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Review

Combination Therapies against COVID-19

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of Coronavirus disease 2019 (COVID-19), which was announced as a pandemic leading to devastating economic and medical burden worldwide. The virus attacks the organ system across the body by binding to its receptor (for example, angiotensin converting enzyme 2) on the surface of the host cell of various organs. The patients present with a variety of pathological symptoms ranging from fever, cough and cytokine storm to acute respiratory distress syndrome (ARDS). Many combination therapies have been developed to combat the disease, via blocking one or more processes of the viral life cycle and/or relieving host complications simultaneously. In this review, the progress of those combination therapies containing at least one small molecule is updated. We believe it'll provide significant inspiration for further development of treatment strategy against SARS-CoV-2, especially its mutant variants.

Keywords: combination therapy; anti-SARS-CoV-2; complication; antiviral; anti-inflammation

1. SARS-CoV-2 Life Cycle

COVID-19 is an infectious disease caused by SARS-CoV-2 virus. It had killed more than 6.12 million of persons worldwide as of 29 March 2022. While the majority of patients with COVID-19 will recover without special treatment, a minority will manifest severe symptoms requiring hospitalization and even suffer serious complications, including ARDS, which may induce multi-organ dysfunction. Considering SARS-CoV-2 is a unprecedented virus and radical treatment against the resulting disease remain to be developed, a clear interpretation of the viral life cycle is essential for designing prophylactic and/or therapeutic strategies that target one or more processes of its life cycle. SARS-CoV-2 affects human starting from entering the nasopharynx by attaching to angiotensin converting enzyme 2 (ACE2) receptor-enriched epithelial cells of the nasal and oral mucosa. Then it goes down and attacks the lung, where the infection mainly takes place. The following processes include endocytosis, replication, transcription, assembly and release of virus [1].

SARS-CoV-2 invades human cells through interaction of the receptor binding domain (RBD) of its spike (S) protein with the host ACE2 on the cell surface [2]. The SARS-CoV-2 S protein belongs to class 1 viral fusion protein and it means that the S protein should be cleaved into S1 and S2 subunits by a fusion protein before functioning normally. S1 contains the receptor binding domain (RBD), which directly binds to the peptidase domain (PD) of ACE2, whereas S2 is responsible for the fusion between the viral envelope and the host cell membrane and internalization by endocytosis with ACE2 [3,4]. Various proteases including cathepsins, trypsin, human airway trypsin-like proteases, furin and transmembrane protease serine protease (2/4) (TMPRSS) have been reported to cleave S protein in a proteolytic way in those harmful coronaviruses since 2003 [5–8]. That a specific furin-like cleavage site was discovered in the S-protein genome sequence of SARS-CoV-2 indicates furin and furin-like proteases may be involved in the S protein cleavage [9]. We have known that SARS-CoV-2 uses TMPRSS2 as well as furin or furin-like proteases, for its interaction with the ACE2 receptor and entry into the host cell. The study by Hoffmann *et al*. [2] proposed a rational role of TMPRSS2 in proteolysis of S protein to S1 and S2 subunits for S protein priming and camostat mesylate, an inhibitor of TMPRSS2, blocked SARS-CoV-2 infection of lung cells. Furthermore, the data showed that both furin and TMPRSS2 could not replace each other functionally and suppression of either of them might interfere virus to bind to host cells. Indeed, many experimental pieces of evidence recently have made clear that these two proteins act synergistically in viral entry and infectivity and shed light on the combination of furin and TMPRSS2 inhibitors as potent antivirals against SARS-CoV-2 [10]. Further processing that the S1 subunit is removed in host cell endosomes is promoted by cathepsins, which eventually assists the fusion of viral envelope with the host membranes, viral RNA release, and replication [11]. Overall, virus S protein, ACE2 and the host proteases play the essential roles in SARS-CoV-2 entry in host cells. Other than ACE2, several other receptors were also discovered including DC-SIGN (also known as CD209), L-SIGN (also known as CD209L or CLEC4M), SIGLEC1 (also known as CD169, sialoadhesin or Siglec-1) [12,13] and neuropilin-1 (NRP1, known to bind furin-

cleaved substrates) [14].

