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Review

Brief review on repurposed drugs and vaccines for possible treatment of COVID-19

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ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the causative agent of the pandemic coronavirus disease 2019 (Covid-19) has claimed more than a million lives. Various *in silico*, *in vitro*, and *in vivo* studies are being conducted to understand the effect of SARS-CoV-2 on the cellular metabolism of humans and the various drugs and drug-targets that may be used. In this review, we discuss protein-protein interactions (PPIs) between viral and human proteins as well as viral targets like proteases. We try to understand the molecular mechanism of various repurposed antiviral drugs against SARS-CoV-2, their combination therapies, drug dosage regimens, and their adverse effects along with possible alternatives like non-toxic antiviral phytochemicals. Ultimately, randomized controlled trials are needed to identify which of these compounds has the required balance of efficacy and safety. We also focus on the recent advancements in diagnostic methods and vaccine candidates developed around the world to fight against Covid-19.

1. Introduction

Coronaviruses belong to the family *Coronaviridae* which in turn consists of two subfamilies *Torovirinae* and *Coronavirinae*. The International Committee of Taxonomy of Viruses (ICTV) classifies the *Coronavirinae* subfamily into four main genera – alpha, beta, gamma, and delta. The severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV) along with some other human coronaviruses like Human coronavirus-Hong Kong university 1 (HCoV-HKU1) and HCoV-OC43, belong to the class of beta coronaviruses which are believed to infect only mammals (Pillaiyar et al., 2020). Several epidemics have emerged in the past due to these coronaviruses. SARS-CoV evolved around 2000–2004 in Guangdong, China and bats were the intermediate hosts. Then, the MERS-CoV emerged in Saudi Arabia in 2012 and infected dromedary camels were responsible for infecting humans directly or indirectly (Kandeel and Al-Nawazi, 2020; Mckee et al., 2020; MERS-CoV, 2020). In December 2019, the coronavirus which apparently emerged from the Hunan seafood market in Wuhan, China and rapidly infected more than 50 people, was first named 2019 novel coronavirus (2019-nCoV). It was then suggested that it was a novel betacoronavirus of *Sarbecovirus* subgenus, and was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by ICTV due to having 79.5% similar genetic sequence to the SARS-CoV (Shereen et al., 2020; Wrobel et al., 2020; Rabi et al., 2020). SARS-CoV-2 spread at a much higher rate than the previous coronavirus outbreaks due to its higher basic reproduction number or R_0 (Rabi et al., 2020). On January 30, 2020, the World Health Organization (WHO) declared this SARS-CoV-2 disease outbreak, Covid-19, as a public health emergency of international concern (Statement on the second meeting of the International Health Regulations, 2005). WHO statistics show more than 87.5 million confirmed Covid-19 cases worldwide which has claimed over 1.9 million lives in 188 countries as of January 9, 2021, and has shown an increasing trend in the number of affected people since its emergence (WHO Coronavirus Disease COVID-19 Dashboard, 2020). It has been very difficult for the countries to control its increasing pandemic potential despite the mandatory quarantines, isolations, and national lockdowns which is affecting the lives of millions of people, overwhelming the healthcare systems, and leading to adverse effects on the global economy (McKee et al., 2020). Therefore, a lot of scientific research using various imaging techniques like electron microscopy and atomic force microscopy as well as other molecular diagnostic methods are being performed to study the SARS-CoV-2 structure and its effect on live cells.

Electron microscopy revealed that SARS-CoV-2 viral particles are

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found clustered within a membrane, separating them from the cell cytoplasm. Black dots observed within the membrane represent cross-sections through the nucleocapsid (Fig. 1A-B) ((Details - Public Health Image Library PHIL, 2020); Goldsmith et al., 2020). It is an enveloped RNA virus that undergoes morphogenesis accompanied by the formation of inclusion bodies filled with membrane-bound, condensed matrix-associated pleomorphic viral particles (Fig. 1C) (Zhu et al., 2020a). It has positive-sense, single-stranded RNA and has a genome size of 30 kb which encodes 14 open reading frames (ORFs). The polyproteins encoded by the 5′ ORF1a/ORF1ab are processed into 16 non-structural proteins (NSP1-16) auto-proteolytically using viral proteases. This leads to the formation of replicase/transcriptase complex which consists of enzymes like protease, primase, polymerase, endonuclease (NSP15), exoribonuclease (NSP14), etc (Gordon et al., 2020). The four structural proteins, the spike protein (S) which is a glycoprotein composed of two subunits S1 and S2, the RNA-binding nucleocapsid (N) protein, the membrane protein (M) spanning the whole membrane, and the envelope protein (E) along with some accessory proteins are present on the 3' end of the viral genome (Fig. 2) (Pillaiyar et al., 2020).

Genome sequencing suggested that although the bat coronavirus, RaTg13, sampled from *Rhinolophus affinis* is 96% similar in structure to the human coronavirus SARS-CoV-2 indicating similar evolutionary roots, the receptor-binding domain in the spike (S) protein diverges due to mutation(s) (Andersen et al., 2020). A mutation leading to the decrease in the stability of the SARS-CoV-2 S protein due to cleavage at the furin-cleavage site helps to facilitate the open conformation required for S to bind strongly to the angiotensin-converting enzyme-2 (ACE2) in humans (Wrobel et al., 2020). Mutations are known to increase the affinity of receptor-binding domain of viral S protein to ACE2 which in turn facilitates the entry of the virus into the target cells (Fig. 2A). ACE2 is mainly located in the cells of the lungs, heart, and kidneys in humans (Rabi et al., 2020; Andersen et al., 2020). It is highly expressed in the type II alveolar cells in the lungs leading to acute respiratory distress syndrome (ARDS), reduced lung function, arrhythmia, and subsequently death (Gordon et al., 2020; Astuti, 2020). People infected with this virus may either be symptomatic or asymptomatic. Symptoms may include not only pneumonia but also disorders of the digestive tract and kidneys due to the expression of ACE2 in proximal tubule cells of the kidney, enterocytes in the colon, and urinary bladder urothelial cell (Astuti, 2020).

The drug discovery process is more difficult due to very little knowledge about the molecular mechanism involved in SARS-CoV-2 infection. To understand this better, a study identified the human proteins associated with the viral proteins using affinity purification-mass spectrometry (AP-MS) and various other techniques after the viral proteins were cloned and expressed in the human kidney and lung cell lines (Gordon et al., 2020). The protein-protein interaction studies, indirect immunofluorescence assay as well as cheminformatic analyses, helped

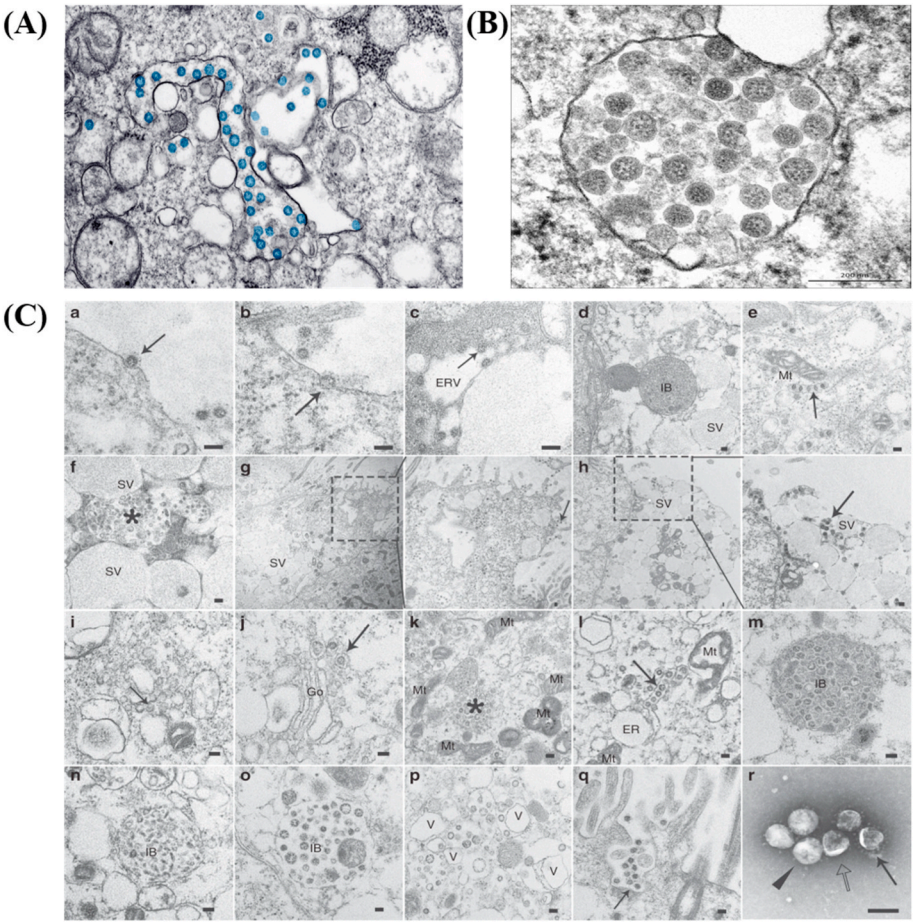

Fig. 1. Transmission electron microscopy images of novel coronavirus SARS-CoV-2. The virus particles are containing black dots which are cross-sections through the nucleocapsid. **A)** Viral particles of SARS-CoV-2 isolated from the first Covid-19 patient in the United States. The spherical viral particles in the cell are colorized blue and are seen clustered within a membrane separating them from the cytoplasm of the cell. The figure is adapted from reference (Details - Public Health Image Library PHIL, 2020). **B)** Viral isolate grown in cell culture shows accumulations of spherical SARS-CoV-2 particles found in membrane-bound areas (vacuoles) in the cisternae of the rough endoplasmic reticulum-Golgi complex (RER– Golgi), where the spikes are located on the inside of the cisternal space and do not touch the cytoplasm of the cell. The spikes are seen with difficulty as a "fuzz" in thin sections of infected cells. The figure is adapted from reference (Goldsmith et al., 2020). **C)** Stages of SARS-CoV-2 morphogenesis in HAE cells show SARS-CoV-2 infected both secretory cells **a**–**h** and ciliated cells **i**–**q** and exhibited similar morphogenetic processes (72 h p.i.). Scale bar: 100 nm. **a-b)** Viral infection begins with the virus attaching (arrow) to the cell membrane followed by fusion (arrow) of the membranes. **c)** Viral particles budding (arrow) into endoplasmic reticulum vesicles (ERV). **d)** Inclusion bodies (IB) in the cytoplasm, filled with viral particles. **e)** Strands of the ER containing viral particle rows (arrow). **f)** IB (star) compressed by secretory vesicles (SV). **g-h)** Viral particles (arrow) are released from the cell along with cytoplasmic components (dashed box) and SV (dashed line box) by exocytosis. **i)** Provirus particles (arrow) in the ciliated cells. **j)** Virus-containing vacuoles (arrow) present in Golgi cisternae (Go). **k)** Viral particle aggregation (star) with matrix not bound by a membrane but enclosed by mitochondria (Mt) in the cytoplasm. **l)** ER with virus particles rows in the cytoplasm. **m-o)** IB filled spherical pleomorphic virus particles. **p)** Virus particles scattered in the vesicle (V)-rich area in the cytoplasm. **q)** Virus particle released from the ciliated cells by exocytosis (arrow). **r)** SARS-CoV-2 particle with distinctive and clear spikes (arrow), without spikes (empty arrow) and with partial spikes (triangle). The figure is adapted from reference (Zhu et al., 2020a).

