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MANAGEMENT OF COVID 19SARS-COV-2 BY USING DIFFERENT APPROACHES OF TREATMENT- A REVIEW

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Abstract

The pandemic of coronavirus disease 2019 (COVID-19) has sparked enormous efforts to create treatment plans that specifically target coronavirus 2 (SARS-CoV-2) and/or human proteins to regulate viral infection, involving thousands of patients in clinical trials and hundreds of possible medications. To date, 11 monoclonal antibodies and a few small-molecule antiviral medications (nirmatrelvir–ritonavir, remdesivir, and molnupiravir) have been approved for the treatment of COVID-19; the majority of these medications must be used within 10 days after the onset of symptoms. Furthermore, already approved immunomodulatory medications, such as glucocorticoids like dexamethasone, cytokine antagonists like tocilizumab, and Janus kinase inhibitors like baricitinib, may be beneficial for treating hospitalized patients with severe or serious COVID-19. Based on accumulating discoveries made since the start of the pandemic and an extensive list of clinical and preclinical inhibitors with anti-coronavirus properties, we present an overview of COVID-19 drug development efforts here. We also talk about the lessons that can be applied to the development of therapeutics to combat COVID-19, long COVID, and pathogenic coronaviruses in future outbreaks. These lessons include drug repurposing strategies, pan-coronavirus drug targets, in vitro assays, animal models, and platform trial design.

1. INTRODUCTION

As the causative agent of coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the seventh coronavirus known to have spilled over from other hosts such as bats and rodents into humans1 . Since its discovery in December 2019 (ref. 2), SARS-CoV-2 has caused more than 6.8 million deaths worldwide (see Related links), making it one of the deadliest viruses in human history.

The impact of the COVID-19 pandemic has been reflected in the extensive efforts to develop prevention and treatment strategies. So far, these efforts have led to multiple successful vaccines in an exceptionally rapid time frame3,4 , as well as the evaluation of a wide range of potential treatments in clinical trials, a few of which have also reached the market (Table 1). Based on lessons learned from six decades of antiviral drug discovery5–8 , two types of anti-SARS-CoV-2 agent can be considered. The first type targets viral proteins (mostly viral enzymes) to block the viral life cycle, which may have high selectivity if the targets lack human homologues, but have the potential risk of drug resistance owing to emerging variants5 . The second type targets host

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proteins involved in the viral life cycle (such as the receptors involved in viral entry9,10), which may exhibit broad-spectrum antiviral activities, but with a low degree of selectivity and potentially poor safety profiles6,7 . In addition, agents that target human proteins such as immune system modulators may be important in addressing harmful host responses to viral infection, such as 'cytokine storm' and thrombosis11.

Efforts to develop COVID-19 drugs have been reviewed extensively over the pandemic12–16, although it has been difficult for such reviews to remain timely for long given the exceptional pace at which new results have been reported. Now, with accumulated evidence in the past 3 years, there is an opportunity to summarize progress broadly, consider remaining needs and challenges, and reflect on the lessons learned. Here, we provide a comprehensive overview of virus-targeted and host-targeted agents against SARS-CoV-2, based on an extensive search identifying more than 700 agents that have been reported with anti-SARS-CoV-2 activities in preclinical and/or clinical studies (Supplementary Tables 1–3). Clinical findings for immunomodulators and anticoagulants are highlighted. We also discuss overarching topics in the discovery and development of such agents, including the strengths and limitations of drug repurposing, suitable disease models and clinical trial strategies. Owing to space limitations, readers are encouraged to consult other reviews about SARS-CoV-2 vaccines4,17, diagnostics18,19, biology and pathogenesis20,21, acute and post-acute syndrome22,23, immunology and inflammation24,25, protein structures and functions26,27, emerging variants28,29 and antiviral drugs against other coronaviruses

Viral targets for antiviral agents

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus from the Coronaviridae family. With a length of ~29.9 kb, the SARS-CoV-2 genome (Fig. 1) is one of the largest among RNA viruses and encodes 16 non-structural proteins (NSP1 to NSP16), four structural proteins (spike, envelope, membrane, nucleocapsid) and nine accessory proteins26. The development of virus-targeted inhibitors aims to block different stages of the SARS-CoV-2 life cycle (Fig. 2), including entry (spike inhibitors), proteolytic processing (main protease inhibitors, papain-like protease inhibitors), RNA synthesis (NSP12 to NSP16 inhibitors) and assembly (nucleocapsid inhibitors). This section focuses on drug development for these viral targets at various stages of the viral life cycle (Fig. 2).

Spike

SARS-CoV-2 spike is a homotrimeric class I fusion glycoprotein on the virion surface that is indispensable for viral entry, making it an attractive antiviral target. In most cases, the spike protein is cleaved by host proteases into a receptor-binding subunit S1 and a membrane-fusion subunit S2, heavily shielded by N-linked and O-linked glycans32. After major conformational rearrangements, the receptor-binding domain (RBD) of SARS-CoV-2 spike binds to angiotensin-converting enzyme 2 (ACE2) on the cell surface with high affinity, almost 22-fold higher than that of SARS-CoV spike for ACE2 (ref. 33). Subsequent structural transitions and proteolytic cleavages drive the postfusion

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conformation of a three-helix bundle that fuses the viral membrane with the host plasma membrane34. Several types of agent have been developed to inhibit the spike–ACE2 interaction or membrane fusion, including neutralizing antibodies, small-molecule inhibitors and peptide inhibitors.

2. Anti-spike antibodies.

More than 100 monoclonal antibodies (mAbs) are marketed for the treatment of human diseases, including a few for viral infections, such as palivizumab for respiratory syncytial virus and ansuvimab for Ebola virus12,35. So far, a handful of anti-SARS-CoV-2 mAbs and mAb cocktails have been approved or granted emergency use authorization (EUA), including bebtelovimab, sotrovimab, regdanvimab, bamlanivimab plus etesevimab, cilgavimab plus tixagevimab, casirivimab plus imdevimab and amubarvimab plus romlusevimab (Table 1). Most of these mAbs and cocktails have been authorized as early treatment options to treat outpatients with mild-to-moderate COVID-19 (ref. 36). With the continuing emergence of SARS-CoV-2 variants, most mAbs are no longer recommended (Box 1) and their efficacy will need to be continually assessed.

More than 300 anti-SARS-CoV-2 mAbs have been reported, including more than 20 candidates in clinical trials (Supplementary Table 2). These candidates were mostly identified through the screening of antibodies from the memory B cells of convalescent patients or humanized mice exposed to SARS-CoV-2 (Fig. 3a). Potent wild-type mAbs can be subsequently engineered with amino acid modifications in the fragment crystallizable (Fc) region to develop mAbs with a longer half-life and enhanced effector functions38. Common Fc modifications include: LALA modification with L234A+L235A; YTE modification with M252Y+S254T+T256E; LS modification with M428L+N434S; TM modification with L234F+L235E+P331S; and GAALIE modification with G236A+A330L+I332E (Fig. 3b). As an example, COV2-2130 and COV2-2196 are two synergistic IgG1κ mAbs engineered with the YTE modification (for half-life extension) and the TM modification (for reduced Fc effector functions), leading to the development of cilgavimab and tixagevimab39. Although all marketed anti-SARSCoV-2 antibodies are IgG1 mAbs (Supplementary Table 2), there is also a growing interest in developing polyclonal antibodies such as SAB-185 and XAV-19, nanobodies such as VHH-E, Nb12 and XG014 and biosynthetic proteins such as ensovibep (Supplementary Table 2). Finally, as a natural source of polyclonal antibodies against SARSCoV-2, high-titre convalescent plasma from COVID-19 survivors might provide passive immunotherapy40,41. However, current evidence does not generally support the efficacy of convalescent plasma in the standard treatment of COVID-19 (refs. 42,43), and its widespread use also poses major challenges, including limited supply, administrative and logistical barriers, and antibody-dependent enhancement of SARS-CoV-2 infection.