In case of the release of the viral genome into the host cell, the SARS-CoV-2 RNA acts as messenger RNA (mRNA) and undergoes translation by using host cell machinery [15]. The viral genome comprises a positive-sense, single-stranded RNA molecule with about 29,900 nucleotides which encode about 31 proteins: nonstructural proteins (NSPs; 16 proteins), structural proteins (4 proteins), and accessory proteins (11 proteins) [16]. The structural proteins consist of S protein, envelope (E) and membrane (M) proteins which form the viral envelope, and nucleocapsid (N) protein which connects with the virus genome. After entering host cell, the NSPs domain is first translated into two polypeptides, both of which are further processed to produce papain like protease (PLpro) (NSP3), main protease (Mpro) (also known as 3-chymotrypsin-like protease (3CLpro); NSP5) [17], and RNA-dependent RNA polymerase (RdRp; NSP12) [18]. Interestingly, the process is promoted by host proteases at the first stage, and then, is strengthened by PLpro and Mpro. The viral RdRp is key for the replication of genetic material. The viral genome and the N structural protein are produced in the host cell cytoplasm, while other viral structural proteins including S, M, and E are finally synthesized in the endoplasmic reticulum and transferred to the Golgi apparatus. The viral RNA–N complex and S, M, and E proteins are then packaged together to form a virion by a budding process. Following packaging, the complete virus particles are transported to the cell surface in vesicles and released by exocytosis [19,20]. These newly released virions continue their life cycle in other healthy cells.

2. Concomitant Symptoms Following Virus Infection

The shortest and the longest average incubation period of SARS-CoV-2 in China are 1.8 days and 12.8 days, respectively, mostly ranging from 3 to 7 days [21,22]. As those with SARS and MERS, most patients with COVID-19 have a specific ground glass appearance on chest computed tomographic (CT) scans [23]. Besides, patients with COVID-19 exhibit similar biopsy features to that seen in SARS-CoV and MERS-CoV patients [24]. Presentations of SARS-CoV-2 frequently emerged include fever (>37.3 °C), coughing, fatigue, muscles ache, runny nose, haemoptysis, headache, diarrhea, and shortness of breath [25]. Most patients showed mild and moderate symptoms, however, some cases in severe condition may suffer ARDS, multi-organ failure, and even death [26]. The statistic data revealed that COVID-19 mortality ranges from 1.4% [27] to 4.3% [28] in different areas or hospitals.

As aforementioned above, SARS-CoV-2 enters into host cells via binding to the receptor ACE2. ACE2 exists generally in many organs and tissues, including nasal epithelium, oral mucosa, kidney, brain, lungs, etc., which indicates SARS-CoV-2 can attack multiple organs aside from the lung [29]. Indeed, SARS-CoV-2 causes a wide variety of symptoms across organ systems in patients with COVID-19. The involvement of the lungs by SARS-CoV-2 might result in ARDS, requiring intubation and admission to the intensive care unit. Individuals with life-threatening SARS-CoV-2 disease demonstrate related cytokine release syndrome (CRS) [30]. CRS appears to be a hazardous factor involved in different inflammatory pathways hastening lung parenchymal impairment and thromboembolism [31]. Severe lung involvement and mortality may be predicted early through lymphocytopenia and elevated signs of inflammatory factors [32]. Particularly, neurogenic pulmonary edema can be seen in invalids with extreme COVID-19 pneumonia. It is also defined as a non-cardiogenic interstitial pulmonary edema with a distribution of the peripheral lung zone that can be found in viral pneumonia [33].