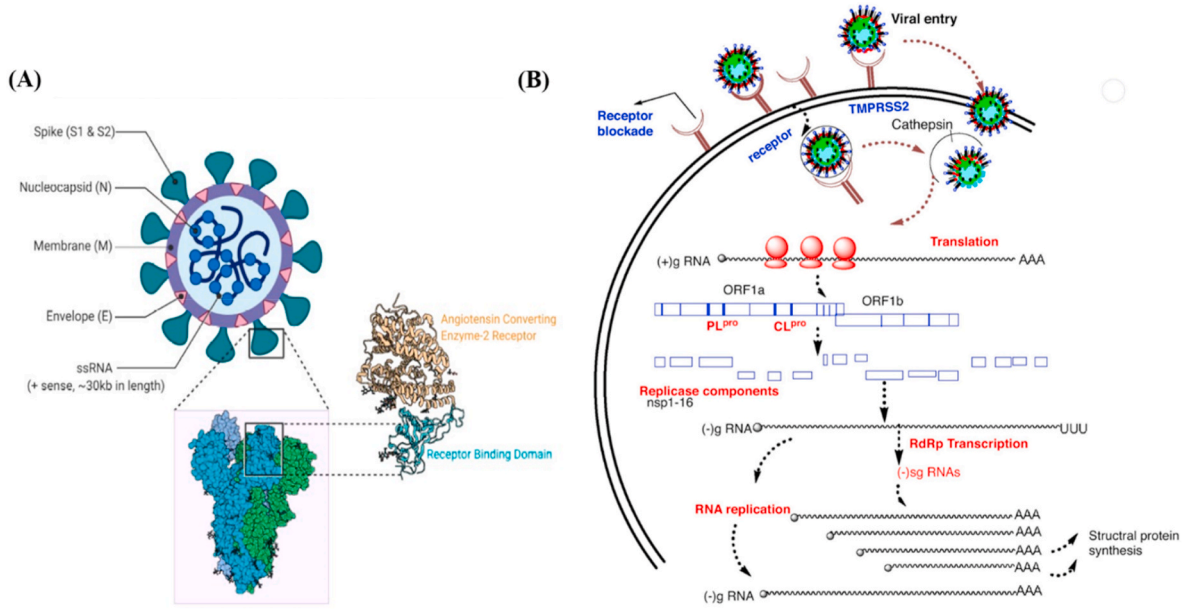

Fig. 2. A) Structure of SARS-CoV-2, a positive-sense, single-stranded RNA virus with 30 kb genome. The four structural proteins are the Spike protein (S) composed of S1 and S2 subunits, the RNA-binding Nucleocapsid (N) protein, the Membrane protein (M) spanning the whole membrane, and the Envelope protein (E). The receptor-binding domain (RBD) in the Spike proteins of SARS-CoV-2 binds to the Angiotensin-Converting Enzyme-2 (ACE2) in humans, this helps in the entry of the virus into the target cells. The figure is adapted from reference (Cascella et al., 2020). **B)** Replication of SARS-CoV-2 involves PPIs between viral and human cellular proteins. The RBD in the viral S protein binds to the host cell membrane receptor. The human cell surface protease TMPRSS2 cleaves ACE2 and thus, helps in S protein activation and entry of the virus into the cell by endocytosis due to conformational changes that lead to membrane fusion. After entering the cells, SARS-CoV-2 releases its genetic material (mRNA) in the cytoplasm of the host cells which undergoes translation to form long polypeptides pp1a and pp1b. These polypeptides encoded by the 5′ ORF1a and ORF1b, are processed into 16 non-structural proteins (NSP1-16) auto-proteolytically using viral proteases like papain-like protease (PL^{pro}) and chymotrypsin-like protease ($3CL^{pro}$) to form the RNA replicase/transcriptase complex (RTC). The viral protease RNA-dependent RNA polymerase (RdRp) encoded by NSP12 as well as other viral proteases also help in the host RNA translation. The figure is adapted from reference (Pillaiyar et al., 2020).

in the development and screening of various antiviral compounds against effective molecular targets of SARS-CoV-2. Several studies suggested various drugs like remdesivir developed by Gilead Sciences, USA which is a nucleotide analog prodrug inhibiting the RNA-dependent RNA polymerase (RdRp) in the virus thus, helping in preventing viral reproduction (Gordon et al., 2020). However, chloroquine is preferred over remdesivir due to its higher availability, and lower cost. However, it may also posses several side effects like cardiotoxicity. Chloroquine affects the terminal glycosylation of the ACE2 and elevates the endosomal pH reducing the virus-receptor binding and thus inhibiting the entry of the virus into the host cell (Vincent et al., 2005). Hydroxychloroquine is known to be a less toxic derivative of chloroquine which helps in reducing the SARS-CoV-2 viral nasopharyngeal carriage within 3–6 days in the Covid-19 patients (Liu et al., 2020; Gautret et al., 2020; Roden et al., 2020). Combination therapy of hydroxychloroquine with azithromycin has shown better results than when either is used alone for the treatment of Covid-19. Further information is required on this combination since it can be used against SARS-CoV-2 and may prevent bacterial superinfections as well however, can also lead to adverse drug reactions like QTc prolongation (Gautret et al., 2020). This review provides an outline of the effect of SARS-CoV-2 on the human cellular metabolism and the various repositioned drugs targeting the viral proteases as well as the protein-protein interactions (PPIs) between the viral and human proteins. It also takes into consideration the various drug dosage regimens, adverse drug reactions, and drug toxicities suggested by various studies, non-toxic antiviral phytochemicals as well as Covid-19 vaccine research and diagnostic methods.

2. Protein-protein interactions between SARS-CoV-2 and human proteins

The SARS-CoV-2 virus enters the human body cells through ACE2 found in the lungs, kidneys, and gastrointestinal tract. The viral uses to the spike glycoprotein (S protein) binds to the ACE-2 receptor on the cell surface of the host. After the binding, the type 2 transmembrane serine protease (TMPRSS2) present on the host cell surface cleaves ACE2, and thus activates the S protein. This leads to conformational changes that result in the fusion of the viral and host cell membrane followed by viral entry into the cells by endocytosis. After entering the cells, SARS-CoV-2 releases its genomic RNA (+sense) in the cytoplasm of the host cells. It then undergoes translation to form long polypeptides pp1a and pp1b. These polypeptides undergo cleavage by viral proteases like papain-like protease (PL^{pro}) and chymotrypsin-like protease ($3CL^{pro}$) to form NSP1−16, which leads to the formation of the RNA replicase/transcriptase complex. The NSPs code for RdRp and the viral proteases as well as interact with the host proteins and block the host immune response by interfering with the host's RNA translation (Fig. 2B) (Rabi et al., 2020; Astuti, 2020; Kumar and Lupoli, 2020).

Apart from the blockage of host innate immune response, SARS-CoV-2 also affects human cellular metabolism by various PPIs. This was identified in a research study by genome annotation followed by cell culture, transfection, and AP-MS. The viral proteins were cloned and expressed in human kidney cell lines HEK-293T/17 and lung cell lines. The protein expression of the virus was verified by western blot and proteomic analysis. AP-MS identified 332 PPIs between SARS-CoV-2 and human proteins related to cellular metabolism, protein expression patterns like transcription, protein transport, and ubiquitination (Chamberlain and Shipston, 2015; Gordon et al., 2020). The PPIs identified by the expression of viral proteins in cell lines helped to study the effect of viral NSPs and ORFs on human cellular metabolism (Gordon et al., 2020). Interaction of viral spike protein (S) with human acyl-transferase complex GOLGA7-ZDHHC5 leads to palmitoylation of the cytosolic tail which facilitates fusion of the viral and host cell membranes (Gordon et al., 2020; Knoops et al., 2008). Host proteins interact with viral NSP8

coding for the signal recognition particle, ORF8 for endoplasmic reticulum (ER) quality control, M for the morphology of ER, and NSP13 for golgins, which help in the reconfiguration of Golgi/ER trafficking during infection with coronavirus. Modifications of endomembrane compartments by NSP6, NSP7, NSP10, and ORF3a help in the replication of coronavirus (Gordon et al., 2020). Interaction of NSP8 and signal recognition particle suggests viral hijacking of the Sec61 translocon which helps to insert SARS-CoV-2 proteins into the host ER membrane (Gordon et al., 2020; Knoops et al., 2008). Sigma receptors in the host interact with viral NSP6 and ORF9c to regulate ER stress response and lipid remodeling; thus, help in viral replication (Gordon et al., 2020; Hellewell et al., 1994; Mitsuda et al., 2011). NSP5 inhibits the transport of histone deacetylase 2 (HDAC2) into the human cell nucleus by cleaving the epigenetic regulator between the nuclear localization sequence and the HDAC domain thus, HDAC2 cannot mediate inflammation and interferon response (Gordon et al., 2020; Barnes 2009; Xu et al., 2019). Viral NSP5 also reacts with human tRNA methyltransferase 1 (TRMT1) leading to dimethylguanosine base modification on the tRNA of both nucleus and mitochondrian (Gordon et al., 2020). NSP5 can also lead to TRMT1 cleavage as well as the removal of zinc finger and nuclear localization signal resulting in viral mitochondrial localization (Gordon et al., 2020).