Table 1 | Therapeutic options for the management of COVID-19 and associated

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diseases

Drug name	Type (delivery route)	Use	Eligible patients	Resistance likelihood[a]	Status
RdRp inhibitors					
Remdesivir (Veklury)	Small molecule (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset, or inpatients	Low	Approved by the FDA, EUA in many countries
Molnupiravir (Lagevrio)	Small molecule (oral)	Tx	Outpatients[b] ≥18 years old and ≤5 days of symptom onset	Low	Approved in the UK, EUA in many countries
JT001 (VV116)	Small molecule (oral)	Tx	Outpatients[b] ≤5 days of symptom onset	Low	Approved in Uzbekistan
M^{pro} inhibitors					
Nirmatrelvir–ritonavir (Paxlovid)	Small molecule (oral)	Tx	Outpatients[b] ≤5 days of symptom onset	Low	Approved in the UK and EU; EUA in many countries
Ensitrelvir (Xocova)	Small molecule (oral)	Tx	Outpatients[b] ≤5 days of symptom onset	Low	EUA in Japan, phase III
Inhibitors that block the spike–ACE2 interaction					
Bebtelovimab	mAb (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset	High (e.g., BQ.1, BQ.1.1)	EUA by the FDA; paused owing to resistance
Regdanvimab (Regkirona)	mAb (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset	High (e.g., Omicron, Gamma, Beta)	EUA in many countries; paused owing to resistance
Sotrovimab	mAb (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset	High (e.g., Omicron)	Approved or EUA in many countries; paused owing to resistance
Amubarvimab and romlusevimab	mAbs (i.v.)	Tx	Outpatients[b] ≤10 days of symptom onset	High (e.g., Omicron)[146]	Approved in China; discontinued
Bamlanivimab and etesevimab	mAbs (i.v.)	Tx	Outpatients[b] ≤10 days of symptom onset	High (e.g., Omicron, beta)	EUA in many countries; paused owing to resistance
		PEP	Certain individuals at high risk of COVID-19		
Casirivimab and imdevimab (REGEN-COV)	mAbs (i.v. or s.c.)	Tx	Outpatients[b] ≤10 days of symptom onset	High (e.g., Omicron)	EUA in many countries, paused owing to resistance
		PEP	Certain individuals at high risk of COVID-19		
Cilgavimab and tixagevimab (Evusheld)	mAbs (i.m.)	PrEP	Certain individuals at high risk of COVID-19	High (e.g., Omicron)	Approved or EUA in many countries, paused owing to resistance
Glucocorticoids					
Dexamethasone	Small molecule (i.v.)	Tx	Inpatients requiring oxygen support	No	Recommended by COVID-19 guidelines
Hydrocortisone	Small molecule (i.v.)	Tx	Inpatients requiring oxygen support	No	Recommended by COVID-19 guidelines
Janus kinase inhibitors					
Baricitinib	Small molecule (oral)	Tx	Inpatients requiring oxygen support	No	Recommended by COVID-19 guidelines
Tofacitinib	Small molecule (oral)	Tx	Inpatients requiring oxygen support	No	Recommended by COVID-19 guidelines
Cytokine antagonists					
Tocilizumab	Anti-IL-6R mAb (i.v.)	Tx	Inpatients receiving systemic corticosteroids and requiring oxygen support	No	Recommended by COVID-19 guidelines
Sarilumab	Anti-IL-6R mAb (s.c.)	Tx	Inpatients receiving systemic corticosteroids and requiring oxygen support	No	Recommended by COVID-19 guidelines
Anakinra	IL-1R antagonist (s.c.)	Tx	Inpatients requiring oxygen support[c]	No	EUA by the FDA; authorized in the EU
Anticoagulants					
Various drugs (such as low-molecular-weight heparin)	Various (i.v., s.c. or oral)	Tx, TP	Non-ICU inpatients with no pregnancy[147]	No	Recommended by COVID-19 guidelines
Anti-C5a inhibitors					
Vilobelimab	mAb (i.v.)	Tx	Hospitalized adults initiated ≤48 hours of oxygen support	No	EUA by the FDA

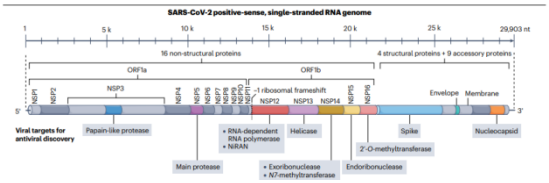

Fig. 1 | The SARS-CoV-2 RNA genome. Antiviral drug targets are indicated beneath the genome map. Accessory proteins are not mapped. NiRAN, nidovirus RdRp-associated nucleotidyltransferase domain; NSP, non-structural protein; ORF, open reading frame; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

antibodies that bind to spike — especially to the RBD — prevent viral entry45. RBD-binding antibodies make up ~90% of neutralizing antibody titres in COVID-19 convalescent plasma46. The other mechanism is antibody effector functions, in which antibodies mediate the destruction of SARS-CoV-2 virions or infected cells via the opsonization pathway, complement-dependent cytotoxicity and/or antibody-dependent cellular phagocytosis and cytotoxicity47. Antibodies developed so far predominantly target the RBD to block the spike–ACE2 interaction (Fig. 3c), except for a few agents such as COV2-3434, S3H3 and 4A8 that target epitopes outside the spike RBD (Supplementary Table 2). RBD-binding antibodies can be categorized into four classes on the basis of the epitope landscape that they target (Fig. 3d). Neutralizing antibodies from different classes that target non-overlapping epitopes could be potentially combined into cocktails with increased potency against SARS-CoV-2 variants (Fig. 3e). Antibody cocktails such as etesevimab (class 1) plus bamlanivimab (class 2) and casirivimab (class 1) plus imdevimab (class 3) have been marketed.

It remains challenging to develop broadly neutralizing antibodies against SARS-CoV-2 variants and multiple distinct sarbecoviruses for several reasons. First, the SARS-CoV-2 spike protein is highly mutable, enabling the emergence of drug-resistance mutations37. Most existing anti-spike antibodies are weakly active or inactive against Omicron variants of concern such as BA.1, BA.1.1, BA.2, BA.4 and BA.5 (refs. 48,49), the spikes of which harbour >30 amino acid substitutions, including at least 15 located in the RBD (Fig. 3e). To counteract neutralization escape, it is important to develop potent antibodies and their combinations that target highly conserved non-overlapping epitopes within or outside the spike RBD. Second, antibody-based therapies might offer clinical benefits to certain patients early in the disease or those with undetectable anti-SARS-CoV-2 antibodies50, but benefits are probably limited for inpatients who have already mounted endogenous antibody responses51. For instance, bamlanivimab (LY-CoV555) was prematurely withdrawn from the ACTIV-3 trial (NCT04501978) owing to its limited benefits for inpatients

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with COVID-19. Whether antibody therapies provide benefits in treating severe COVID-19 remains under investigation. Third, serum titres of anti-SARS-CoV-2 antibodies decrease over time52, and RBD-targeted antibodies may protect for only a few months46. Broadly neutralizing super-antibodies53 and antibodies encoded via lipid-nanoparticle-encapsulated mRNA54 may have the potential to provide longer-term protection against variants of concern. Lastly, the need for intravenous or intramuscular infusion of neutralizing antibodies, their strict storage and distribution requirements, and high production costs are important factors that limit the accessibility of neutralizing antibodies to patients living in resource-limited regions with poor medical facilities.

3. Anti-spike small molecules, peptides and engineered proteins.

Small molecules such as clofazimine that potentially inhibit SARSCoV-2 spike have been investigated (Supplementary Table 1) but so far, no results from late-stage clinical trials have been reported. Potential small-molecule inhibitors of the spike–ACE2 interaction such as MU-UNMC-2, P2119, P2165, H69C2, DRI-C23041 and AB-00011778 (Supplementary Table 1) need further optimization before they could progress towards clinical trials.