SARS-CoV-2 is a potent inducer of inflammatory cytokines. The virus can activate immune cells and induce the secretion of inflammatory cytokines and chemokines directing to pulmonary vascular endothelial cells. Surge of cytokines can cause a cytokine storm and extensive inflammation all over the body of patients. Organ damage following SARS-CoV-2 invasion may be attributed to the cytokine storm or cytokine cascade [34]. Although respiratory failure from ARDS is the utmost reason for death, several death cases were reported to die from circulatory failure as a result of myocardial damage, which indicated the infection of SARS-CoV-2 might cause fulminant myocarditis [35]. Neurological syndromes caused by COVID-19 include anosmia and dysgeusia, ataxia, headache, dizziness, and unconsciousness [36]. The neuron-invading potency and possible role of SARS-CoV-2 in the patients with acute respiratory failure was first proposed by Li *et al*. [37]. Although COVID-19 is characterized by respiratory presentations, the relative virus can also attack the alimentary system. Most patients suffer diarrhea, nausea, or vomiting [38]. SARS-CoV-2 is also considered a causative agent for thyroiditis or thyrotoxicosis. And SARS-CoV-2 may be a potential trigger for autoimmune thyroid disease [39]. Once infected by SARS-CoV-2, various key organs across the body can be attacked, and possibly result in multiple organ involvement. More complications about this viral infection can be read in another review [33].

3. Combination Therapy as a Treatment Strategy Blocking One or More Processes of the Viral Life Cycle and Relieving the Complications

The whole viral lifetime involves attachment and entry to the host cell, translation, replication and release, during which time the patients manifest various symptoms ranging from cough to cytokine storm, even multi-organ dysfunction, etc. In order to combat the disease COVID-19 efficiently, not only the stages of the viral life cycle, but the complications should be considered for potential ther-

apeutic intervention. As long as immunologic complications like macrophage activation syndrome (MAS) occur, anti-viral monotherapy is not enough and additional anti-inflammatory treatment should be added. Early detection and proper treatment of MAS and cytokine storm will reduce the incidence and mortality in COVID-19 patients. Therefore, a combination therapy with dual or multiple drugs encompassing one or more targets could be given more favor. Ideally, the combined use of drugs should have at least dual functions: inhibiting or killing the virus and relieving the complicated symptoms of infected patients. The former function is performed by anti-viral drugs which block RNA synthesis and virus invasion, and bind to receptor proteins on the surface of cells, and cell cycle protein, etc. The latter function is served mainly by anti-inflammatory drugs which control cytokine production, break down the basement membrane, regulate outer mitochondrial membrane permeability, stimulate activated B-cell and T-cell proliferation, etc. Other drugs serving the latter function include anti-oxidant, immunomodulator and relating symptom-relieving drugs.

Combination therapies have the advantage to improve treatment efficiency while decreasing concentration of individual drug. For example, remdesivir at 5.05 μM combined with omipalisib at 0.25 μM exhibited 79% viral inhibition, while the concentrations of single drug, remdesivir or omipalisib to achieve the same inhibition rate were about 20.18 μM or 1.97 μM, respectively [40]. And the lower drug concentration may reduce the adverse effect of each drug emerged when used alone. The limited efficacy of some drugs may also be enhanced by combination therapy. Furthermore, if the drugs selected for combined use target different objects (exhibit different mode of actions), combination of these drugs might enhance the overall activity by simultaneously engaging two or more pathways [40].

Drug combination therapy has been proved useful in treating virus infection disease [41]. Recently it also showes some application potential on COVID-19 patients. Some studies demonstrate that combination therapy for COVID-19 outpatients might decrease hospitalization and death by 89%. Particularly, in some cases, combination therapy displays excellent outcome. COVID-19 patients sometimes may suffer several devastating conditions, such as cytokine cascade, organ damage, and thrombosis. McCullough and co-authors recommended that combination therapy should be a vital standard for management of those with these devastating conditions [42]. It is exciting that combination therapy is effective against some new SARS-CoV-2 variants, which makes this treatment strategy invaluable especially when the SARS-CoV-2 virus presents fast-mutating characteristics.