Similarly, immune signaling pathways in the human cells are targeted by the SARS-CoV-2 viral proteins affecting the innate immune response of the host. NSP13 interacts with TANK-binding kinase 1 (TBK1) as well as proteins of transducin-like enhancer family to modulate interferon signaling and Nuclear Factor-Kappa B (NF-KB) inflammatory response along with ORF9c (Gordon[a] et al., 2020; Ryzhakov et al., 2007; Xia et al., 2011). The viral nucleocapsid (N) protein interacts with host mRNA proteins like stress granule related factors (Gordon[a] et al., 2020; Ivanov et al., 2019). Manipulation and suppression of stress granules that induce innate immune response help in viral replication (Raaben et al., 2007). Two E3 ubiquitin ligases, MIB1 and TRIM59 bound by NSP9 and ORF3a respectively also regulate the innate immune signaling during antiviral response (Gordon et al., 2020; Kondo et al., 2012; Li et al., 2011). Viral ORF6 leads to interferon signaling antagonism by interacting with host NUP98-RAE1 responsible for interferon induction (Gordon et al., 2020; Faria et al., 2005). Reduced immune response due to the viral infection leads to reduced lung function, severe pneumonia, arrhythmia, and subsequent death in humans (Gordon et al., 2020). The viral proteins also affect human cellular metabolism by interacting with the host proteins at the epigenetic level. SARS-CoV-2 ORF10 binds to Cullin 2 (CUL2) RING E3 ligase complex, $CUL2^{ZYG11B}$, where ZYG11B can degrade exposed N-terminals of ORF10. Alternatively, ORF10 can hijack the ubiquitination pathway of the complex to degrade the restriction factors, and thus help in replication and pathogenesis of the virus (Gordon et al., 2020; Gordon et al., 2020). Viral transmembrane protein E mimics the histone and binds to the bromodomain-containing proteins, BRD2 and BRD4, in the host thus disrupting BRD-histone binding which induces changes in gene transcription and host's protein expression, in turn, helping the virus to replicate (Gordon et al., 2020; Faivre et al., 2020).

Due to limited knowledge, effective antivirals with high efficacy for combating Covid-19 has been difficult to develop. These PPIs between the SARS-CoV-2 and human proteins will provide knowledge about the molecular mechanism of SARS-CoV-2 infection and replication. The interaction between the virus and the human host was targeted and studied by AP-MS, cheminformatic analyses, *in vitro*, and *in vivo* antiviral screening to formulate therapies targeting the dependency of SARS-CoV-2 on the host and for drug repurposing (Gordon et al., 2020).

3. Repurposed drugs for Covid-19 and their mechanism of action

Drug repurposing is also known as drug repositioning which helps to identify new uses of investigational or approved drugs different from their original use. Drug repurposing has proved to be more advantageous than the development of an entirely new drug for any disease due to huge costs, time-consuming research, and approval processes concerned with drug discovery. The PPIs between viral and human proteins as well as the viral proteases can be screened against various cheminformatic databases. Molecular docking and simulation, as well as literature search, can help to find various approved or candidate drugs that can target SARS-CoV-2 viral interactions with human host proteins (Fig. 3). Various repurposable drug candidates targeting these host proteins help to interfere with the replication of SARS-CoV-2 and disease progression post-infection. Besides, it would be more difficult for the virus (population) to develop resistance mutations against the antivirals if there is a large antiviral drug repertoire (Sadegh et al., 2020). Apart from these *in silico* models, *in vitro* models like various lung and kidney cell lines can also be used to study drug action, drug dosage regimens, as well as toxicities, and adverse drug reactions associated with the drug or drug candidates. Some repurposable drugs can also be screened in Covid-19 patients by supplementing them with other approved antiviral drugs (Table 1). Combination therapy can increase the efficiency of the treatment. These repurposable drugs can also help in relieving some of the symptoms of Covid-19 patients and increasing their survival rates (Gautret et al., 2020; Arshad et al., 2020; "Solidarity" clinical trial for COVID-19 treatments, 2020).

3.1. Drugs screened by in silico and in vitro studies

The PPIs between SARS-CoV-2 and human proteins as well as other molecular targets can be targeted by various repurposed drugs (Fig. 3A). Cheminformatic literature search and specialist knowledge revealed 69 drugs, some of which are approved and some clinical and preclinical candidates from other research, with the potential to target the interacting viral and host proteins (Fig. 3B-C) (Gordon[a] et al., 2020; Gordon et al., 2020). The CoVex software can also be used to map the SARS-CoV-2 and human PPIs and identify repurposable drugs (Fig. 3) (Sadegh et al., 2020). These drugs include bafilomycin A1 which inhibits V1-ATPase responsible for inhibiting autophagy and transport of endosomes. Its subunits react with the viral NSP6 and M impacting the viral life cycle. Some drugs target the epigenetic regulators in the host like valproic acid, which is an approved drug, and apicidin, a preclinical candidate, that inhibit HDAC2. Clinical candidates like ABBV-744 and CPI-0610 inhibit BRD2/4 (Gordon et al., 2020). Similarly, a natural product like WDB002, obtained from a soil-inhabiting *Streptomyces* bacterium, is known to first target a human protein called FKBP12, further forming a complex with the human centrosomal protein, CEP250, which interacts with viral NSP13 (Shigdel et al., 2020). Likewise, mycophenolic acid, ribavirin, and merimepodib seem to play a role in inhibiting inosine-5′-monophosphate dehydrogenase 2 (IMPDH2) which interacts with viral NSP14 for the biosynthesis of human purine in viral replication. Sapanisertib and rapamycin are inhibitors of the mTORC1 pathway, repressing the mTOR translation in viral synthesis and replication (Gordon[a] et al., 2020). Silmitasertib, particularly, reduces stress granule disassembly by CK2 inhibition thus increasing stress granules required for innate immune response in viral replication (Table 1) (Gordon[a] et al., 2020; Reineke et al., 2017). Another small molecule drug is pevonedistat that can be used to inhibit the NEDD8-activating enzyme (NAE) that helps in the neddylation of Cullin 2 (CUL2) required for ubiquitin transfer to the substrate; thus, hijacking the ubiquitination pathway and helping in the replication of the virus (Gordon[a] et al., 2020; Soucy et al., 2009). Antibiotics like chloramphenicol, tigecycline, and linezolid also have an off-target effect against host mitochondrial ribosomes which react with viral protein NSP8 (Gordon[a] et al., 2020; Mckee et al., 2006).

These 69 ligands were then investigated for antiviral activity against SARS-CoV-2 infected Vero E6 cells by cell viability, plaque-forming, and off-target assays. Two main types of antiviral molecules against SARS-CoV-2 were found, molecules binding to sigma-1 and sigma-2 receptors, and protein biogenesis inhibitors (Gordon et al., 2020). It was

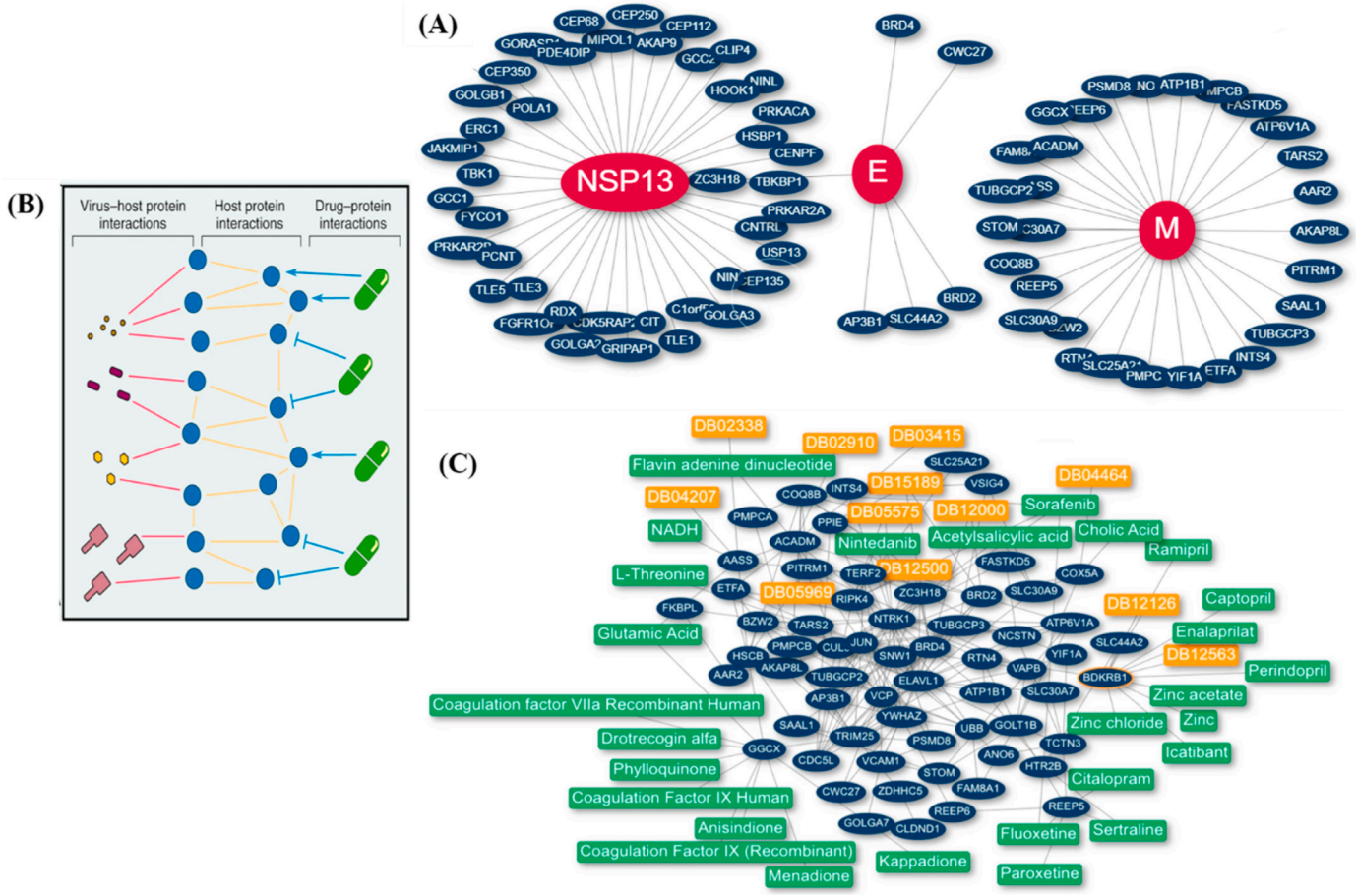

Fig. 3. SARS-CoV-2 virus-host interactome to identify repurposable drugs studied using the CoVex software. **A)** Protein-protein interactions (PPIs) between the human proteins (blue nodes) and some of the SARS-CoV-2 structural (E, M) and non-structural (NSP13) proteins (pink nodes). PPI map constructed using CoVex software (Sadegh et al., 2020). **B)** SARS-CoV-2 life cycle is controlled by protein interactions between the virus and host proteins. Identification of repurposable drug candidates (green drugs) targeting the host proteins (blue circles) can help to interfere with the replication of the virus and disease progression post-infection. The figure is adapted from reference (Sadegh et al., 2020). **C)** Drug–protein–protein interaction network map obtained using the viral proteins Spike, E and M with multi-Steiner tree followed by closeness centrality on the CoVex software. Blue nodes are human protein targets. Green nodes are the approved drugs and orange nodes are the non-approved candidate drugs. Interactions between proteins and drugs are represented by lines. The figure is adapted from reference (Sadegh et al., 2020).

