapsulated in a lipid microparticle capsule [75]. A phase 1 study with mRNA-1273 vaccine, which encodes the stabilized perfusion S protein (2 injections) led to production of neutralizing antibodies in all participants (NCT04283461) [75]. It was also associated with adverse events, resembling symptoms of mild COVID-19 in a significant number of the recipients [75].

Given the transient nature of mRNA-induced immunogenicity, repeated injection is often necessary to induce a sufficient immune response. A potentially superior approach is the use of self-amplifying mRNA (SAM) constructs, whereby a replicase mediates prolonged transcription of the viral mRNA and hence, persistent presentation of the antigen to the immune system (reviewed in [74]). The SAM approach enables generation of a large amount of antigen from a small amount of mRNA vaccines because of continuous generation of the antigen. A major limitation of the SAM platform is size constraints of the inserts used to produce antigen.

Two RNA constructs, one presenting the replicate and the other target antigen, also have been developed, which might offer the advantage of persistent antigen expression upon a single injection [76]. Likewise, dendritic cells transfected with the viral RNA could potently activate antigen-specific T cells and confer robust immunogenicity. Finally, nucleoside-modified mRNA vaccines are highly effective in presenting antigen and eliciting an immune response. In accord with the diversity of approaches and potential efficacy of the mRNA-based vaccines, there are enormous efforts by several academic centers and the industry to develop mRNA-based vaccines, utilizing various renditions of the approach. Several mRNA-base vaccines already have been tested for safety and immunogenicity in provoking neutralizing antibodies (Phase 1 and 2 clinical studies). Enrolment into large-scale phase 3 clinical efficacy clinical trials has already been started.

7.2. DNA-based vaccines

The approach utilizes a vector, such as plasmids, replication-deficient adenoviruses, lentiviruses, or replication-competent vesicular stomatitis virus, to transfer a SARS-CoV-2 gene and express a viral protein, typically the S protein, to elicit immunogenicity. DNA vaccines have many characteristics of desirable vaccine as they are relatively easy to manufacture in large and high quality at a relatively low cost, relatively safe, and stable at room temperature. The DNA vaccines could be delivered by intramuscular or intradermal inoculation and even electroporation. DNA vaccines have been developed for several infectious diseases and have been shown to be well-tolerated and immunogenic. They are also being tested for safety and immunogenicity against SARS-CoV-2. Preliminary studies in rhesus macaques upon expression of several viral S immunogens have shown have humoral and cellular responses, including production of neutralizing antibodies and IFN-g producing CD4+ and CD8+ T cells [77]. The immune response following DNA vaccination was effective in reducing viral RNA levels upon inoculation of the immunized monkeys with SARS-CoV-2 virus [77]. Likewise, vaccination with a recombinant adenovirus that expressed the full-length S protein led to dose-dependent antibody and T cell responses in the majority of the vaccinated individuals, which were peaked at ~ 4 weeks [78]. No serious side effect was reported, but mild to moderate side effects were common [78]. A phase 2 study with this vaccine is planned. One caveat with the vector-based vaccines is immunogenicity of the vectors. Replication-deficient adenoviruses are known to be immunogenic and elicit host-immune reaction and shutdown of the transgene expression, which were amply demonstrated in the early days of gene therapy [79,80].

7.3. Peptide-based vaccines

Typically a synthetic viral peptide or a fusion recombinant peptide is delivered to the host via intramuscular or subcutaneous injection to provoke the immune response. The approach is relatively safe, offers the option of choosing the best desirable epitope as

an antigen, and provokes a robust immune response, particularly when used in conjunction with adjuvants.

Full-length or domains of the S, M, and N proteins of SARS-CoV-2 are the candidate antigens, as they are effective in producing host antibodies, at least in the case of SARS-CoV. To enhance immunogenicity of the viral peptides, often an adjuvant or an epitope that is recognized by the T or B cells are fused with the viral protein. Likewise, multi-epitope peptides might confer a superior immune response. Repeated administration is often required to induce a sufficient humoral and cellular immune response. A recently developed technique is delivery of a fragment of viral S protein through microneedle array, as opposed to conventional subcutaneous injection [81]. Similarly, fusion proteins, comprised of a fragment of viral S or M protein and an adjuvant, such as aluminum, are being developed and tested to induce vaccination. Over a dozen programs are advancing various peptide-based vaccine platforms from the preclinical studies to early phase 1 and 2 clinical studies in humans.