Thermostable designed ankyrin repeat proteins (DARPins) such as ensovibep, FSR16m and FSR22 (Supplementary Table 1) target SARS-CoV-2 spike to block the spike−ACE2 interaction. The phase III ACTIV-3/TICO trial, which was terminated early, showed no clinical benefits of ensovibep (MP0420) in hospitalized patients with COVID-19 (ref. 62). However, the phase II/III EMPATHY trial showed clinical benefits of ensovibep in outpatients with symptomatic COVID-19 (NCT04828161). In general, DARPins could provide antiviral options, although optimization may still be needed.

Papain-like protease (part of NSP3)

Papain-like protease (PLpro) is a cysteine protease that cleaves not only the pp1a and pp1ab polyproteins to release viral proteins NSP1, NSP2 and NSP3 (Supplementary Fig. 1a), but also removes host ubiquitin and ubiquitin-like interferon-stimulated gene 15 (ISG15) from signalling proteins to suppress innate immune responses63. The PLpro catalytic site contains a classical catalytic triad (Cys111–His272–Asp286) that preferentially cleaves the tetrapeptide motif LXGG↓XX in adjacent viral proteins (NSP1–NSP2, NSP2–NSP3, NSP3–NSP4) and the C-terminal tails of cellular ubiquitin and ISG15 (Supplementary Fig. 1b). Nearly 15 Å away from the catalytic site, a flexible β-hairpin loop, known as blocking loop 2 (BL2), controls substrate access to the catalytic site (Supplementary Fig. 1b).

More than 30 potent PLpro inhibitors have been reported (Supplementary Table 1). Based on their mechanisms of action, anti-PLpro agents can be classified into three groups: class (i), covalent inhibitors that form a C–S thioether linkage with the catalytic cysteine; class (ii), non-covalent inhibitors that block the entry of PLpro substrates into the catalytic site; and class (iii), non-covalent inhibitors that target allosteric binding pockets (Supplementary Fig. 1c). In class (i), covalent inhibitors such as VIR250 and VIR251 (ref. 64) are peptidomimetics that mimic the tetrapeptide motif LXGG to inhibit PLpro peptidase activity. The ‘featureless’ two glycine residues of the LXGG motif make the development of potent peptidomimetic inhibitors difficult because only peptide

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substrates with two glycine residues at the P1 and P2 positions can be accommodated within the substrate-binding pocket65. In class (ii), non-covalent inhibitors such as GRL0617, F0213, acriflavine, Jun9-72-2, Rac3k and XR8-24 (Supplementary Table 1) occupy the BL2 groove to block the channel that is used by substrates to access the catalytic site65. Most of these inhibitors seem to only inhibit SARS-CoV and SARS-CoV-2, but not MERS-CoV, because of sequence and structural dissimilarities at the BL2 groove (Supplementary Fig. 1b). An exception is F0213, which inhibits both SARS-CoV-2 and MERS-CoV PLpro, probably by targeting a narrow substrate-binding pocket adjacent to the BL2 groove66. In class (iii), non-covalent inhibitors such as HE9 target an allosteric pocket (30 Å away from the catalytic site) to block the binding of ISG15 and ubiquitin to PLpro (Supplementary Fig. 1c). Zn-ejector drugs such as disulfiram block an allosteric Zn2+-binding pocket of PLpro (ref. 67). The selectivity and toxicity of allosteric PLpro inhibitors should be evaluated extensively because Zn ejectors may interfere with Zn-containing proteins in humans, and the ISG15-binding pocket is also present in human ubiquitin-specific peptidase 18 (USP18).

PLpro inhibition blocks viral protein maturation and restores human immune responses68. However, the design of selective PLpro inhibitors is challenging, partly because PLpro is structurally similar to a large family of human deubiquitinating enzymes (DUBs) and DUB-like proteases that also recognize ubiquitin or ubiquitin-like proteins69. DUB and DUB-like proteases are under investigation as therapeutic targets for other human diseases, but no inhibitors of these proteins have yet been approved70. Future development may focus on non-covalent PLpro inhibitors in class (ii) with extensive optimizations that enhance selectivity and potency, and reduce cross-reactions with homologous DUB and DUB-like proteases in humans.

4. Main protease (NSP5)

The SARS-CoV-2 main protease (Mpro), also known as 3C-like protease, is a cysteine protease that cleaves the pp1a and pp1ab polyproteins to release viral proteins NSP4 to NSP16 (Fig. 4a). Inhibition of Mpro-mediated proteolytic cleavage prevents the maturation of key viral enzymes such as NSP12 and NSP13, thereby blocking subsequent viral replication (Fig. 2). The SARS-CoV-2 Mpro homodimer has a strong preference for hydrolysing glutamine residues at the P1 position of the cleavage motif Gln↓(Ser/Ala/Asn) (Fig. 4a). Although there is no known host protease with a primary cleavage site identical to that of Mpro71, some human cysteine proteases (for example, cathepsins B, K, L and S) can also cleave at the C-terminal side of Gln residues72; therefore, potential cross-specificity needs to be considered when developing Mpro inhibitors.

Each subunit of the Mpro homodimer possesses a catalytic dyad that is formed by a nucleophilic cysteine at position 145 (Cys145) and a nearby histidine residue at position 41 (His41)73,74. The Mpro catalytic dyad catalyses the formation of a covalent carbon–sulfur bond between the Cys145 thiolate and the main-chain carbonyl of the substrate's P1 glutamine75 (Fig. 4b). Similar to the structure-based rational design of HIV-1 and hepatitis C virus (HCV) protease inhibitors5,76, various small-molecule Mpro inhibitors have been designed to maximize drug–

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receptor interactions, particularly via the formation of extensive interactions with backbone atoms from the S1′, S1, S2, S3 and S4 subsites of Mpro (Fig. 4b). More than 100 Mpro inhibitors have been reported, including leading candidates such as nirmatrelvir, ensitrelvir and SIM0417 (Supplementary Table 1). Mpro inhibitors can be classified into four groups (Fig. 4c) based on their mechanisms of action: class (i), peptidomimetic inhibitors that covalently bind to the Mpro catalytic pocket; class (ii), non-peptidomimetic inhibitors that block the Mpro catalytic pocket via covalent interactions; class (iii), orthosteric inhibitors that occupy the Mpro substrate-binding pocket through non-covalent reversible interactions; and class (iv), non-covalent inhibitors that target allosteric sites, mostly to impair Mpro dimer formation.

In class (i), peptidomimetic covalent inhibitors commonly bear an electrophilic warhead such as a nitrile (for example, nirmatrelvir), a ketone (for example, PF-00835231), an α-ketoamide (for example, compound 13b-K), an aldehyde (for example, compound 18p) or a Michael acceptor (for example, compound N3) to form a covalent bond with the catalytic Cys145 of Mpro (Supplementary Table 1). Nirmatrelvir is a peptidomimetic that harbours a nitrile warhead that covalently bonds with Cys145 to achieve reversible inhibition77 (Fig. 4c). Nirmatrelvir inhibits all seven human coronavirus (HCoV) wild types78 and various SARS-CoV-2 variants in cell culture79, and substantially reduces viral loads in mice and hamsters80.