also found that RNA helicase, eukaryotic initiation factor-4A (eIF4A) inhibitor, zotatifin as well as the eukaryotic translation elongation factor-1A (eEF1A) inhibitor, ternatin-4, help to reduce the rate of mRNA translation in SARS-CoV-2. PS3061, a Sec61 inhibitor, blocks SARS-CoV-2 replication by inhibiting SARS-CoV-2 protein insertion into the ER (Gordon et al., 2020; Carelli et al., 2015) Drugs that show antiviral activity by acting as ligands of the sigma receptor include haloperidol, chloroquine, clemastine, cloperastine, progesterone, and astemizole (Gordon et al., 2020; Gordon et al., 2020; Hellwell et al., 1994). It was shown that sigma-1 receptor agonist, dextromethorphan exhibits proviral activity against SARS-CoV-2, and ligands like PB28 have high selectivity against off-target proteins like hERG ion channels but very high efficacy for sigma receptors. PB28 has also shown higher antiviral activity than hydroxychloroquine (Table 1) (Gordon et al., 2020). However, *in silico* and *in vitro* studies of these candidates do not promise clinical performance but put forward promising candidates that can be investigated further. Drug repurposing pharmacological studies will require genetic-based approaches like pharmacogenomics to determine the functional relevance of the PPIs between human and viral proteins for host-directed therapies.

3.1.1. *Virus-based anti-CoV therapeutics targeting PPIs*

Studies summarise the various virus-based anti-CoV therapeutics that can be developed by understanding the genomics and targeting specific viral enzymes and host receptors of various coronaviruses like SARS-CoV. This can give an insight into new SARS-CoV-2 targets apart from the PPIs between the virus and the host due to the 79.5% genetic similarity between SARS-CoV and SARS-CoV-2 (Pillaiyar et al., 2020; Rabi et al., 2020). RNA helicase eIF4A inhibitor, silvestrol, a non-toxic inhibitor of viral mRNA translation, helps to inhibit the structural and non-structural protein expression as well as viral replicase/transcriptase complex formation in CoVs (Pillaiyar et al., 2020; Müller et al., 2018). PL^{pro} is an important target for antiviral therapy in coronaviruses including SARS-CoV-2 because it is involved in deubiquitination and innate immune response evasion by the virus in addition to its proteolytic activity (Mielech et al., 2014). Various SARS-CoV PL^{pro} inhibitors identified are thiopurine compounds, zinc ion as well as naphthalene inhibitors (Báez-Santos et al., 2015). 8-(Trifluoromethyl)-9H-purin-6--amine, as well as benzotriazole and *B. papyrifera* derivatives can inhibit both $3CL^{pro}$ and PL^{pro} CoV proteases (Park et al., 2017). Non-peptide inhibitors of SARS-CoV $3CL^{pro}$ include decahydroisoquinoline (Shimamoto et al., 2015). SK40 with isoserine backbone can be used against the mutant protease, R188I, in SARS-CoV where the mature SARS-CoV $3CL^{pro}$ undergoes degradation (Konno et al., 2017). SSYA10-001 is a small 1,2,4-triazole derivative that can inhibit NSP13 or the viral helicase of SARS-CoV, and thus not allow its replication (Adedeji et al.,

Table 1
Antiviral drugs identified by various *in silico, in vitro*, and *in vivo* studies, their predicted mode of action against the SARS-CoV-2 virus and drug dosage regimens.

Drug name	System used for antiviral screening	Effect	Drug dosage regimen	Reference
Chloroquine	Vero E6 cells, cheminformatic literature search (ChEMBL, ZINC),	Inhibits endosomal maturation by pH elevation, impaired terminal glycosylation of ACE2, reduces PICALM expression, Sigma receptor modulator	EC_{50} = 1.13 μM; Patients aged between 18-65 years and weight more than 50 kg - 500 mg BID for 7 days, Weight less than 50 kg – 500 mg BID for days 1–2 and then 500 mg once a day for days 3–10.	Vincent et al. (2005); Liu et al. (2020); Gordon et al. (2020); Hu et al. (2020); Wang et al. (2020); Gao and Hu (2020)
Hydroxychloroquine	Vero E6 cells	Inhibits endosomal maturation by pH elevation, blocks T-cell stimulation reduced cytokine storm in Covid-19 patients, disrupt interaction between DNA/RNA and TLRs	EC_{50} = 4 μM; 200 mg BID for 5 days; 400 mg BID on day 1 and 200 mg BID from days 2–5; 800 mg BID or 600 mg BID for 1–2 days followed by 400 mg BID or 200 mg TID maintenance dose.	Liu et al. (2020); Gautret et al. (2020); Arshad et al. (2020); Zhou et al. (2020); Chen et al. (2020); Garcia-Cremades et al. (2020)
Remdesivir	Vero E6, Huh-7 cells	Inhibits viral RdRp	200 mg on day 1 and 100 mg daily from days 2–10	Liu et al. (2020); Wang et al. (2020); Biegel et al. (2020)
Silvestrol	MRC-5 cells, PBMCs	Inhibits RNA helicase eIF4A, viral mRNA translation, structural and non-structural protein expression and viral replication-transcription complex formation	Clinical trials not completed	Pillaiyar et al. (2020); Müller et al. (2018)
Saracatinib	Huh-7 cells	Inhibits SFK	Clinical trials not completed	Pillaiyar et al. (2020); Shin et al. (2018)
Favipiravir	Vero E6 cells	Inhibits viral RdRp	1600 mg BID on day 1 and 600 mg bid from days 2–14	Oestereich et al. (2014); Wang et al. (2020); Cai et al. (2020)
Ribavirin	Vero cells, LLC-MK2 cells, cheminformatic literature search (ChEMBL, ZINC)	Inhibits IMPDH2	Clinical trials not completed	Gordona et al. (2020); Falzarano et al. (2013)
Azithromycin	Covid-19 patients in hospitals (clinical trials) to study combination therapy	Supplemented with hydroxychloroquine, leads to a rapid decline in nasopharyngeal viral load, decreases mucus secretion, acts on bronchial epithelial cells, blocks endocytosis by accumulating in lysosomes, inhibits vacuolar ATPase immunomodulatory effects	Combination therapy of 200 mg hydroxychloroquine TID for 10 days and 500 mg azithromycin on day 1 followed by 250 mg per days for days 2–5; Combination therapy of 400 mg of hydroxychloroquine BID on day 1 followed by 200 mg BID from days 2–5 and 500 mg azithromycin on day 1 followed by 250 mg per days for days 2–5.	Gautret et al. (2020); Arshad et al. (2020); Gielen et al. (2010), Tyteca et al. (2002), Oliver and Hinks et al. (2020)
Ritonavir	Covid-19 patients (clinical trials) to study combination therapy	Inhibits viral protease, increases ribavirin efficiency in combination therapy, lower ARDS, lower death rate	Combination therapy of 400 mg lopinavir and 100 mg ritonavir BID for 14 days	Pillaiyar et al. (2020); De Wilde et al. (2014); Chu et al. (2004); Cao et al. (2020); Lim et al. (2020)
Lopinavir	Screening of FDA approved drugs library *in silico*, Covid-19 patients (clinical trials)	Inhibits SARS-CoV-2 $3CL^{pro}$, increases ribavirin efficiency in combination therapy, lower ARDS, lower death rate		
Dexamethasone	Covid-19 patients (clinical trials)	Lower SOFA score, improved PaO2/FiO2, antifibrosis and anti-inflammation in ARDS patients	20 mg once daily from days 1–5 followed by 10 mg once daily from days 6–10	Villar et al. (2020)
Chlorpromazine	Screening of FDA approved drugs library *in silico*	Inhibits chathrin-mediated viral endocytosis	Clinical trials not completed	Pillaiyar et al., 2020; De Wilde et al. (2014)
Bafilomycin A	Cheminformatic literature search (ChEMBL, ZINC), Vero E6 cells	Inhibits V1-ATPase, thus increases autophagy	Clinical trials not completed	Gordon et al., 2020; Gagliardi et al. (1998)
Valproic Acid, Apicidin	Cheminformatic literature search (ChEMBL, ZINC), Vero E6 cells	Inhibits HDAC2	Clinical trials not completed	Gordon et al., 2020; Krämer et al. (2003); Khan et al. (2008)
Mycophenolic acid, Merimepodib	Cheminformatic literature search (ChEMBL, ZINC), Vero E6 cells	Inhibits IMPDH2	Clinical trials not completed	Gordon et al., 2020; Sintchak and Nimmesgern (2000)
Silmitasertib	Cheminformatic literature search (ChEMBL, ZINC), Vero E6 cells	Inhibits CK2, thus increases stress granule	Clinical trials not completed	Gordon et al., 2020; Pierre et al. (2011)
Haloperidol, Clemastine, Cloperastine, PB28	Cheminformatic literature search (ChEMBL, ZINC), Vero E6 cells	Sigma receptor modulator	Clinical trials not completed	Gordon et al. (2020), Hellewell et al. (1994)
Ternatin-4	Cheminformatic literature search	Inhibits elongation factor eEF1A, reduces viral mRNA translation	Clinical trials not completed	Gordon et al. (2020); Carelli et al. (2015)