7.4. Attenuated, inactivated, and nonreplicating virus

Attenuated and inactivated pathogens are the classic antigens for vaccine generation, dating back to vaccination against smallpox by Edward Jenner who coined the term vaccination. The virus is typically inactivated upon treatment with formalin or other chemicals or upon exposure to ultraviolet light. In the case of live attenuated virus, viral genome is deoptimize to reduce its pathogenicity while maintaining its immunogenicity against multiple viral antigen. The use of live attenuated or inactivated virus for vaccination is somewhat compounded by the potential pathogenicity due to an inadequate inactivation or attenuation. There is also the risk of live attenuated virus evolving into a more pathogenic strain due to mutagenesis or recombination with the wild type virus.

Preclinical studies in *Rhesus macaques* immunized with three injections of purified inactivated SARS-CoV-2 (treated with b-propiolactone) induced an effective immune response, as evidenced by decreased viral load and protection against infection upon challenge of the monkey with SARS-CoV-2 [82]. Inactivated SARS-CoV-2 vaccines are currently being tested in early phase clinical trials.

8. Concluding remarks

The pace of advances in developing specific drugs to targets SARS-CoV-2 and vaccine to prevent COVID-19 is quite remarkable, facilitated largely by the existing basic science discoveries about coronaviruses, as well as technological advances in molecular biology and genetics and nucleic acid-based vaccine development. ClinicalTrials.Gov lists over 150 vaccine studies against SARS-CoV-2, which utilize a variety of platforms ranging from inactivated whole virion to specific RNA vaccines to non-specific BCG vaccine. There is, however, no concrete evidence for the success of targeted therapy or efficacy of the potential vaccines. The data on the effectiveness of existing targeted therapies, ranging from monoclonal antibodies against ACE2 to nucleotide analogues are equivocal at best. Likewise, a large number of vaccine candidates are likely to drop out from competition after the initial studies, and a considerable number are likely to fail in the phase 3 studies. Only those built upon robust basic science principles and phase 1-2 data will have the chance of succeeding in phase 3 efficacy clinical trials. It might be necessary to identify additional drug and vaccine targets and to screen chemical libraries to find suitable compounds, followed by optimization and testing in preclinical and phase 1-3 clinical trials. At the clinical level, it is important to identify and test the drugs or vaccines in the target groups, such as the elderly who face the brunt of mortality from COVID-19, and define robust clinical endpoints for efficacy of the interventions. Despite the challenges ahead, developing specific anti-SARS-CoV-2 therapies and effective vaccines against COVID-19 are global priorities and are likely essential for the successful elimination of COVID-19. The progress toward these goals is most remarkable, largely stemming from important basis science discoveries. It merits noting that typically the clinical impacts of the fundamental basic science discoveries are initially unclear. It is such discoveries, however, that are expected to pave the way for the successful eradication of COVID-19. Thus, when uncovering the secrets of nature, one should never be concerned with the immediate clinical implications. The fundamental discoveries are foundation for the successful cures of diseases.

Declaration of competing interest

None.