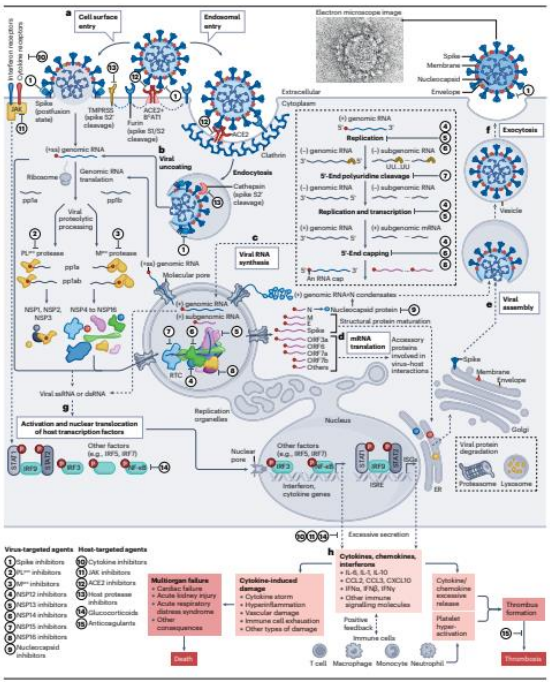

Fig. 2 | The life cycle of SARS-CoV-2 and drug targets.a, Entry of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) into host cells. The viral spike protein binds to angiotensin-converting enzyme 2 (ACE2) (in complex with the sodium-dependent neutral-amino-acid transporter B0 AT1) on the membrane surface. Spike is cleaved by furin at the S1/S2 cleavage site and subsequently cleaved at the S2′ site by transmembrane protease serine subfamily (TMPRSS) proteases in the cell surface entry pathway, or cathepsins in the endosomal entry pathway172 (see Fig. 7 for further details). b, Viral uncoating. The (+ss) genomic RNA is released from the viral particle into the host cell. The genomic RNA is translated into open reading frame 1a (ORF1a) and ORF1ab polyproteins (pp1a and pp1ab), which are subsequently cleaved by papain-like protease (PLpro) and the main protease (Mpro) to release 16 non-structural proteins (NSPs). c, Viral RNA synthesis. Several NSPs assemble into the replication–transcription complex (RTC) that replicates

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and translates viral genomic RNA in replication organelles. d, Viral mRNA translation. Structural proteins are sorted into the endoplasmic reticulum (ER) and Golgi apparatus for maturation. Accessory proteins modulate virus–host interactions and viral pathogenesis. e, Viral assembly. The genomic RNA is packed with viral nucleocapsid (N) for viral assembly, along with structural proteins. f, Viral release by exocytosis. g, Viral RNA triggers host immune signalling pathways, which involve activation of transcription factors to produce cytokines such as interleukin (IL)-6, chemokines such as C–C motif chemokine ligand 2 (CCL2) and C–X–C motif chemokine ligand 10 (CXCL10), and interferons such as interferon-α (IFNα). h, Excessive production and secretion may result in cytokine-induced damage, multiorgan failure, thrombosis or death (see reviews elsewhere11,280,281). Immune cells also provide positive feedback to release more cytokines, chemokines and interferons187. Ps in red circles denote phosphorylation sites. dsRNA, doublestranded RNA; E, envelope protein; IRF, interferon regulatory factor; ISG, interferonstimulated gene; ISRE, interferon-stimulated response element; JAK, Janus kinase; M, membrane protein; NF-κB, nuclear factor-κB; ssRNA, single-stranded RNA; STAT, signal transducer and activator of transcription; UU...UU, polyuridines. The electron micrograph image of SARS-CoV-2 (contributed by C.S. Goldsmith and A. Tamin) was retrieved from the CDC Public Health Image Library.

RNA-dependent RNA polymerase (NSP12)

SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), encoded by NSP12, is a highly conserved holoenzyme involved in viral RNA replication and transcription. After Mpro-mediated proteolytic processing, mature NSP12 coordinates with other non-structural proteins (NSP7 to NSP10, NSP13 to NSP16) in the viral replication–transcription complex93 (Fig. 5a), which catalyses template unwinding, RNA synthesis, RNA proofreading and RNA capping27. By targeting key components of the replication–transcription complex (Fig. 5b), a series of antiviral agents can be developed to block RNA synthesis (NSP12 RdRp inhibitors), template unwinding (NSP13 helicase inhibitors), RNA proofreading (NSP14 exoribonuclease inhibitors), uridine cleavage (NSP15 endoribonuclease inhibitors) and RNA capping (NSP9 inhibitors, NSP12 NiRAN inhibitors, NSP13 ATPase inhibitors, NSP14 guanineN7-methyltransferase inhibitors and NSP16 2′-O-methyltransferase inhibitors). NSP12 inhibitors will be described in this section, while other inhibitors will be addressed later.

NSP12 is composed of an N-terminal nidovirus RdRp-associated nucleotidyltransferase domain (NiRAN) for viral RNA capping and other activities94,95, a dynamic interface domain and a C-terminal RdRp domain for viral RNA synthesis (Fig. 6a). During viral RNA capping, the NiRAN domain acts as a guanylyltransferase that adds a guanosine 5′-triphosphate to the 5′-end of viral RNA96 (Fig. 5b). The NiRAN active site can be blocked by nucleos(t)ide analogues such as remdesivir triphosphate95 and bemnifosbuvir triphosphate97 (Fig. 6b). However, it is a challenge to develop potent NiRAN inhibitors with high specificity and low toxicity because the NiRAN domain

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shares significant structural homology with many human kinases, such as insulin receptor kinase, spleen tyrosine kinase and O-mannose kinase98. The SARS-CoV-2 RdRp active site is the key drug target. Similar to HIV reverse transcriptase inhibitors99, RdRp inhibitors can be classified into nucleos(t)ide analogues (such as remdesivir, molnupiravir, favipiravir and bemnifosbuvir) and non-nucleos(t)ide analogues (such as suramin) with different mechanisms of action (Fig. 6c).

Box 1

COVID-19 treatment guidelines and resources

COVID-19 guidelines

- World Health Organization (WHO): Clinical management of COVID-19
- Pan American Health Organization (PAHO): Coronavirus Disease (COVID-19)
- European Centre for Disease Prevention and Control: Treatment and pharmaceutical prophylaxis of COVID-19
- US National Institutes of Health (NIH): COVID-19 Treatment Guidelines
- Australian Department of Health and Aged Care: COVID-19 treatments
- Government of Canada: Coronavirus (COVID-19)
- National Health Commission of the People's Republic of China: Diagnosis and treatments for COVID-19
- Federal Ministry of Health (Germany): Together against Corona
- Indian Council of Medical Research: COVID-19 guidelines and information on research study
- Government of Japan: COVID-19 information and resources
- Government of the United Kingdom: COVID-19 guidance and support
- Infectious Diseases Society of America (IDSA): COVID-19 real-time learning network
- American Academy of Pediatrics (AAP): Critical updates on COVID-19 in children
- European Respiratory Society (ERS): Management of hospitalised adults with coronavirus disease-19 (COVID-19)

Labelling resources and authorization of COVID-19 therapies and vaccines

- US Food and Drug Administration (FDA): Coronavirus Disease 2019 (COVID-19)
- European Medicines Agency (EMA): Treatments and vaccines for COVID-19

Other drug-related databases and resources

- BMJ Best Practice: Online COVID-19 treatment algorithm
- FDA guidance documents: Conduct of COVID-19 clinical trials
- The Liverpool COVID-19 drug interaction database
- SARS-CoV-2 drug binding pockets

To improve its intracellular delivery and bypass the rate-limiting first phosphorylation step, remdesivir was synthesized as a monophosphoramidate prodrug of its parent nucleoside (GS-441524)101 through the addition of an amino acid ester and aryloxy-substituted phosphoryl group102. This prodrug approach, called 'ProTide' (Fig. 6d), has been used in two FDA-approved antivirals: the HIV reverse transcriptase inhibitor tenofovir alafenamide and the HCV NS5B polymerase inhibitor sofosbuvir. After it diffuses across the cell membrane, remdesivir undergoes a series of metabolic conversions to generate the active metabolite remdesivir triphosphate

During the 2014–2016 outbreak of Ebola virus, remdesivir was identified as a potent anti-Ebola inhibitor102, but its anti-Ebola use was not pursued because of limited benefits in Ebola-infected patients104. Soon after the COVID-19 outbreak, its anti-SARS-CoV-2 potential was demonstrated in preclinical and clinical studies105. As an analogue of ATP, remdesivir triphosphate competes with natural ATP substrates to be efficiently incorporated into nascent RNA chains, causing delayed chain termination at the post-translocation position −3 (ref. 106) (Fig. 6d). This blockade is mediated by a steric clash of the 1′-cyano group of remdesivir triphosphate with the side chain of Ser-861 (Fig. 6d) near the RdRp catalytic site107. The 1′-cyano group and C-linked nucleobase of remdesivir are crucial for its anti-coronavirus activity.