In this review, we aim to summarize the outcome of the combination therapies against COVID-19. We focus on the combination therapies that contain at least one small molecule, such as remdesivir, umifenovir, or hydroxychloroquine (HCQ). While those combination therapies with both or more biomacromolecules, like antibody, nanobody, convalescent plasma or some other therapeutic proteins like interferon, are not included. You may read another relative review for this kind of combination therapies [43]. We deem that this review would provide an option for the scientific and rational therapeutic alliance against COVID-19.

4. Progress of Combination Therapy with at Least One Small Molecule

4.1 Combination Therapy with Small Molecules

In this section, we will summarize those combination therapies with two or more small molecules. Each combination therapy may comprise two virus-directed antivirals, one virus-directed antiviral and another host-directed, one antiviral and another complication-relieving drug (anti-inflammatory, antioxidant, etc.), one antiviral and its pharmacokinetic enhancer, or other antimicrobials, etc. And according to the composition, they are categorized to 6 groups, all of which will be presented in the next 6 chapters, respectively. Many studies of combination therapy involve remdesivir, so we give a brief introduction of it first. Remdesivir is a nucleotide analog and the high resemblance between its triphosphate form and adenosine triphosphate (ATP) enables it to function as a competing inhibitor of RNA synthesis (RdRp inhibitor). Remdesivir shows great potential in inhibting all the coronaviruses including SARS-CoV-2 [44]. Beigel *et al.* [45] completed a double-blind trial and they found that about half patients (total 1062 participants) treated with remdesivir had a shorter recovery time. Their data also indicate that remdesivir may prevent deterioration of the disease. Given the positive results of the trial, remdesivir became the first antiviral to be authorized by the Food and Drug Association for emergency use for hospitalized adult patients at the risk of serious illness [46]. Following the approval, the clinical performance of the drug is also closely supervised and updated with the new evidence [47]. It was found that remdesivir could lead to renal failure or liver dysfunction during therapeutic process of COVID-19 [48,49]. Furthermore, a solidarity trial guided by the World Health Organization (WHO) demonstrated remdesivir had little or no benefit on hospitalized patients with COVID-19 [50]. Thus, on 20 November 2020, WHO recommended against its use in spite of state of illness of hospitalized patients. Nonetheless, recently, on 22 April 2022 WHO updated the conditional recommendation for the use of remdesivir in patients with non-severe COVID-19 at the highest risk of hospitalization. There have been many clinical trials on remdesivir in a completed, terminated or recruiting stage, which were designed in combination with other agents such as Interferon beta-1b, Interferon beta-1a, Tocilizumab, Lopinavir/Ritonavir, Merimepodib, DWJ1248, baricitinib, or dexamethasone

Table 1. Combination therapies with antivirals acting on SARS-CoV-2.

Drugs for Combination	Targets or action mechanisms	Results	Reference
MG-101 + Sitagliptin	Mpro + PLpro	Improved the antiviral effect against SARS-CoV-2 Delta variant	[51]
MG-101 + Lycorine or Nelfinavir	Mpro	Enhanced anti-SARS-CoV-2 activity	[51]
GC376 + remdesivir	Mpro + RdRp	Additive antiviral activity	[52]
Molnupiravir + nirmatrelvir	RdRp + Mpro	Synergisitic antiviral activity	[53]
An indole derivative+ remdesivir	Mpro	Synergistic activity	[54]
Corilagin + remdesivir	RdRp (with different mechanisms)	Additive inhibitory effect	[55]
SOF + DCV	RdRp	Clinical recovery rate was increased and hospitalization length reduced	[56]
SOF + DCV; SOF + DCV + ribavirin	RdRp	Favoring this combination therapy	[57,58]
VEL + SOF + the national standard of care*	Mpro + RdRp	(1) Safe; (2) did not improve the clinical status or reduce mortality	[59,60]
Linoleic acid (LA) + remdesivir	SARS-CoV-2 S glycoprotein	Synergistic in inhibiting SARS-CoV-2 replication	[61,62]
Glycyrrhizin (GR) + boswellic acid (BA)	SARS-CoV-2 S glycoprotein	(1) Reduction of systemic inflammation; (2) reduced risks of hospitalization and mortality, being safe, well tolerated, and widely available	[63–65]
Cepharanthine (CEP) + nelfinavir (NFV)	SARS-CoV-2 S protein + Mpro	Synergistic to limit SARS-CoV-2 proliferation	[66]
NAC + CBS or BSS	Cysteine enzymes (proteases) including PLpro, Mpro, helicase (Hel) and ACE2	Significantly diminished the viral load of lung and the pathologic condition	[67]