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References

[1] Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270–3.
[2] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020;579:265–9.
[3] Bartlam M, Yang H, Rao Z. Structural insights into SARS coronavirus proteins. Curr Opin Struct Biol 2005;15:664–72.
[4] Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281–92 e6.
[5] Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, et al. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020;581:221–4.
[6] DeDiego ML, Alvarez E, Almazan F, Rejas MT, Lamirande E, Roberts A, et al. A severe acute respiratory syndrome coronavirus that lacks the E gene is attenuated in vitro and in vivo. J Virol 2007;81:1701–13.
[7] Gordon DE, Jang GM, Bouhaddou M, Xu J, Obernier K, White KM, et al. A SARS–CoV-2 protein interaction map reveals targets for drug repurposing. Nature 2020;583:459–68.
[8] Totura AL, Baric RS. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. Curr Opin Virol 2012;2:264–75.
[9] Marian AJ. The discovery of the ACE2 gene. Circ Res 2013;112:1307–9.
[10] Jaimes JA, Millet JK, Whittaker GR. Proteolytic cleavage of the SARS-CoV-2 spike protein and the role of the novel S1/S2 site. iScience. 2020;23:101212.
[11] Bestle D, Heindl MR, Limburg H, Van Lam van T, Pilgram O, Moulton H, et al. TMPRSS2 and furin are both essential for proteolytic activation of SARS–CoV-2 in human airway cells. Life Sci Alliance 2020:3.
[12] Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;181:271–80 e8.
[13] Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S. TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein. J Virol 2014;88:1293–307.
[14] Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, et al. SARS-CoV-2 receptor ACE2 is an interferon-stimulated gene in human airway epithelial cells and is detected in specific cell subsets across tissues. Cell 2020;181:1016–35 e19.
[15] Lukassen S, Chua RL, Trefzer T, Kahn NC, Schneider MA, Muley T, et al. SARS–CoV-2 receptor ACE2 and TMPRSS2 are primarily expressed in bronchial transient secretory cells. EMBO J 2020;39:e105114.
[16] Vaarala MH, Porvari KS, Kellokumpu S, Kyllonen AP, Vihko PT. Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. J Pathol 2001;193:134–40.
[17] Guzik TJ, Mohiddin SA, Dimarco A, Patel V, Savvatis K, Marelli-Berg FM, et al. COVID-19 and the cardiovascular system: implications for risk assessment, diagnosis, and treatment options. Cardiovasc Res 2020;116:1666–87.
[18] Del Valle DM, Kim-Schulze S, Hsin-Hui H, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature helps predict COVID-19 severity and death. medRxiv. 2020, 2020.05.28.20115758.
[19] Kwok KO, Lai F, Wei WI, Wong SYS, Tang JWT. Herd immunity - estimating the level required to halt the COVID-19 epidemics in affected countries. J Infect 2020;80:e32–ee3.
[20] Ellinghaus D, Degenhardt F, Bujanda L, Buti M, Albillos A, Invernizzi P, et al. Genomewide association study of severe Covid-19 with respiratory failure. N Engl J Med 2020, NEJMoa2020283.