Bemnifosbuvir (AT-527, RO7496998), initially designed for HCV inhibition, is a prodrug of the guanosine nucleotide analogue AT-9010 that shows a dual mechanism of inhibition against SARS-CoV-2 RdRp and NiRAN97. The efficacy and safety of oral bemnifosbuvir versus placebo among high-risk outpatients with COVID-19 are currently being evaluated in the phase III SUNRISE-3 trial (NCT05629962). Galidesivir, an adenosine C-nucleoside analogue, showed limited benefit in a phase I study of 24 inpatients with moderate-to-severe COVID-19 (NCT03891420) and has not progressed further. Of the other NSP12 inhibitors (such as 6-72-2a, 5-iodotubercidin, HeE1-2Tyr, 5-hydroxymethyltubercidin) with in vitro activity (Supplementary Table 1), some have obvious limitations that need to be addressed. For example, sangivamycin is an experimental N-nucleoside analogue that exhibits in vitro activity against many SARS-CoV-2 variants133, but targets cellular proteins, including protein kinase C and histone H3-associated protein kinase133. Suramin blocks the binding of viral RNA template strand within the RdRp catalytic site (Fig. 6c) and inhibits SARS-

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CoV-2 replication in Vero E6 cells134. However, suramin has never been recommended for antiviral use because of its poor bioavailability and strong side effects (the negatively charged suramin binds to many human proteins with positively charged surfaces).

Helicase (NSP13)

SARS-CoV-2 helicase, a member of the 1B helicase superfamily, has RNA unwinding and adenosine 5′-triphosphatase (ATPase) activities136. The helicase structure includes an N-terminal zinc-binding domain that coordinates zinc ions; a C-terminal RNA-binding ATPase with two RecAlike domains; and stalk and 1B domains that bridge the N-terminal and C-terminal domains94. Dynamic structures of the helical stalk and the 1B domain are unlikely to be druggable, but the RNA-binding site and the ATPase active site (Supplementary Fig. 2) have been identified as conserved drug-binding pockets13.

A few small-molecule leads have been reported to inhibit helicase RNA unwinding (such as FPA-124 and 2-phenylquinoline derivatives) and/or ATPase activity (such as 5645-0263 and ranitidine bismuth citrate) (Supplementary Table 1). However, further optimization to address issues such as off-target toxicity and limited potency is needed, because SARS-CoV-2 and some host helicases such as human DDX helicases138 share similar substrate-binding structures and functions, including double-stranded RNA unwinding and NTPase hydrolysis. Although no helicase inhibitor has yet entered clinical trials for COVID-19, potent helicase inhibitors have been developed for other infectious diseases139, including amenamevir, which is approved in Japan for herpes zoster treatment, and pritelivir, which is in a phase III trial enrolling immunocompromised patients infected with aciclovir-resistant herpes simplex virus (NCT03073967).

NSP14 exoribonuclease and guanine-N7-methyltransferase

SARS-CoV-2 NSP14 has an N-terminal exoribonuclease domain and a C-terminal guanine-N7-methyltransferase domain (Supplementary Fig. 3a) that conduct viral RNA proofreading and capping, respectively140,141. The exoribonuclease domain of NSP14 interacts with its cofactor NSP10 to act as a 3′–5′ exoribonuclease that rectifies viral RNA mispairing by removing misincorporated nucleotides or nucleotide analogues from the 3′-end of the nascent RNA strand141. This proofreading mechanism is indispensable for maintaining the replication fidelity of the long SARS-CoV-2 genome142. It also limits the effectiveness of nucleos(t)ide inhibitors such as ribavirin because NSP14 exoribonuclease rapidly excises ribavirin 5′-monophosphate from the viral RNA143. NSP14 inhibitors that target the exoribonuclease active site have been identified, such as compound#79, A-2 and B-1 (Supplementary Table 1), but off-target effects of these inhibitors should be carefully evaluated because of the structural similarity between NSP14 and human DEDD exonucleases.

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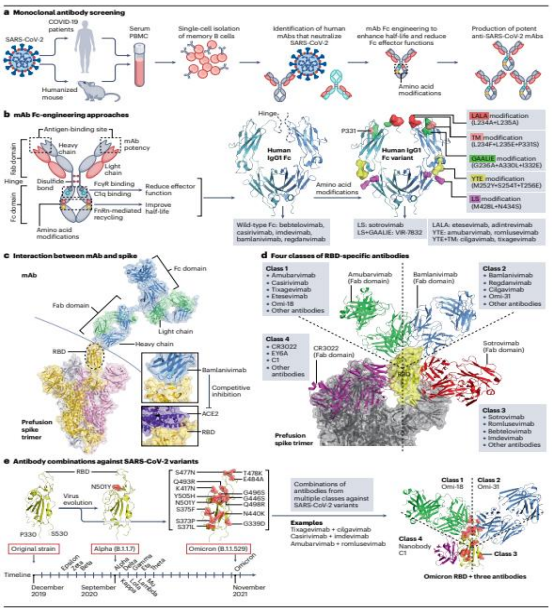

Fig. 3 | Development of anti-spike monoclonal antibodies. a, Screening of monoclonal antibodies (mAbs). Peripheral blood mononuclear cells (PBMCs) are collected from convalescent donors or humanized mice exposed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Single-cell cultures of plasma or memory B cells are prepared to screen potent mAbs. b, Fragment crystallizable (Fc)-engineering approaches that modify amino acids in the Fc domain of mAbs to enhance the half-life and reduce the effector functions38. Common modifications such as LALA, TM, LS, YTE and GAALIE are indicated, with their wild-type residues visualized using the structure of human IgG1 Fc (PDB: 4X4M), and their applications in selected mAbs are shown below. c, Structure of a mAb in complex with the prefusion spike trimer. Bamlanivimab (PDB: 7KMG) blocks the binding of angiotensin-converting enzyme 2 (ACE2) to the receptor-binding domain (RBD) of spike (PDB: 6M17). d, Four antibody classes can be defined based on their interactions with the spike RBD. Representative antibodies from each class are shown, including amubarvimab (class 1, PDB: 7CDI), bamlanivimab (class 2, PDB: 7KMG), S309 (class 3, PDB: 7TNO) and CR3022 (class 4, PDB: 6W41). e, Antibodies from multiple classes can be combined to inhibit SARS-CoV-2 variants. SARS-CoV-2 variants harbour amino acid substitutions in the RBD (for example, 15 mutations in the Omicron variant) that potentially hamper antibody potency. The timeline of the earliest documented samples provided by the WHO (see Related links) shows viral evolution from the SARS-CoV-2 original strain to more than ten variants to date, including Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), Epsilon (B.1.427/B.1.429), Zeta (P.2), Eta (B.1.525), Theta (P.3), Iota (B.1.526), Kappa (B.1.617.1), Lambda (C.37), Mu (B.1.621) and Omicron (B.1.1.529). A three-antibody combination of Omi-18 from class 1, Omi-31 from class 2 and nanobody C1 from class 4 can simultaneously target one RBD of an Omicron variant (PDB: 7ZFB). Fab, fragment antigen binding.

Endoribonuclease (NSP15)

NSP15 is a uridine-specific endoribonuclease (Supplementary Fig. 4a) that cleaves 5′-polyuridine tracts in negative-sense viral RNA (Supplementary Fig. 4b) to evade activation of host immune responses146. The active site of NSP15 Mn2+-dependent endoribonuclease, which cleaves viral RNA substrates and produces the 2′,3′-cyclic phosphodiester and 5′-hydroxyl termini146,147, is a potential drug-binding pocket (Supplementary Fig. 4c). Inhibitors identified so far (Supplementary Table 1), such as tipiracil148, a uracil derivative approved for the treatment of colorectal cancer, have

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weak activities against SARS-CoV-2 (Supplementary Fig. 4d), and further optimizations are needed. In theory, NSP15 endoribonuclease can be an attractive antiviral target owing to the lack of close human homologues.