* The national standard of care comprises hydroxychloroquine and lopinavir/ritonavir as well as supportive care.

(https://www.clinicaltrials.gov/ct2/results?cond=COVID19&term=remdesivir&cntry=&state=&city=&dist=).

4.1.1 Antivirals Acting on SARS-CoV-2

SARS-CoV-2 proteases Mpro and PLpro are promising targets for antiviral drug development for their vital roles in coronavirus replication. MG-101, a Mpro inhibitor, significantly improved the antiviral activity against SARS-CoV-2 Delta variant, when combined with PLpro inhibitor Sitagliptin. Similarly, enhanced anti-SARS-CoV-2 activity was also observed in combination therapy of two Mpro inhibitors, such as MG-101 plus Lycorine HCl or Nelfinavir. MG-101, combined with Lycorine HCl or Nelfinavir mesylate, led to a 3–4 log reduction in virus titer at 1 μM concentration of each drug compared with blank group. Overall, these experimental data suggest that the double suppression of Mpro and PLpro or suppressing the same protease with multiple drugs is an attractive approach to combat COVID-19 [51] (Table 1, Ref. [51–67]). GC376, a pre-clinical inhibitor against feline infectious peritonitis (corona) virus (FIPV), was shown to be a potent inhibitor of Mpro in Vero cells. Moreover, combination of GC376 with remdesivir could reinforce antiviral activity, indicating additive effect of the combination of RdRp inhibitor and protease inhibitor (Table 1) [52]. Molnupiravir, a prodrug of the nucleoside derivative N-hydroxycytidine (NHC), targets viral RNA polymerase and becomes the first authorized oral antiviral for COVID-19, though some mild adverse effects emerged including nausea, diarrhea, headache and insomnia, etc. [68,69]. Most recently, it was found to exhibit synergisitic antiviral activity against Omicron infection in Calu-3 cells when combined with nirmatrelvir, a SARS-CoV-2 Mpro inhibitor [53]. Hattori *et al.* [54] discovered a small molecule compound with an indole moiety targeting Mpro. This compound blocked virus replication without viral breakthrough and exerted synergistic anti-SARS-CoV-2 activity in combination with remdesivir. Corilagin is an ellagitannin with a hexahydroxydiphenoyl group bridging over the 3-O and 6-O of the glucose core, as a medicinal herbal agent found in Euphorbia fischeriana, Euphorbia hyssopifolia, and other organisms. It was reported to function as non-nucleoside inhibitor of SARS-CoV-2 RdRp, possibly via inhibiting the conformational change of RdRp, a different mechanism compared with remdesivir. When combined with remdesivir, it exhibited additive inhibition against SARS-CoV-2 RdRp [55]. Sofosbuvir (SOF) and daclatasvir (DCV) are clinically approved direct-acting RdRp inhibitors against hepatitis C virus (HCV). Some *in silico* and *in vitro* studies suggest that SOF and DCV also have high affinity for SARS-CoV-