(*continued on next page*)

Table 1 (*continued*)

Drug name	System used for antiviral screening	Effect	Drug dosage regimen	Reference
	(ChEMBL, ZINC), Vero E6 cells			
5,7,3′,4′-tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone	Molecular docking of predicted SARS-CoV-2 $3CL^{pro}$ structure against phytochemical library	Very high binding affinity, very high docking score due to strong hydrogen bond formation	Clinical trials not completed	ul Qamar et al. (2020)
Methyl rosmarinate, Myricitrin	Molecular docking of predicted SARS-CoV-2 $3CL^{pro}$ structure against phytochemical library	High docking score, closer interaction with Cys-His catalytic dyad residues	Clinical trials not completed	ul Qamar et al. (2020)

Table note: ACE2: Angiotensin-Converting Enzyme-2 in humans; ARDS: Acute Respiratory Distress Syndrome; BID: Twice a day; $3CL^{pro}$: Chymotrypsin-like protease; ChEMBL: Chemical database maintained by the European Bioinformatics Institute (EBI) of the European Molecular Biology Laboratory; CK2: Casein kinase 2 is a human protein kinase; Covid-19: Coronavirus disease 2019; Cys-His: Cysteine-Histidine is a catalytic dyad. eEF1A: Eukaryotic translation elongation factor-1A in humans; eIF4A: Eukaryotic initiation factor-4A which is a RNA helicase in humans; HDAC2: Histone deacetylase in humans; IMPDH2: Inosine-5′-monophosphate dehydrogenase 2; PaO2/FiO2: Ratio between oxygen partial pressure in arterial blood and inspired oxygen fraction; PICALM: Phosphatidylinositol binding clathrin assembly protein which is a cargo-selecting clathrin adaptor; RdRp: RNA-dependent RNA polymerase of the virus; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; Sec61: Translocon on the ER membrane of the host; SFK: Src-family of tyrosine kinases; SOFA: Sequential Organ Failure Assessment Score is an important variable to predict disease severity; TID: Thrice a day; TLR: Toll-like receptors; V1-ATPase: Vacuolar ATPase with V1 complex that hydrolyses ATP; Vero E6, Huh-7, MRC-5, PBMC, LLC-MK2: Various cell lines used for *in vitro* viral studies; ZINC: A Free Database of Commercially Available Compounds for Virtual Screening.

2014). The host innate interferon response is important for controlling viral replication. SARS-CoV replication *in vitro* is inhibited by recombinant interferon-α and interferon-β (Cinatl et al., 2003). Rapamycin, trametinib, everolimus, imatinib, selumetinib, and dasatinib block the ABL1 and AKT-mTOR pathways which help to block viral entry and post-entry events in SARS-CoVs (Kindrachuk et al., 2015). N-(2-aminoethyl)-1-aziridineethanamine can inhibit cell-cell fusion as well as ACE2 catalytic activity (Huentelman et al., 2004). Small molecules like SSAA09E2, discovered for blocking viral entry, blocked the interaction between viral S protein and ACE2. SSAA09E1 blocked cathepsin L and SSAA09E3 prevented the fusion of host cellular membrane and viral membrane (Adedeji et al., 2013). Hexamethylene amiloride is a viroporin inhibitor that reduces the activity of the E ion channels in SARS-CoV (Wilson et al., 2006).

3.1.2. *Anti-CoV therapeutics inhibiting CoV replication*

Apart from virus-based anti-CoV therapeutics, various repositioned drugs inhibiting the entry and replication of various coronaviruses that can also be used against SARS-CoV-2 are summarised in different research studies (Pillaiyar et al., 2020). Seven broad-spectrum inhibitors with low 50% effective concentration (EC_{50}) values: emetine, lycorine, phenazopyridine, mycophenolate mofetil, pyrvinium pamoate, monensin sodium, and mycophenolic acid, suppressed replication of various coronaviruses in a dose-dependent manner (Shen et al., 2019). The antipsychotic drug, chlorpromazine, can inhibit the clathrin-mediated endocytosis of the virus. Similarly, loperamide helps to reduce intestinal motility but it can also inhibit CoV replication (Pillaiyar et al., 2020; De Wilde et al., 2014). A combination therapy of two anticancer drugs, saracatinib, which inhibits Src-family of tyrosine kinases (SFKs), screened using Huh-7 cells, and gemcitabine can help to treat CoV diseases (Pillaiyar et al., 2020). E-64-D inhibits cathepsin which is required for the fusion step during the viral entry (Bosch et al., 2008). Dasatinib and imatinib mesylate are known to inhibit the ABL-1 pathway which is important for viral replication. Likewise, DNA synthesis inhibitors like toremifene citrate and gemcitabine hydrochloride can also be used in antiviral therapy against coronavirus (Pillaiyar et al., 2020). All these drugs and small molecules inhibiting the replication of different coronaviruses can potentially be used against SARS-CoV-2. These repurposed drugs and their combination therapies might prove to be very beneficial for Covid-19 treatment.

3.1.3. *Mechanism of repurposed drugs like chloroquine and hydroxychloroquine*

Apart from summarising the repurposed drugs, some studies conducted *in vitro* assays to find out the drug action mechanism of the repurposed drug (Table 1). The mechanism of action of chloroquine against SARS-CoV-2 is predicted to be the same as it is for SARS-CoV. Viral antigens were visualized in SARS-CoV-infected Vero E6 cells by indirect immunofluorescence and it was suggested that chloroquine can have both prophylactic and therapeutic advantages: it could prevent viral infection in-vitro when it was added 24 h before viral infection and was effective when added 3–5 h post-infection as well. Immunoprecipitation analyses showed that cellular trafficking, N-glycosylation, and other enzymatic processes are regulated by the low acidic pH of the vesicles. Chloroquine increases the pH of the endocytic vesicles, thus it affects viral entry. Chloroquine reduces the biosynthesis of the viral spike protein by reducing the level of Golgi-modified pro-spike leading to a reduction in viral replication (Vincent et al., 2005). NH_4Cl can reduce the transduction of viruses with SARS-CoV spike protein and affect the terminal modifications of the N-glycosylated chains of ACE2 which leads to less efficient ACE2-SARS-CoV interaction (Yang et al., 2004). Protein expression of under-glycosylated ACE2 on the surface of the cell and its poor affinity to viral spike proteins may be the primary mechanism for the prevention of viral infection by pre-treatment of drugs. Elevation of pH in endosomes affecting the virus-endosome fusion may be the mechanism for treatment with chloroquine after viral infection (Vincent et al., 2005).

Studies suggest that chloroquine can be used to study interactions of nanoparticles like a virus with the host cells to obtain information about the changes induced by chloroquine on cellular uptake of SARS-CoV-2 (Hu and Wolfram, 2020). SARS-CoV-2 shape (spherical) and size (60–140 nm) are within the same range as most other synthetic nanoparticles (Zhu et al., 2020b). Chloroquine reduces the accumulation of nanoparticles like a virus in macrophages as well as expression of cargo-selecting clathrin adaptor, phosphatidylinositol binding clathrin assembly protein which affects membrane curvature leading to inhibition of endocytosis of SARS-CoV-2 viral nanoparticle into the host cell (Wolfram et al., 2017; Miller et al., 2015). Prevention of lysosome acidification by chloroquine leads to interference in the fusion of endocytic vesicles and membrane receptor recycling which is necessary for cellular entry of SARS-CoV-2 (Hu and Wolfram, 2020; Haladyj et al., 2018). It also inhibits the fusion of viral envelope with the endosome because of the inactivated viral surface spike protein which is required to be activated for the fusion. The activation of spike protein is done by endosomal proteases like cathepsins which are activated only by the acidification of endosomes and chloroquine is known to inhibit this acidification (Hu and Wolfram, 2020).

Hydroxychloroquine is another repurposed drug for Covid-19 which

is found to be less toxic than chloroquine for inhibition of SARS-CoV-2 infection *in vitro* (Vero E6 cells) (Liu et al., 2020). Hydroxychloroquine can reduce the inflammatory response and cytokine storm (aggressive inflammatory response with the release of a large amount of pro-inflammatory cytokines) in patients severely infected with SARS-CoV-2 by blocking T-cell stimulation, and thus decreasing cytokine production. It can also disrupt the interaction between DNA/RNA and the toll-like receptors to inhibit the expression of pro-inflammatory genes (Liu et al., 2020; Zhou et al., 2020). In a study, a cytotoxicity assay was conducted on Vero E6 cells to find the 50% cytotoxic concentration (CC_{50}) as well as dose-response curves to find EC_{50} for chloroquine and hydroxychloroquine. Immunofluorescence revealed that cells treated with chloroquine and hydroxychloroquine had more virions with early endosome antigen 1 (EEA1) EEs, and very few were co-localized with late endosomal-lysosomal protein $LAMP1^{+}$ ELs. In untreated cells, ELs were higher than EEs (Liu et al., 2020). Thus, they concluded that transport of SARS-CoV-2 from EEs to ELs, required for the release of the viral genome for replication is blocked by chloroquine and hydroxychloroquine (Mingo et al., 2015). This also changed their morphology, abnormally increasing the size of EEs. It can be said that the failure of transport of virions to the final releasing site is due to inhibition of endosomal maturation by pH elevation caused due to chloroquine and hydroxychloroquine (Liu et al., 2020).

Apart from chloroquine, arbidol (umifenovir) can also be used to treat Covid-19 prophylactically. It interferes with clathrin-mediated endocytosis and interacts with viral glycoprotein aromatic residues which are required for cellular recognition and fusion of the virus (Teissier et al., 2011; Kadam and Wilson 2017). A combination therapy of darunavir, which is a viral protease inhibitor, and cobicistat, which enhances pharmacokinetics of darunavir by inhibiting cytochrome P450, can be used to treat Covid-19 (Chen et al., 2020; Santos et al., 2019). Initially, Vero E6 cells were used to test favipiravir which is a Covid-19 experimental drug used against the Ebola virus previously. Favipiravir inhibits viral RdRp just like the nucleotide analog prodrug, remdesivir (Oestereich et al., 2014; Wang et al., 2020). These repurposed drugs screened by various *in silico* and *in vitro* antiviral screening systems have paved a path towards combating Covid-19.