2′-O-methyltransferase (NSP16)

SARS-CoV-2 NSP16 and its activator NSP10 form a heterodimeric 2′-O-methyltransferase complex (Supplementary Fig. 5) that efficiently converts viral RNA from the Cap-0-RNA into the Cap-1-RNA configuration for evading activation of pattern recognition receptors144,149. Despite their anti-SARS-CoV-2 activities in cell culture, S-adenosyll-homocysteine (SAH) and SAM derivatives such as sinefungin usually have poor membrane permeability (due to their zwitterionic nature) and high toxicity (due to human methyltransferases such as cap-specific mRNA (nucleoside-2′-O-)-methyltransferase 1 (CMTR1)), therefore limiting their clinical applications.

Nucleocapsid

The SARS-CoV-2 nucleocapsid is a flexible and multivalent protein (Supplementary Fig. 6) with multiple functions including viral genome packaging153 and suppression of innate antiviral immunity154. However, it remains a challenge to develop nucleocapsid inhibitors. First, drug-binding pockets in nucleocapsid, unlike those of viral proteases and RdRp, seem to be structurally dynamic153, thereby making it difficult for stable drug binding. Second, the nucleocapsid is the most abundant SARS-CoV-2 protein and folds into the intricate ribonucleoprotein complex with viral RNA. Third, current nucleocapsid inhibitors are inferior to protease and RdRp inhibitors regarding antiviral potency and binding affinity. Despite active research in the past 30 years, no antiviral nucleocapsid inhibitor has been approved.

Other viral targets

Non-structural proteins such as NPS1, NSP6, NPS7 and NSP9 have also been explored as antiviral targets (Supplementary Table 1). Nevertheless, the specificity and potency of their potential inhibitors, mostly obtained from drug repurposing, requires further improvement and evaluation. SARS-CoV-2 accessory proteins (such as open reading frame 3a (ORF3a), ORF6 and ORF9b) are involved in a wide variety of functions, including viral replication, immune evasion, autophagy and/or apoptosis155. Although a few compounds have been screened for inhibition of SARS-CoV-2 accessory proteins (Supplementary Table 1), such proteins are generally dispensable for the viral life cycle.

Host targets for antiviral agents

SARS-CoV-2 hijacks host factors (n > 300) to complete its viral life cycle10, including key cellular proteins such as ACE2. Despite their limitations concerning drug selectivity and safety, antiviral agents that target conserved human proteins have the potential advantage of broad-spectrum activities against emerging variants and multiple viruses6,9 . A successful example is ibalizumab, an FDA-approved mAb that binds to the T cell surface glycoprotein CD4 to inhibit viral entry of HIV-1 and HIV-2 multidrug-resistant strains161. This section introduces important host proteins that have been explored as antiviral targets to interrupt the viral entry or replication of SARS-CoV-2 (Fig. 2).

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Angiotensin-converting enzyme 2 (ACE2)

ACE2 is the primary entry receptor (Fig. 7a) for some human coronaviruses such as SARS-CoV-2, SARS-CoV and HCoV-NL63 (ref. 162). At the initial stage of viral entry into host cells, the N-terminal domain

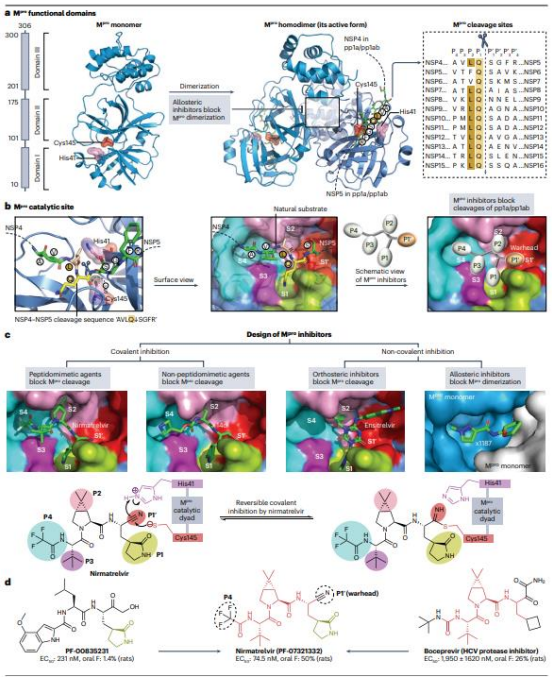

Fig. 4 | Structure of the SARS-CoV-2 main protease and its drug-binding pocket. a, The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) main protease (Mpro) functional domains, protein structures and cleavage sequences. The Mpro homodimer is the active form (PDB: 7VU6). The cleavage sequence 'AVLQ↓SGFR' between adjacent NSP4 and NSP5 in the pp1a and pp1ab polyproteins is localized across the Mpro catalytic dyad formed by Cys145 and His41 (PDB: 7DVP). Mpro cleavage sequences from the reference genome are shown on the right. b, Mpro catalytic site in the pre-cleavage state with the NSP4–NSP5 cleavage sequence (PDB: 7DVP). Mpro inhibitors with P1′ warhead, P1, P2, P3 and P4 moieties can be developed to maximize the drug–receptor interactions at the S1′, S1, S2, S3 and S4 subsites of Mpro, respectively. c, Four classes of Mpro inhibitors and the drug-binding pockets of nirmatrelvir (PDB: 7VH8), 14c (PDB: 7T4B), ensitrelvir (PDB: 7VU6) and x1187 (PDB: 5RFA). Reversible covalent inhibition of nirmatrelvir via the catalytic dyad Cys145–His41 is also shown77. The drug-binding pocket of x1187 is captured at the dimer interface (see panel a). d, Development of nirmatrelvir from PF-00835231 (ref. 78). Nirmatrelvir and boceprevir share identical structures at the backbone and P2/P3 moieties. The half-maximal effective concentration (EC50) values of PF-00835231, nirmatrelvir and boceprevir against SARS-CoV-2 USA_WA1/2020 and oral bioavailability (oral F) in rats were obtained from the literature.

of the ACE2 dimer163 on the cell surface is recognized by the spike RBD (Fig. 7b). SARS-CoV-2 infection subsequently decreases ACE2 expression and weakens ACE2-mediated regulation of the human renin–angiotensin system, resulting in pulmonary hypertension, inflammation and cardiovascular complications.

Cellular proteases

Cellular proteases, such as furin, transmembrane protease serine 2 (TMPRSS2), cathepsin proteases, ADAM10 and ADAM17, cleave and prime SARS-CoV-2 spike for viral entry168–170. Membrane fusion of SARSCoV-2 virions into host cells depends on proteolytic cleavage of viral spike via a two-step process (Fig. 7a): first, furin-mediated cleavage at the S1/S2 site (684AR↓SV687) to release the receptor-binding subunit S1 and the membrane-fusion subunit S2

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(ref. 171); and second, proteolytic cleavage at the S2′ site (814KR↓SF817), located within the membranefusion subunit S2, to release the fusion peptide of spike that anchors the host cell membrane172. Cleavage at the S2′ site is processed by TMPRSS2 or TMPRSS13 during rapid cell–membrane fusion173 or endosomal cathepsin proteases (primarily cathepsin B and L) during slow endosomal internalization174. Omicron replicates quickly in the human bronchus175, but it is less pathogenic, probably because the Omicron spike has an altered preference for cathepsin-mediated endosomal entry.

Other host proteins

In addition to ACE2 and cellular proteases, many host proteins such as farnesoid X receptor178, bromodomain-containing protein 2 (ref. 179), caspase-6 (ref. 180), CD147 (ref. 181), eukaryotic translation elongation factor 1A182, glycogen synthase kinase 3 (ref. 183) and transmembrane protein 16F184 have been explored for the development of anti-SARSCoV-2 agents (Supplementary Table 3). Most such agents are in preclinical development, with a few having entered clinical trials. Sabizabulin, a microtubule disruptor with potential antiviral and anti-inflammatory activities, reduced all-cause mortality by day 15, 29 and 60 in a small cohort of high-risk adults hospitalized with moderate-to-severe COVID-19 (ref. 185). Meplazumab, a humanized anti-CD147 IgG2 mAb, plus standard care reduced the 29-day mortality and viral loads in a phase II/III study of inpatients with severe COVID-19 (ref. 186). In theory, inhibitors targeted at conserved host proteins overcome drug resistance, but their efficacy and safety profiles require evaluation. Future development of host-targeted inhibitors may focus on host proteins that are indispensable for the viral life cycle.