[21] Levantovsky R, van der Heide V. Shared CD8(+) T cell receptors for SARS-CoV-2. Nat Rev Immunol 2020. doi:10.1038/s41577-020-00433-7. (Online ahead of print).
[22] Snyder TM, Gittelman RM, Klinger M, May DH, Osborne EJ, Taniguchi R, et al. Magnitude and dynamics of the T-cell response to SARS-CoV-2 infection at both individual and population levels. medRxiv. 2020, 2020.07.31.20165647.
[23] Nguyen A, David JK, Maden SK, Wood MA, Weeder BR, Nellore A, et al. Human leukocyte antigen susceptibility map for severe acute respiratory syndrome coronavirus 2. J Virol 2020;94(13):e00510–20.
[24] Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York city area. JAMA 2020;323(20):2052–9.
[25] Karagiannidis C, Mostert C, Hentschker C, Voshaar T, Malzahn J, Schillinger G, et al. Case characteristics, resource use, and outcomes of 10 021 patients with COVID-19 admitted to 920 German hospitals: an observational study. Lancet Respir Med 2020;S2213-2600(20):30316–17.
[26] Perrotta F, Corbi G, Mazzeo G, Boccia M, Aronne L, D'Agnano V, et al. COVID-19 and the elderly: insights into pathogenesis and clinical decision-making. Aging Clin Exp Res 2020;32(8):1599–608.
[27] Marquez EJ, Chung CH, Marches R, Rossi RJ, Nehar-Belaid D, Eroglu A, et al. Sexual-dimorphism in human immune system aging. Nat Commun 2020;11:751.
[28] Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Impact of sex and gender on COVID-19 outcomes in Europe. Biol Sex Differ 2020;11:29.
[29] Gal-Oz ST, Maier B, Yoshida H, Seddu K, Elbaz N, Czysz C, et al. ImmGen report: sexual dimorphism in the immune system transcriptome. Nat Commun 2019;10:4295.
[30] Lin B, Ferguson C, White JT, Wang S, Vessella R, True LD, et al. Prostate-localized and androgen-regulated expression of the membrane-bound serine protease TMPRSS2. Cancer Res 1999;59:4180–4.
[31] Liu H, Gai S, Wang X, Zeng J, Sun C, Zhao Y, et al. Single-cell analysis of SARS–CoV-2 receptor ACE2 and spike protein priming expression of proteases in the human heart. Cardiovasc Res 2020;116:1733–41.
[32] Tucker NR, Chaffin M, Bedi KC Jr, Papangeli I, Akkad AD, Arduini A, et al. Myocyte-specific upregulation of ACE2 in cardiovascular disease: implications for SARS-CoV-2-mediated myocarditis. Circulation 2020;142:708–10.
[33] Akhmerov A, Marban E. COVID-19 and the heart. Circ Res 2020;126:1443–55.
[34] Buja LM, Wolf DA, Zhao B, Akkanti B, McDonald M, Lelenwa L, et al. The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. Cardiovasc Pathol 2020;48:107233.
[35] Barth RF, Buja LM, Parwani AV. The spectrum of pathological findings in coronavirus disease (COVID-19) and the pathogenesis of SARS-CoV-2. Diagn Pathol 2020;15:85.
[36] Inciardi RM, Adamo M, Lupi L, Cani DS, Di Pasquale M, Tomasoni D, et al. Characteristics and outcomes of patients hospitalized for COVID-19 and cardiac disease in Northern Italy. Eur Heart J 2020;41:1821–9.
[37] Shi S, Qin M, Cai Y, Liu T, Shen B, Yang F, et al. Characteristics and clinical significance of myocardial injury in patients with severe coronavirus disease 2019. Eur Heart J. 2020;41:2070–9.
[38] Bilaloglu S, Aphinyanaphongs Y, Jones S, Iturrate E, Hochman J, Berger JS. Thrombosis in hospitalized patients with COVID-19 in a New York City health system. JAMA 2020;324(8):799–801.
[39] Martin-Rojas RM, Perez-Rus G, Delgado-Pinos VE, Domingo-Gonzalez A, Regalado-Artamendi I, Alba-Urdiales N, et al. COVID-19 coagulopathy: an in-depth analysis of the coagulation system. Eur J Haematol 2020. doi:10.1111/ejh.13501.
[40] Al-Samkari H, Karp Leaf RS, Dzik WH, Carlson JCT, Fogerty AE, Waheed A, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. Blood 2020;136:489–500.
[41] Mezalek ZT, Khibri H, Ammouri W, Bouaouad M, Haidour S, Harmouche H, et al. COVID-19 associated coagulopathy and thrombotic complications. Clin Appl Thromb Hemost 2020;26:1076029620948137.
[42] Monteil V, Kwon H, Prado P, Hagelkruys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. Cell 2020;181:905–13 e7.
[43] Brouwer PJM, Caniels TG, van der Straten K, Snitselaar JL, Aldon Y, Bangaru S, et al. Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. Science 2020;369:643–50.
[44] Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 2020;323(16):1582–9.
[45] Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci U S A 2020;117:9490–6.
[46] Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. JAMA 2020.
[47] Gharbharan A, Jordans CCE, GeurtsvanKessel C, den Hollander JG, Karim F, Mollema FPN, et al. Convalescent plasma for COVID-19. A randomized clinical trial. medRxiv. 2020;2020 07.01.20139857.
[48] Hatesuer B, Bertram S, Mehnert N, Bahgat MM, Nelson PS, Pohlmann S, et al. Tmprss2 is essential for influenza H1N1 virus pathogenesis in mice. PLoS Pathog 2013;9:e1003774.
[49] Yamamoto M, Matsuyama S, Li X, Takeda M, Kawaguchi Y, Inoue JI, et al. Identification of nafamostat as a potent inhibitor of middle east respiratory syndrome coronavirus S protein-mediated membrane fusion using the split-protein-based cell-cell fusion assay. Antimicrob Agents Chemother 2016;60:6532–9.
[50] Jang S, Rhee JY. Three cases of treatment with nafamostat in elderly patients with COVID-19 pneumonia who need oxygen therapy. Int J Infect Dis 2020;96:500–2.