3.2. Drugs screened by in vivo studies in Covid-19 patients

The effects of combination therapies are required to be tested in Covid-19 patients ("Solidarity" clinical trial for COVID-19 treatments, 2020). Some antiviral drugs for Covid-19 patients were supplemented with interferons, which are signaling proteins released in response to viral infection by the host cell; corticosteroids, that help to reduce inflammation; and other repurposable approved drugs to increase their efficiency which would lead to higher survival rates of the patients (Table 1). However, the ability of corticosteroids to suppress the innate host defense against the virus must be considered. Therefore, clinical trials were conducted for these combination therapies. Viral protease inhibitors like ritonavir and lopinavir, were known to help increase ribavirin efficiency. (Chu et al., 2004). Similarly, combination therapy of corticosteroid and interferon-α-1 improved the saturation of oxygen (Loutfy et al., 2003). Corticosteroid therapy in Covid-19 patients has the potential to show higher survival rates by suppressing the cytokine storm of interferons, interleukins (IL) and tumor necrosis factor (TNF) caused in response to acute viral respiratory infections which lead to tissue injury (Tang et al., 2020). Early administration of the corticosteroid, dexamethasone, in patients with moderate-to-severe ARDS, could reduce the lung-protective mechanical ventilation duration and overall mortality (Villar et al., 2020). SARS-CoV-2 infected patients also experience ARDS. Studies suggest that corticosteroids can attenuate the systemic and pulmonary damage in ARDS patients due to their anti-fibrotic and anti-inflammatory properties (Rhen and Cidlowski, 2005). Corticosteroids improved the PaO2/FiO2 (Ratio between oxygen partial pressure in arterial blood and inspired oxygen fraction) above 200 mm Hg within 24 h. Dexamethasone increased the survival of patients treated with extracorporeal lung support. Patients treated with dexamethasone experienced lower Sequential Organ Failure Assessment score from day 3 of treatment initiation. Consequently, dexamethasone increased the number of ventilator-free days by more than 4 days and increased the 60-day survival for ARDS patients by 15%. The number of ICU deaths due to multiple organ failure in dexamethasone-treated patients decreased by almost half compared to the control group (Villar et al., 2020). An editorial also focused on the cytokine storm of TNF-α, IL-6, and IL-12 in the case of Covid-19 and its role in acute lung injury (ALI) and also the ability of one cytokine to induce other cytokines for increasing the pro-inflammatory response as seen in the case of TNF-α inducing IL-6 levels. Cytokine storm is also complemented with the generation of anaphylatoxins like C3a and C5a which induce innate immune cells to release the proinflammatory cytokines. Oncologists have used monoclonal antibodies (mAbs) to interrupt cytokine storm in cancer treatment. Thus, IL-6 inhibition with the anti-IL-6R mAb (tocilizumab) is predicted to enhance the treatment of Covid-19 as it helps to reduce C5aR1 and C5aR2 in blood along with a decrease in C-reactive protein (Buonaguro et al., 2020).

In another study, supplementing azithromycin along with hydroxychloroquine in Covid-19 patients, helped to clear the virus more efficiently, leading to a rapid decline in the viral load in the nasopharynx (Gautret et al., 2020). Azithromycin also decreases mucus secretion and increases the function of the lungs by acting directly on bronchial epithelial cells (Gielen et al., 2010). Furthermore, it targets granulocytes, it blocks viral endocytosis by accumulating in lysosomes and thus, affecting adhesion and apoptosis of neutrophils (Tyteca et al., 2002; Oliver and Hinks et al., m 2020). It can also inhibit vacuolar ATPase required for lysosome-autophagosome fusion, by inhibiting autophagy. Azithromycin also exhibits anti-inflammatory properties via suppression of IL1-beta, IL2, and TNF thus, it helps to deal with the aggravated immune response in the case of Covid-19. Azithromycin inhibits the T-cells via inhibition of NF-KB activation and calcineurin signaling. The immunomodulatory properties of azithromycin have shown effective response in various respiratory diseases like asthma and Chronic Obstructive Pulmonary Disease (Oliver and Hinks, 2020). Clinical trials showed that these drugs screened in the Covid-19 patients helped to increase the efficiency of the other repurposed drugs in combination therapies, relieve the symptoms, and increase the survival rates of patients. These drugs screened by *in silico*, *in vitro*, and *in vivo* studies require to undergo large scale randomized controlled trials for testing efficacy and safety.

4. Repurposed drug dosage regimens and adverse drug reactions

Dosage requirements or the drug dosage regimens of the various repurposed drugs and the combination therapies are being studied *in vitro* and *in vivo* to find out about drug toxicities and adverse drug reactions. Results of various studies about the activity of hydroxychloroquine and chloroquine on coronavirus were summarised (Colson et al., 2020). The culture tests of chloroquine on Vero E6 cells to treat viral SARS-CoV-2 infection revealed EC_{50} to be 1.13 μM (Wang et al., 2020). A study says that in patients with mild to severe form of Covid-19 pneumonia, with 500 mg of chloroquine two times a day, the therapeutic concentration could be reached (Gao et al., 2020). Earlier research done with hydroxychloroquine for long-term treatment revealed that a dose of 600 mg/day could help to reach a concentration of 1 μg/mL in the body (Lagier et al., 2014). The optimum dose of hydroxychloroquine for SARS-CoV-2 still needs to be assessed which might include a loading dose followed by a maintenance one (Colson et al., 2020).

4.1. Chloroquine dosage

Various clinical trials for the dose optimization of chloroquine and

hydroxychloroquine supplemented with other antivirals yielded a variety of results (Table 1). A study summarised the dosage requirements of chloroquine and hydroxychloroquine which are cheap and easily available and have the potential to curb the global pandemic, Covid-19. Emergency use authorization was issued by the Food and Drug Administration (FDA) for the use of these two drugs to treat Covid-19. The study summarised that the seventh edition of the "Guidance of Coronavirus Disease 2019" of China recommended a chloroquine phosphate dosage of 500 mg twice a day for 7 days for patients aged between 18 and 65 with more than 50 kg of body weight, and for the ones less than 50 kg, a dosage of 500 mg two times a day for the first two days and then a single dose of 500 mg per day for the next 3–7 days (Gao and Hu 2020). Chloroquine showed better results than lopinavir/ritonavir in decreasing the hospitalization duration of Covid-19 patients, bringing body temperature back to normal and conversion to virus-negative since the chloroquine-treated patients tested negative by day 7, 10, or 14 after treatment (Huang et al., 2020). Some adverse effects seen in these patients include nausea, diarrhea, rash, cough, and abdominal pain.

4.2. Hydroxychloroquine and azithromycin dosage

One of the studies revealed that a patient group receiving 200 mg hydroxychloroquine sulfate twice a day for 5 days along with the standard treatment of oxygen therapy, immunoglobulins, and other antivirals reduced the time taken by the body to return to normal temperature and decreased the cough remission time (Chen et al., 2020). Early administration of hydroxychloroquine in patients at the initial viral replication phase showed more benefit than the patients with the drug administered at the later hyperimmune response phase (Arshad et al., 2020). Although treatment with hydroxychloroquine significantly reduced the viral load in Covid-19 patients, the addition of azithromycin to it showed more efficient results in virus elimination (Table 1). This was indicated in a study where patients were administered with 200 mg hydroxychloroquine sulfate 3 times a day for 10 days. Few of these hydroxychloroquine-treated patients received 500 mg of azithromycin on the first day and then 250 mg per day for the next 4 days to prevent superinfection by bacteria. Results at day 6 post inclusion showed that 57% of patients treated only with hydroxychloroquine were cured whereas 100% of the patients treated with the combination of azithromycin and hydroxychloroquine were cured. Results also showed that the effect of the drugs was higher in patients with respiratory tract infections symptoms like bronchitis, pneumonia, and pharyngitis than in asymptomatic patients. One of the patients treated only with hydroxychloroquine was PCR-positive for the virus even after the treatment; therefore, azithromycin was administered which cured the infection at the earliest (Gautret et al., 2020). Another study did a multi-hospital assessment for this hypothesis. First, treatment only with hydroxychloroquine took place that involved 400 mg twice on day 1 followed by 200 mg twice a day from days 2–5 and 13.5% of people died in this group. Later, an only-azithromycin treatment followed which included administration of 500 mg once on day 1 and 250 mg once a day for the next 4 days where the mortality was 22.4%. The combination therapy of the two drugs was administered to the patients with very low cardiac risk factors which led to a 20% mortality. Additionally, O_2 saturation and Modified Sequential Organ Failure Assessment score were recorded which are important variables to predict disease severity and mortality. Results affirmed that hydroxychloroquine alone or in combination therapy with azithromycin led to higher survival of Covid-19 patients (Arshad et al., 2020).

4.3. Corticosteroids and other drugs dosage

Corticosteroid therapy in Covid-19 patients also showed higher survival rates. 20 mg dexamethasone once daily was administered to the patients from day 1–5 followed by 10 mg once a day from day 6–10 (Villar et al., 2020). Corticosteroid therapy's main adverse effect is seen to be hyperglycemia which can be controlled by controlling the dose and the period of treatment (Maerz and Akhtar, 2011). Remdesivir dosage regimen of 200 mg on day 1 and 100 mg from days 2–10, as well as favipiravir dosage regimen of 1600 mg twice on day 1 and 600 mg twice daily from days 2–14, have shown effective results in the clinical trials for Covid-19 treatment (Biegel et al., 2020; Cai et al., 2020). Combination therapy of 400 mg lopinavir and 100 mg ritonavir twice daily for 14 days have proven to be effective in the randomized controlled trials but they have shown some gastrointestinal adverse effects in the Covid-19 patients (Table 1) (Cao et al., 2020).

4.4. Safety considerations

Various safety considerations need to be accounted for the use of azithromycin and hydroxychloroquine in the treatment or prophylaxis of Covid-19. A study reported the results of a few studies that the treatment of systemic lupus erythematosus using hydroxychloroquine led to QT interval prolongation and torsade de pointes provocation (Roden et al., 2020; Morgan et al., 2013). Hydroxychloroquine is known to bind to the hERG protein important for the electrical activity regulation in the heart which in turn explains the cardiac side effects of hydroxychloroquine (Gordon et al., 2020). Azithromycin is also known to cause severe retinopathy, arrhythmia, QT prolongation, higher risk for sudden death and to provoke polymorphic ventricular tachycardia. Risk groups for this include the females and older age groups (Gautret et al., 2020; Kim et al., 2005). FDA Perspective also supported the results of some studies that proved that the administration of azithromycin makes the patient vulnerable to prolongation of QTc interval and torsade de pointes (Mosholder et al., 2013). Various electrophysiologic studies suggested that the combinatorial effect of both hydroxychloroquine and azithromycin can provoke proarrhythmia (Zhang et al., 2017). Very limited data is available about the safety of these combination therapies. Many randomized clinical trials to study their combined effect on QT or risk of arrhythmia are being initiated to find out more about the adverse drug reactions (Roden et al., 2020).