2 RdRp. While other studies show little or no effect on preventing SARS-CoV-2 infection. Recently, Sadeghi *et al*. [56] reported promising outcomes in a clinical trial using the combination therapy SOF/DCV on moderate or severe COVID-19 patients. They found that SOF/DCV treatment increased 14-day clinical recovery rates and reduced hospitalization length compared with standard care alone [56]. Two similar SOF/DCV clinical trials, though in notably small scale, were also performed and provided preliminary data favoring the SOF/DCV combination or SOF/DCV/ ribavirin triple therapy [57,58]. Velpatasvir (VEL) is known as an inhibitor of HCV NS5A protein. Recently it was also reported to be tailored to A chain and B chain active sites of the SARS-CoV-2 3CLpro [59]. A single-center, randomized controlled trial study was conducted to evaluate the efficacy of the SOF/VEL combination plus the national standard of care (hydroxychloroquine and lopinavir/ritonavir as well as supportive care) in patients with moderate to severe COVID-19 illness. And the outcome showed SOF/VEL was safe, however, combining SOF/VEL with the standard of care did not provide any benefit for the clinical improvement or mortality reduction [60]. Linoleic acid (LA) is an inflammatory response modulator, isolated from the traditional meal *Vicia faba* [61]. Toelzer *et al*. [62] resolved a 2.85-angstrom cryo–electron microscopy structure in which the receptor binding domains of SARS-CoV-2 S glycoprotein tightly binded LA in three composite binding pockets. LA binding stabilized a locked S conformation, leading to reduced ACE2 interaction *in vitro*. In human Caco-2 ACE2+ cells, LA supplementation synergized with remdesivir in inhibiting SARS-CoV-2 replication [62]. Recently, Li *et al*. [63] experimentally proved that glycyrrhizin (GR) inhibited SARS-CoV-2 infection through interaction with S protein and blocks attachment of recombinant S protein to host cells. Also, boswellic acid (BA) is reported to exhibit a high affinity for the functional S protein of SARS-CoV-2 [64]. A randomized clinical trial was conducted to explore the effect of GR/BA versus placebo on hospitalized patients with moderate SARS-CoV-2 infection. And a potent decline in serum C-reactive protein levels was observed in the GR/BA group in comparison with the placebo group, which reflected reduction of systemic inflammation by GR/BA treatment. Though many superior profiles were still observed including the reduced risks of hospitalization and mortality, credible safety, good tolerance, and extensive availability, with this combination, it's limited by lack of group receiving GR or BA alone to compare with the combination group (ClinicalTrials.gov number, NCT04487964) [65]. The anti-inflammatory drug cepharanthine (CEP) and human immunodeficiency virus (HIV) protease inhibitor nelfinavir (NFV) were discovered more potent than remdesivir and other drugs currently in clinical trial through screening a group of authorized drugs in a SARS-CoV-2 infection cell assay. Further study demonstrated that cepharanthine inhibited SARS-CoV-2 entry through interfering with S protein engagement to its ACE2 receptor, while nelfinavir suppressed viral replication partly by inhibition of SARS-CoV-2 main protease. Consistent with their different modes of action, synergistic effect of this combination therapy (CEP/NFV) to limit SARS-CoV-2 proliferation was highlighted over a wide range of concentrations [66]. Bismuth drugs colloidal bismuth subcitrate (CBS) or bismuth subsalicylate (BSS), usually used in the treatment of gastroduodenal disorders, in combination with *N*-acetyl-L-cysteine (NAC) significantly diminished the viral load of lung and the pathologic condition in Syrian hamster infection model. The mechanism of this combination involved NAC plays a vital role in preventing the hydrolysis of bismuth drugs *via* forming stable coordination compound [Bi(NAC)3], and optimizing the pharmacokinetic profiles of CBS. Besides, the cocktail also demonstrated broad-spectrum antiviral activities against key viral cysteine enzymes/proteases such as PLpro, Mpro, helicase (Hel) and ACE2 (Table 1) [67].