[51] Cortegiani A, Ippolito M, Ingoglia G, Iozzo P, Giarratano A, Einav S. Update I. A systematic review on the efficacy and safety of chloroquine/hydroxychloroquine for COVID-19. J Crit Care 2020;59:176–90.
[52] Cavalcanti AB, Zampieri FG, Rosa RG, Azevedo LCP, Veiga VC, Avezum A, et al. Hydroxychloroquine with or without Azithromycin in Mild-to-Moderate Covid-19. N Engl J Med 2020, NEJMoa2019014.
[53] Chorin E, Dai M, Shulman E, Wadhwani L, Bar-Cohen R, Barbhaiya C, et al. The QT interval in patients with COVID-19 treated with hydroxychloroquine and azithromycin. Nat Med 2020;26:808–9.
[54] Wang Q, Wu J, Wang H, Gao Y, Liu Q, Mu A, et al. Structural basis for RNA replication by the SARS-CoV-2 polymerase. Cell 2020;182(2):417–428.e13.
[55] Jockusch S, Tao C, Li X, Anderson TK, Chien M, Kumar S, et al. A library of nucleotide analogues terminate RNA synthesis catalyzed by polymerases of coronaviruses that cause SARS and COVID-19. Antiviral Res 2020;180:104857.
[56] Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19 - preliminary report. N Engl J Med 2020;383(10):994.
[57] Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020;395:1569–78.
[58] Antinori S, Cossu MV, Ridolfo AL, Rech R, Bonazzetti C, Pagani G, et al. Compassionate remdesivir treatment of severe Covid-19 pneumonia in intensive care unit (ICU) and Non-ICU patients: clinical outcome and differences in post-treatment hospitalisation status. Pharmacol Res 2020;158:104899.
[59] Spinner CD, Gottlieb RL, Criner GJ, Arribas Lopez JR, Cattelan AM, Soriano Viladomiu A, et al. Effect of remdesivir vs standard care on clinical status at 11 days in patients with moderate COVID-19: a randomized clinical trial. JAMA 2020, e2016349.
[60] Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with favipiravir for COVID-19: an open-label control study. Engineering (Beijing) 2020.
[61] Yamamura H, Matsuura H, Nakagawa J, Fukuoka H, Domi H, Chujoh S. Effect of favipiravir and an anti-inflammatory strategy for COVID-19. Crit Care 2020;24:413.
[62] Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, Agostini ML, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020;12(541):eabb5883.
[63] Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of Lopinavir-Ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020;382:1787–99.
[64] Griffin S. Covid-19: Lopinavir-Ritonavir does not benefit hospitalised patients, UK trial finds. BMJ. 2020;370:m2650.
[65] Chi Y, Ge Y, Wu B, Zhang W, Wu T, Wen T, et al. Serum cytokine and chemokine profile in relation to the severity of coronavirus disease 2019 (COVID-19) in China. J Infect Dis 2020;222(5):746–54.
[66] Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A 2020;117:10970–5.
[67] Biran N, Ip A, Ahn J, Go RC, Wang S, Mathura S, et al. Tocilizumab among patients with COVID-19 in the intensive care unit: a multicentre observational study. Lancet Rheumatol 2020.
[68] Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol 2020;2:e474–ee84.
[69] Group RC, Horby P, Lim WS, Emberson JR, Mafham M, Bell JL, et al. Dexamethasone in hospitalized patients with Covid-19 - preliminary report. N Engl J Med 2020.
[70] Eckerle LD, Becker MM, Halpin RA, Li K, Venter E, Lu X, et al. Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication is revealed by complete genome sequencing. PLoS Pathog 2010;6:e1000896.
[71] Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell 2020;182(4):812–827.e19.
[72] Grubaugh ND, Hanage WP, Rasmussen AL. Making sense of mutation: what D614G means for the COVID-19 pandemic remains unclear. Cell 2020;182(4):794–5.
[73] Liu L, Wang P, Nair MS, Yu J, Rapp M, Wang Q, et al. Potent neutralizing antibodies directed to multiple epitopes on SARS-CoV-2 spike. Nature 2020;584(7821):450–6.
[74] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov 2018;17:261–79.
[75] Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. N Engl J Med 2020, NEJMoa2022483.

[76] Walsh EE, Frenck R, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. RNA-based COVID-19 vaccine BNT162b2 selected for a pivotal efficacy study. medRxiv. 2020, 2020.08.17.20176651.
[77] Yu J, Tostanoski LH, Peter L, Mercado NB, McMahan K, Mahrokhian SH, et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science 2020;369(6505):806–11.
[78] Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet 2020;395:1845–54.
[79] Yang Y, Nunes FA, Berencsi K, Furth EE, Gonczol E, Wilson JM. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. Proc Natl Acad Sci U S A 1994;91:4407–11.
[80] Michou AI, Santoro L, Christ M, Julliard V, Pavirani A, Mehtali M. Adenovirus-mediated gene transfer: influence of transgene, mouse strain and type of immune response on persistence of transgene expression. Gene Ther 1997;4:473–82.
[81] Kim E, Erdos G, Huang S, Kenniston TW, Balmert SC, Carey CD, et al. Microneedle array delivered recombinant coronavirus vaccines: immunogenicity and rapid translational development. EBioMedicine 2020;55:102743.
[82] Gao Q, Bao L, Mao H, Wang L, Xu K, Yang M, et al. Development of an inactivated vaccine candidate for SARS-CoV-2. Science 2020;369:77–81.