Furthermore, various pharmacodynamic and pharmacokinetic models as well as *in vitro* studies were conducted to find out the effect of chloroquine and hydroxychloroquine on QTc prolongation to optimize the drug dosage for treatment of Covid-19. Another study predicted that higher doses of hydroxychloroquine (>400 mg twice a day) are more efficient in the reduction of transmissibility, viral suppression, and cure within 7 days. The effect of a low dose regimen could not be differentiated from that of a placebo (Garcia-Cremades et al., 2020). The viral kinetic model of another study showed a 0.006 msec increase in QT interval due to a 1nM increase in chloroquine concentration that led to an increase in the rate of viral decline (Ursing et al., 2020). Clinical Pharmacokinetics/Pharmacodynamics models showed a 50% viral load decline for plasma hydroxychloroquine concentrations estimated around 4 μM. The optimal dose for hydroxychloroquine should be below 7.5 μM as more than 1% of the patients experienced an increase in QTc interval by >60 msec. 800 mg hydroxychloroquine twice a day for 10 days has been known to be very efficient in decreasing the viral loads to 9% but is associated with significant QTc prolongation. Dosing regimens like 800 mg twice a day or 600 mg twice a day for 1–2 days followed by 400 mg twice a day or 200 mg thrice a day maintenance dose help in reaching the EC_{50} levels faster and thus, prove to be more beneficial than extending treatment duration with lower doses (Garcia-Cremades et al., 2020). Hydroxychloroquine leads to inhibition of the inward rectifier K+ channels. This is associated with the prolongation of QTc (Kazama et al., 2012). Docking studies suggest that this inhibition occurs due to the binding of nitrogen and quinoline ring present in hydroxychloroquine with the potassium channels (Rodríguez-Menchaca et al., 2008). Studies suggest that chloroquine maximum dose of 1200 mg per day for 2–6 weeks would not lead to cardiac toxicity (Munster et al., 2002). Based on this data as well as other Pharmacokinetics/Pharmacodynamics models used in the experiment, it is expected that

hydroxychloroquine 400–600 mg twice a day for 10 days or less would not lead to cardiac toxicity in patients without any QTc prolongation risk (Tisdale et al., 2013). Hydroxychloroquine may lead to other toxicities like retinopathy and gastrointestinal toxicity but doses up to 1200 mg were well tolerated in patients with rheumatologic disease as well as cancer (Munster et al., 2002). More randomized clinical trials and various Pharmacokinetics/Pharmacodynamics models are required to find the drug dosing regimens as well as toxicities and adverse drug reactions of various other repurposed drugs for Covid-19. New molecules and new drugs need to be discovered with lesser side effects to combat Covid-19.

5. Alternative antiviral drugs from medicinal plants

Due to the toxic effect of these repositioned drugs, alternative drugs are gaining rapid popularity. Earlier, polyphenols like 3'-(3-methylbut-2-enyl)-3′,4,7-trihydroxyflavane, 4-hydroxyisolonchocarpin, broussochalcone A, broussochalcone B, papyriflavonol A, kazinol A, kazinol B, kazinol F, kazinol J, and broussoflavan A isolated from *Broussonetia papyrifera* have displayed antiviral activity against SARS CoV PL^{pro} (Park et al., 2017). According to recent studies, a bioactive compound obtained from *Artemesia annua* seemed to be an appealing alternative to fight against SARS-CoV-2. However, this new drug awaits clinical trial (Haq et al., 2020). New drug targets like SARS-CoV-2 protease $3CL^{pro}$ are screened against anti-viral phytochemicals which are derived from medicinal plants and may help to combat Covid-19. A study targeted the viral protease $3CL^{pro}$ in SARS-CoV-2 which cleaves the polypeptide produced by the virus to generate various NSP required for the replication of the virus (ul Qamar et al., 2020; Anand et al., 2003). The 3D structure of $3CL^{pro}$ predicted by homology modeling consisted of 3 domains. The substrate-binding site that is situated between domains I and II has a Cys-His catalytic dyad. Based on earlier studies, a library of 32, 297 antiviral natural compounds was screened against the homology model of SARS-CoV-2 $3CL^{pro}$. The results helped to identify nine natural, non-toxic, druggable compounds that could bind to the catalytic dyad and receptor binding site of SARS-CoV-2 $3CL^{pro}$ (Table 1). 5,7,3′, 4′-Tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone, extracted from *Psorothamnus arborescens* showed a very high docking score with the highest binding affinity due to the formation of strong hydrogen bonds (Fig. 4) (ul Qamar et al., 2020). The other screened phytochemicals displayed much closer interactions with catalytic dyad residues than other drugs proposed by drug repurposing studies that target SARS-CoV-2 $3CL^{pro}$, like colistin, nelfinavir, and prulifloxacin, used as controls in the study) (ul Qamar et al., 2020; Li et al., 2020). Along with the isoflavone, two other phytochemical complexes, methyl rosmarinate extracted from *Hyptis atrorubens*, and myricitrin extracted from *Myrica cerifera* were very stable when subjected to molecular docking simulations (Fig. 4). Hydrogen bonds responsible for stabilizing interactions between proteins also remained stable throughout. In the future, *in-vivo* and *in-vitro* analyses are needed for the transformation of the potential inhibitors into clinical drugs. This would help to develop novel, non-toxic, natural anti-Covid-19 drugs in the future) (ul Qamar et al., 2020).

6. Recent advancements in diagnosis, treatment and vaccines of Covid-19

The recent spread of coronavirus SARS-CoV-2 has shown that clinical management and treatment of infected patients are lacking. Currently, the maximum mortality rate is due to respiratory failure from ARDS. The major clinical treatment consists of symptom management and oxygen therapy with mechanical ventilation for patients with respiratory failure. The convalescent plasma containing SARS-CoV-2-specific neutralizing antibodies has also been used to treat a small number of Covid-19 patients (Cao, 2020). Recently it was reported that patients with severe Covid-19 exhibit cytokine storm syndrome. Therefore, identification (decreasing platelet counts or increasing ferritin) and treatment of hyperinflammation by drug repurposing using existing, approved therapies is recommended to reduce the rising mortality (Mehta et al., 2020).

6.1. Diagnostic methods

For identification and testing, the *in vitro* diagnostic assays for Covid-19 use real-time reverse transcriptase-polymerase chain reaction (RT-PCR) that generally takes a few h and are expensive. Cepheid, USA designed a Covid-19 test kit and reduced the assay duration to 45 min. Abbott ID Now™ Covid-19 test is the point-of-care *in vitro* diagnostic assay that can detect SARS-CoV-2 in just 5 min. It uses the isothermal nucleic acid amplification technology to detect viral RNA from SARS-CoV-2 qualitatively. Immunoassays like enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay, and chemiluminescent immunoassay which help to detect immunoglobulin G (IgG) and immunoglobulin M (IgM) can also be used for Covid-19 diagnosis (Vashist, 2020). SARS-CoV-2 DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) assay is a rapid (<40 min) CRISPR–Cas12-based lateral flow assay which is pending approval and is faster than the US Centers for Disease Control and Prevention SARS-CoV-2 real-time RT–PCR assay (Broughton et al., 2020). The CovidNudge test is another sensitive and rapid point-of-care test that does not require laboratory handling or sample pre-processing. The nasopharyngeal swabs are inserted directly into a cartridge which contains the reagents required for RT-PCR reactions, including technical replicates of SARS-CoV-2 gene targets. The overall sensitivity of this test compared to the laboratory-based testing was 94% and the specificity was 100% (Gibani et al., 2020).

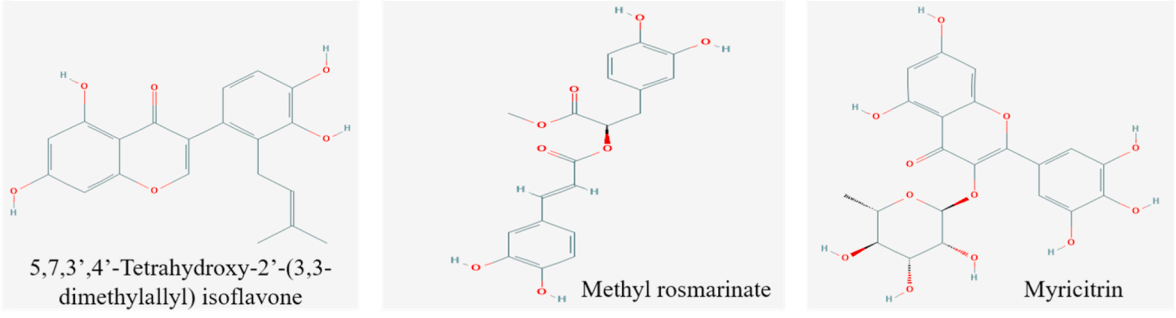

Fig. 4. Structures of non-toxic anti-viral phytochemicals screened against the homology model of SARS-CoV-2 $3CL^{pro}$, which were very stable when subjected to molecular docking simulations, due to hydrogen bonds. 5,7,3′,4′-Tetrahydroxy-2'-(3,3-dimethylallyl) isoflavone extracted from *Psorothamnus arborescens* (PubChem CID – 11610052). Methyl rosmarinate extracted from *Hyptis atrorubens* (PubChem CID - 6479915). Myricitrin extracted from *Myrica cerifera* (PubChem CID - 5281673).