Targeting immune and inflammatory responses and coagulation

SARS-CoV-2 infection induces dysfunctional immune responses and aggressive inflammatory responses that are often associated with severe manifestations, including fatal systemic inflammation, cytokine storm, multiorgan damage and acute respiratory distress syndrome24,187,188. Repurposing a wide range of marketed immunomodulators and anticoagulants, as well as some investigational drugs, has been extensively explored to mitigate dysfunctional responses in patients with COVID-19 (refs. 189,190). However, many of these agents have not resulted in meaningful beneficial effects in clinical trials (Supplementary Table 4), and this section focuses on those with conclusive evidence. Limitations of drug repurposing in the context of COVID-19 are discussed later.

Systemic corticosteroids Systemic

corticosteroids are widely available and affordable antiinflammatory and immunosuppressive drugs for the treatment of autoimmune and inflammatory diseases191. Corticosteroids can be

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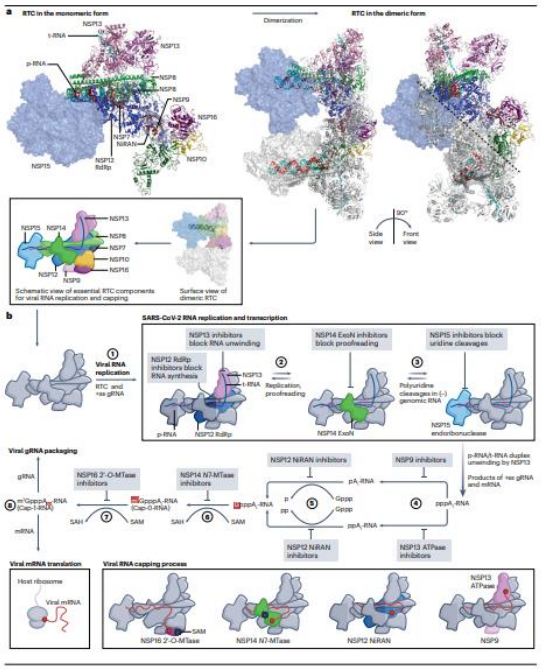

Fig. 5 | The SARS-CoV-2 replication–transcription complex and its drug targets. a, Model of the severe acute respiratory virus syndrome coronavirus 2 (SARS-CoV-2) replication–transcription complex (RTC) in complex with the product RNA (p-RNA) and template RNA (t-RNA), based on the superimposition of protein structures from the PDB codes 7EGQ, 7JYY, 7RDY and 7TQV. The exact structure of the SARS-CoV-2 RTC is yet to be discovered. The monomer is shown on the left, and two views of the active dimeric RTC are shown on the right. A schematic view of the essential RTC components is shown below. b, RTC activities and mechanisms of action of drugs that target it. Step 1: the RTC initiates viral RNA replication after unwinding the viral genomic RNA (gRNA). Step 2: non-structural protein 12 (NSP12) RNA-dependent RNA polymerase (RdRp) and NSP14 exoribonuclease (ExoN) mediate RNA synthesis and proofreading, respectively. Step 3: NSP15 cleaves uridines in the viral single-stranded RNA/double-stranded RNA (ssRNA/dsRNA), especially the long polyuridine tracts at the 5′-end of negative gRNA, to avoid host immune defences146. The products of ss gRNA and mRNA undergo a four-step process (steps 4–8) to complete viral RNA capping for immune evasion93,94. Step 4: NSP13 ATPase hydrolyses and releases the γ-phosphate of the 5′-triphosphate of viral RNA94. An alternative pathway is mediated by the RNAylated NSP9 (refs. 277,283). Step 5: NSP12 nidovirus RdRp-associated nucleotidyltransferase domain (NiRAN) transfers a covalently linked guanosine 5′-monophosphate to the 5′-diphosphate end of viral RNA96. Step 6: the NSP14 guanine-N7- methyltransferase (N7-MTase) domain uses S-adenosylmethionine (SAM) as the methyl donor to produce the intermediate Cap-0-RNA structure (m7 GpppA1- RNA)284. Step 7: NSP16 2′-O-methyltransferase (2′-O-MTase) uses SAM as the methyl donor to produce the cap-1-RNA structure (m7 GpppA1m-RNA)149. Step 8: the capped RNA genome is translocated for viral packaging, while the other capped mRNAs are translocated to host ribosomes for translation. Schematic RTC models involved in the steps of the viral RNA capping process are shown at the bottom of the figure.

classified into glucocorticoids (such as dexamethasone, hydrocortisone, prednisolone and methylprednisolone) and mineralocorticoids (such as fludrocortisone). The former exert anti-inflammatory action and immune regulation with clinical benefits for inpatients with severe COVID-19 (refs. 192,193). The latter bind to cellular mineralocorticoid receptors to regulate the salt and water balance192 and probably have no beneficial effect on COVID-19 outcomes.

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5. Outlook and challenges

Treatment strategies for COVID-19

To date, more than ten antiviral agents have been marketed for COVID-19 treatment (Table 1). The treatment window of antiviral agents is probably limited to the viral phase of SARS-CoV-2 infection (Fig. 8). For outpatients with mild-to-moderate COVID-19, early antiviral treatment needs to reduce the risk of progression to severe COVID-19. However, current marketed antiviral agents, except for oral nirmatrelvir–ritonavir and molnupiravir, are delivered by injection often in hospitals and infusion centres, limiting their practical administration in outpatient and resource-limited settings. In addition to antiviral agents, over-thecounter medications such as acetaminophen (paracetamol) are also effective for relieving COVID-19 symptoms such as fever, although they cannot eliminate coronaviruses245.

During the early stages of COVID-19, treatment with antiinflammatory drugs and immunomodulators might be harmful owing to the suppression of immune responses and increased viral loads. But for hospitalized patients with severe or critical COVID-19, a synergistic benefit might be achieved using antiviral agents plus supportive interventions such as immunomodulators, anticoagulants and/or anti-inflammatory drugs (Table 1). For instance, dexamethasone and remdesivir provide a synergistic benefit for hospitalized patients requiring oxygen support246. Nevertheless, drug−drug interactions should be cautiously evaluated because many antiviral and non-antiviral drugs are frequently administered, especially for severely ill adults with pre-existing diseases. Pre-existing use of some drugs such as mTOR inhibitors that increase human susceptibility to SARS-CoV-2 infection247 should be discontinued, at least during the period of viral infection. Antiviral drug resistance, often induced by amino acid substitutions in the drug-binding pockets, poses a challenge to the development of potent antiviral drugs against SARS-CoV-2 variants5 . Clinical use of many mAbs (Table 1) is no longer recommended because of drug resistance conferred by Omicron variants36. Some mutations may confer resistance to nirmatrelvir (such as E166V92) and remdesivir (such as V792I248). These mutations are currently at a low prevalence in global SARS-CoV-2 isolates, but special care should be taken for high-risk populations such as severely immunocompromised patients with high titres of SARS-CoV-2 for a prolonged period248,249. Based on lessons learned from HIV and HCV therapies, it could be possible to develop dual-drug or triple-drug combinations (such as a polymerase inhibitor and a protease inhibitor plus a pharmacokinetic booster) that effectively inhibit multiple viral targets to maximize the chance of efficacy and to reduce the risk of drug resistance, although drug−drug interactions and accumulated adverse effects must be evaluated99,250. The potential of such combinations to provide additional clinical benefits requires investigation.

Drug repurposing for COVID-19 treatment

Given the urgency of identifying therapies for COVID-19 and the typical lengthy timelines of traditional drug development, drug repurposing — involving the screening of approved drugs or clinical-stage candidates251 originally developed

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for other human diseases — has been extensively pursued to identify agents that either inhibit SARS-CoV-2 or mitigate the consequences of viral infection. The hope is that prior knowledge of PK/PD and safety profiles, delivery route, and formulation of the repurposing candidates could accelerate and de-risk their development. Drug repurposing is conceptually appealing in response to an emergent outbreak, but its implementation faces challenges. Here, we highlight lessons learned from the repurposing efforts so far First, repurposing candidates must be rigorously assessed in preclinical and clinical studies (Fig. 9), particularly when the biological rationale for repurposing a given candidate (for example, hydroxychloroquine) is not clear. In some cases, the rationale may be relatively strong when the principal mechanisms of drug action are similar between the original and the new applications. For example, repurposing RNA polymerase nucleos(t)ide drugs such as remdesivir and molnupiravir to inhibit viral RNA synthesis would be expected to have a relatively high probability of success, but it remains a trial-and-error endeavour to identify nucleos(t)ide analogues that escape the SARSCoV-2 proofreading mechanism252. By contrast, repurposing DNA polymerase inhibitors such as tenofovir to inhibit the RNA synthesis of SARS-CoV-2 is doomed to failure because of their different mechanisms of action. Moreover, HIV protease inhibitors such as lopinavir should not be repurposed for SARS-CoV-2 treatment253 owing to the lack of similarity between the drug-binding pockets in HIV and SARSCoV-2 proteases. Except for nucleos(t)ide inhibitors such as tenofovir, ribavirin and lamivudine, other virus-targeted inhibitors have not been approved by the FDA to treat more than one infectious disease5,6 . They are not good repurposing candidates because their chemical structures are often designed to target a particular drug-binding pocket with high selectivity, and thus they are unlikely to have a similar level of potency against an unrelated target. Importantly, cationic amphiphilic drugs (such as hydroxychloroquine, azithromycin and

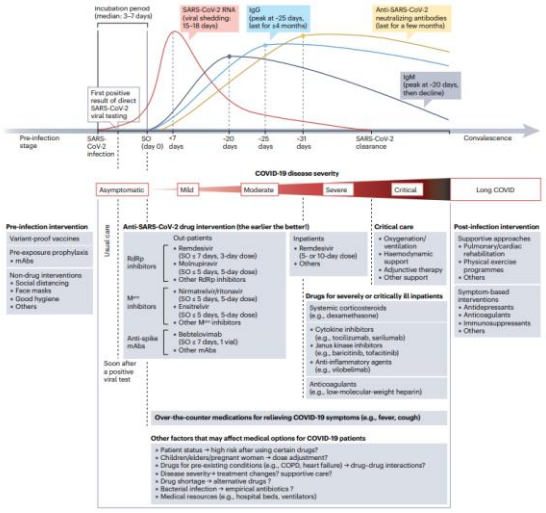

Fig. 8 | Therapeutic strategies for COVID-19 and future coronavirus outbreaks. a, Therapeutic interventions at various stages of coronavirus disease 2019 (COVID-19). At the pre-infection stage, variant-proof vaccines, pre-exposure prophylaxis and nonpharmaceutical interventions can be considered. Once severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is diagnosed, anti-SARS-CoV-2 therapies are ideally administered to outpatients as soon as possible so that the viral load is significantly reduced at an early stage. SARS-CoV-2 viral load usually peaks within the first week after symptom onset (SO) and SARS-CoV-2 RNA shedding in the upper respiratory tract has a mean duration of approximately 17 days287. Levels of anti-SARS-CoV-2 immunoglobulin M (IgM), IgG and

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neutralizing antibodies peak at approximately 20, 25 and 31 days, respectively288. At the advanced stage of COVID-19 progression, immunomodulators, anticoagulants, anti-inflammatory drugs and/or critical care can be considered for severely or critically ill inpatients under certain conditions (Box 1). Post-infection interventions might be needed for some survivors experiencing persistent symptoms after COVID-19 infection289. COPD, chronic obstructive pulmonary disease; mAbs, monoclonal antibodies; Mpro, main protease; RdRp, RNA-dependent RNA polymerase.

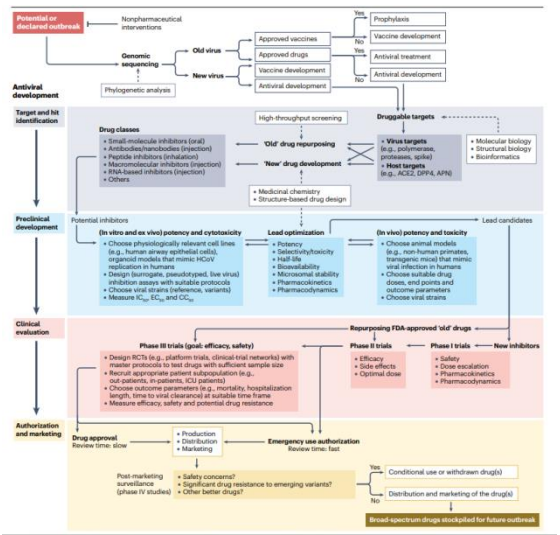

Fig. 9 | Strategies to combat coronavirus outbreaks. The early stage of a potential or declared outbreak requires immediate nonpharmaceutical interventions to prevent pathogen spread. Genomic sequencing can be used to identify coronavirus strains, which guide the clinical use and/or development of vaccines and antiviral drugs. Steps involved in antiviral development are shown, which typically progress from target and hit identification to preclinical development, clinical evaluation and drug authorization and marketing. ACE2, angiotensin-converting enzyme 2; APN, aminopeptidase N; CC50, half-maximal cytotoxic concentration; DPP4, dipeptidyl peptidase 4; EC50, half-maximal effective concentration; HCoV, human coronavirus; IC50, half-maximal inhibitory concentration; ICU, intensive care unit; RCT, randomized control trial.

Second, we suggest that future repurposing efforts focus on candidates that meet the following criteria: a well-defined molecular mechanism of action that has a plausible therapeutic rationale; a high ity from robust preclinical studies in suitable disease models and/or clinical case studies; and lack of severe toxicity (or if toxicity occurs, it should be remediable). Ideally, repurposed inhibitors should have a low likelihood of drug resistance, or if resistance emerges, it should be addressable. Large libraries of approved or clinical-stage agents with diverse bioactivity might be useful for antiviral drug screening, but such open-access libraries are currently lacking.

Third, bearing in mind that most repurposing candidates tested for COVID-19 failed to show clinical benefits, precautions should be taken to avoid off-label use of existing drugs until robust evidence is available. Candidates that failed to show significant clinical benefits in COVID-19 trials include, but are not limited to, various drugs developed against other viruses, such as lopinavir–ritonavir, tenofovir disoproxil fumarate, tenofovir alafenamide, emtricitabine, baloxavir marboxil, sofosbuvir, daclatasvir and velpatasvir; and a wide range of therapies developed for other human diseases, such as (hydroxy)chloroquine, acetylcysteine, allogeneic mesenchymal cells, aspirin, atorvastatin, almitrine, aviptadil acetate, azithromycin, camostat mesylate, astegolimab, brensocatib, canakinumab,

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gimsilumab, siltuximab, otilimab, ciclesonide, clopidogrel, colchicine, dapagliflozin, fluticasone furoate, fluvoxamine, efmarodocokin alfa, imatinib, ivermectin, metformin, nicotine, nitazoxanide, pirfenidone, losartan, ticagrelor, telmisartan, vitamin C, vitamin D3 and zinc gluconate (Supplementary Table 4).

6. CONCLUSIONS

Three classes of antiviral medications, including mAbs targeting spike and small-molecule inhibitors of NSP12 RdRp and NSP5 protease, have been approved thus far as a result of COVID-19 drug discovery efforts. Additionally, several therapies that target host proteins to enhance clinical outcomes have also been approved (Table 1 and Box 1).

To combat COVID-19, cost-effective and easily implemented therapeutic approaches are still required. These may involve investigating combination therapy to overcome medication resistance brought on by new variations. In order to battle SARS-CoV-2 variations, pan-coronavirus inhibitors and their combinations—ideally administered orally or through inhalation—should be developed. It would also be good to have access to these medicines in order to be ready for any future pathogenic coronavirus outbreaks.

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