6.2. Need for newer therapeutics

For the treatment of Covid-19, although various studies suggest that chloroquine can be employed because it can inhibit SARS-CoV-2 infected kidney-derived cell-line Vero cells, a recent study reported that chloroquine could not block the SARS-CoV-2 infected TMPRSS2-positive lung cell line Calu-3. This suggests that chloroquine does not target a lung cell viral activation pathway, and thus is unlikely to protect us against SARS-CoV-2 (Hoffmann et al., 2020; Haq et al., 2020). Another study presented that hydroxychloroquine exhibited antiviral activity against the virus-infected kidney cells Vero E6 but when used against SARS-CoV-2 infection in non-human primates (macaques), neither hydroxychloroquine alone nor the combination therapy of azithromycin and hydroxychloroquine showed any significant effect as a prophylactic or therapeutic agent (Maisonnasse et al., 2020). On 4 July 2020, WHO accepted the recommendation from the Solidarity Trial's International Steering Committee to discontinue the use of lopinavir/ritonavir and hydroxychloroquine arms for the trial because the interim results indicated very little or no reduction in the mortality of hospitalized Covid-19 patients when compared to standard of care. This decision does not apply to the non-hospitalized patients or in other studies of lopinavir/ritonavir and hydroxychloroquine as pre- or post-exposure prophylaxis for Covid-19 "Solidarity" clinical trial for COVID-19 treatments, 2020. Various preclinical and clinical trials are underway to test the efficacy of the different repurposed drug combination therapies for Covid-19.

Nanoparticles like liposomes, lipid-polymeric nanoparticles, dendrimers, etc. can be used for the delivery of multiple drugs with different physicochemical properties in the case of Covid-19 drug combination therapies (Chauhan et al., 2020). Formulations of dexamethasone nanomedicine may help to sustain the anti-inflammatory, anti-fibrotic, and anti-edema drug activity because nanoparticles have the potential to accumulate in the macrophages. Evidence from earlier studies about nanomedicine indicate that pulmonary delivery of the dexamethasone liposomes might be more efficient in targeting alveolar macrophages than free dexamethasone to interfere in the (sub)acute phase of Covid-19 (Lammers et al., 2020). Engineered nanocarriers can be loaded with antigenic moieties and administered orally or by intranasal routes to humans overcoming the tissue barriers and targeting the key locations like lymph nodes for effective immunization (Chauhan et al., 2020). Prophylactic antiviral CRISPR in human cells (PAC-MAN) is CRISPR-Cas13-based antiviral strategy, known to effectively degrade RNA from SARS-CoV-2 sequences (Abott et al., 2020).

6.3. Vaccines for Covid-19

Covid-19 vaccines being developed around the world are mainly of two different forms; classical vaccine platforms which include live-attenuated viruses, whole-inactivated viruses, protein subunits, and virus-like particles; as well as next-generation vaccine platforms like viral DNA vaccines, RNA vaccines, viral vectors, and antigen-presenting cells (Fig. 5A) (Van Riel and de Wit, 2020). Nanotechnology also benefits the modern vaccines because nanomaterials can mimic viral structures, act as adjuvants and are ideal for targeted antigen delivery (Shin et al., 2020). New techniques like immunoinformatics to identify potential vaccine candidates or a prefusion version of viral protein have been beneficial for Covid-19 vaccine research (Dance, 2020). However, the prefusion SARS-CoV-2 S has some stability issues which can be covalently stabilized by an intermolecular disulfide bond in a closed conformation to enhance SARS-CoV-2 S resistance to proteolysis and thus, it can be used in vaccine design, serology, and immunology studies (McCallum et al., 2020). Covid-19 vaccines developed using various platforms are taking much less time in the various development stages when compared to the classical vaccines developed earlier for other diseases (Fig. 5B) (Calina et al., 2020). As of 8 January 2021, out of hundreds of vaccines developed by researchers around the world, 172 vaccines are under preclinical trials and 63 vaccines are undergoing human clinical trials to produce a safe and effective vaccine at the earliest to combat Covid-19 (Available at who.int). In the US, the National Institutes of Health (NIH) and Moderna Inc's experimental mRNA vaccine mRNA-1273 encoding the virus spike protein to attach to the human host, showed promising human clinical trial results, therefore it was approved for use in Canada and has emergency use authorization in the US, Israel and the European Union (COVID-19 therapies and vaccine landscape, 2020) In mRNA-1273, prefusion-stabilizing mutations improved the immunogenicity and induced immune response against both wild-type (D614) and mutant (D614G) SARS-CoV-2 (Corbett et al., 2020). Moderna's mRNA vaccine incorporates nanotechnology since it is based on a lipid nanoparticle platform (Shin et al., 2020). AstraZeneca's coronavirus vaccine ChAdOx1 nCoV-19 (AZD1222) based on chimpanzee adenovirus, ChAdOx1 developed by the University of Oxford which is undergoing Phase III human clinical trials at most places, has shown promising results in phase I and II trials with peaked spike-specific T-cell responses by the 14th day and anti-spike IgG responses by day 28. Neutralizing antibody responses were seen in 100% participants after the second dose of the vaccine. This vaccine has emergency use authorization in United Kingdom, Argentina and India (Draft Landscape of COVID-19 Candidate Vaccines, 2020; Coronavirus Vaccine Tracker, 2020; Folegatti et al., 2020). COVAXIN^{TM} or BBV152, India's first Covid-19 vaccine developed by Bharat Biotech in

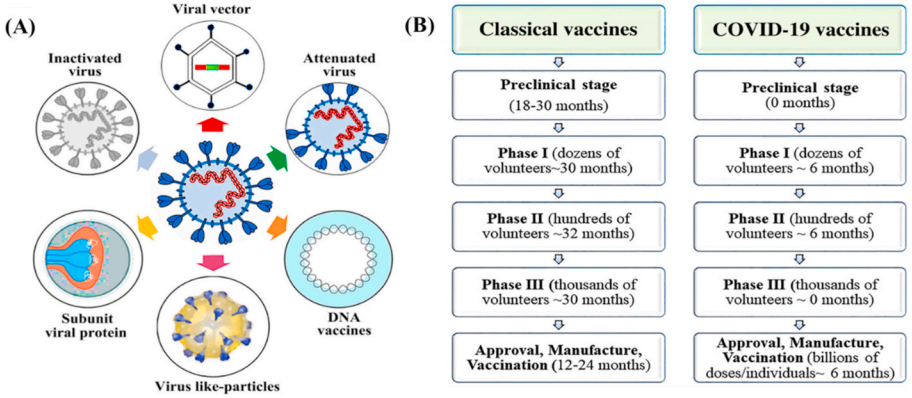

Fig. 5. A) A representation of the classical vaccine platforms (live-attenuated viruses, whole-inactivated viruses, protein subunits, and virus-like particles) and the next-generation vaccine platforms (viral DNA vaccines, viral vectors) used for Covid-19 vaccine development for humans. The figure is adapted from reference (Calina et al., 2020). **B)** Stages of development of Covid-19 vaccines compared with classical vaccines. The figure is adapted from reference (Calina et al., 2020).

collaboration with the National Institute of Virology (NIV) and Indian Council of Medical Research (ICMR) was approved by the Drug Controller General of India (DCGI) for phase III human clinical trials and has emergency use authorization in India. It is a whole-virion inactivated vaccine which means it cannot replicate in humans but will trigger an antibody response (Draft Landscape of COVID-19 Candidate Vaccines, 2020; Coronavirus Vaccine Tracker, 2020). Among these, Comirnaty or BNT162 which is a RNA-based vaccine with 3 lipid nanoparticle platform-mRNAs developed by Pfizer and BioNTech, was the first Covid-19 vaccine to receive emergency use authorization in the US by the FDA. It is approved for use in many countries like Canada, Switzerland and Bahrain (Draft Landscape of COVID-19 Candidate Vaccines, 2020; Coronavirus Vaccine Tracker, 2020).

In the future, research has to be carried out in different parts of the world using various *in vitro*, *in vivo,* and *in silico* models to study viral replication and pathogenesis. UV-C light of 200–230 nm wavelength can be used to limit virus transmission in indoor spaces (Garcia de Abajo et al., 2020). Various imaging and molecular diagnostic tools like atomic-force microscopy, fluorescence microscopy, electron microscopy, and other computational biology tools can be used to study SARS-CoV-2 efficiently and treat Covid-19 (Kaniyala Melanthota et al., 2020).

7. Conclusion

The emergence of SARS-CoV-2 from China in December 2019 led to the Covid-19 pandemic which has affected millions of people all over the world. This disease leads to pneumonia and ARDS which may progress to multi-organ failure and ultimately death. Various studies identified the PPIs between the ORFs and NSPs of SARS-CoV-2 and human proteins, the viral proteases which can be targeted, and other novel viral targets using AP-MS, cheminformatic analyses, and indirect immunofluorescence assays. These viral proteins and their interactions could help us to understand the effect of SARS-CoV-2 on human cellular metabolism as well as to identify and formulate various drugs and vaccines to combat Covid-19. Various *in silico* studies on cheminformatics software, *in vitro* studies on lung and kidney cell lines, and *in vivo* studies in model organisms and humans (Covid-19 patients) were performed to test the efficiency of various repurposed drugs and their combination therapies. Toxicity testing was also done for the repurposed drugs identified to find out the adverse drug reactions as well as to formulate the drug dosage regimens of the drugs and their different combinations. Due to cardiac toxicities and QTc prolongation caused due to a few repurposed drugs, a library of new molecules like anti-viral phytochemicals was screened against the homology model of SARS-CoV-2 $3CL^{pro}$ by molecular docking simulations. The availability of these prophylactic and therapeutic drugs and their safety is of great concern for the treatment of Covid-19. Randomized controlled trials are necessary to test the efficacy and safety of the drugs identified by *in silico* and *in vitro* studies. Inadequate knowledge about the true number of affected individuals in developing countries due to Covid-19 tests being done only on individuals with strong symptoms is another major concern. Time-efficient and cost-effective Covid-19 diagnostic assays like *in vitro* diagnostic assays using RT-PCR and immunoassays need to be developed and used worldwide to fight the pandemic. Newer diagnostic methods, novel anti-viral drug research, classical and next-generation vaccines being developed around the world are the only ways to combat Covid-19. Newer techniques like immunoinformatics will be helpful to identify potential vaccine candidates and we shall be able to combat the pandemic in a much better and efficient way in the future. The large-scale and efficient production of vaccines, the duration of antibody response to the vaccine, as well as lack of masks, gloves, and ventilators in the underdeveloped and developing countries, is another major concern which the scientific and the medical community has to face. However, a thorough understanding of the current situation and development in the research regarding the effect of SARS-CoV-2 on human cellular metabolism, the repurposed drugs and their toxicities as well as the various vaccine candidates is necessary to accelerate the research for the development of a novel anti-SARS-CoV-2 drug and an efficient vaccine.

Declaration of competing interest

The authors declare no conflict of interest.

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