4.1.2 Antivirals Acting on SARS-CoV-2 and Host Cell

As aforementioned, some host proteases facilitate SARS-CoV-2 entry in host cell and the virus rely on host translation system for replication, thus it's also vital to block host relative protease and translation associated enzymes for combating SARS-CoV-2. IMU-838, a developmental dihydroorotate dehydrogenase (DHODH) inhibitor in phase II for autoimmune disease, showed enhanced *in vitro* anti-SARS-CoV-2 activity when combined with remdesivir [70] (Table 2, Ref. [2,40,70–88]). Biering *et al*. [71] identified compound B02, a human RAD51 inhibitor, exhibiting antiviral synergy with remdesivir after screening a library of FDA-approved and well-studied preclinical and clinical chemicals (Table 2). Besides, a synergy between remdesivir and emetine (an anti-protozoan drug against ameobiasis) was noticed that remdesivir at 6.25 μM in combination with emetine at 0.195 μM may achieve 64.9% inhibition in viral yield [72]. Baricitinib, a Janus kinase inhibitor, plus remdesivir showed better outcomes than remdesivir alone in reducing recovery time and accelerating clinical improvement among COVID-19 patients. Furthermore, severe adverse events were also reduced in the combination group (ClinicalTrials.gov number, NCT04401579) [73]. A living guideline from the WHO published 14 January 2022 strongly recommended the use of baricitinib as an alternative to interleukin-6 (IL-6) receptor blockers, in combination with corticosteroids, in patients with severe or critical COVID-19 (https://www.who.int/teams/health-care-readiness/covid-19/therapeutics). Of note, baricitinib, used alone or as a combination with remdesivir, may bring about some adverse effects, such as liver injury [89] and transient leukocytopenia [90]. Tipifarnib is a mighty farnesyltransferase inhibitor with the potential as an anticancer remedy and has completed phase III clinical trial [74]. Omipalisib (also termed GSK2126458) is a powerful in-

Table 2. Combination therapies with antivirals acting on SARS-CoV-2 and host cell.

Drugs for Combination	Targets or action mechanisms	Results	Reference
IMU-838 + remdesivir	DHODH + RdRp	Enhanced *in vitro* anti-SARS-CoV-2 activity	[70]
B02 + remdesivir	Human RAD51 + RdRp	Antiviral synergy	[71]
Emetine + remdesivir	A protein synthesis inhibitor + RdRp	64.9% inhibition in viral yield	[72]
Baricitinib + remdesivir	Janus kinase + RdRp	Reducing recovery time and accelerating improvement	[73]
Baricitinib + corticosteroids	Janus kinaser + anti-inflammation	Strongly recommended by WHO	https://www.who.int/teams/health-care-readiness/covid-19/therapeutics
Tipifarnib + Omipalisib (GSK2126458)	Farnesyltransferase + phosphoinositide 3-kinases	Strong synergistic effects	[40,74,75]
Omipalisib + remdesivir; tipifarnib + remdesivir	Phosphoinositide 3-kinases + RdRp; Farnesyltransferase + RdRp	Strong synergistic effects	[40]
Calpeptin + remdesivir	Cysteine proteinase + RdRp	Enhanced the anti-SARS-CoV-2 activity	[76]
Lenvatinib + remdesivir	Host RTK + RdRp	Exhibited striking synergistic effect	[77]
Camostat + enzalutamide or ARD-69	TMPRSS2 serine protease + antiandrogen or androgen receptor degrader	More efficacious in blocking the entry	[78]
Raloxifene + tilorone	A heparin/HS-binding drug + a pan-antiviral agent	Synergistic against SARS-CoV-2-induced cytopathic effect	[79]
Fluoxetine + GS-441524	Acid sphingomyelinase + RdRp	Synergistic antiviral effect	[80]
HCQ + azithromycin	An alkalinizing lysosomatropic drug + antibiotic	(1) Synergic; (2) a better clinical status and a quicker virus eradication; (3) no clinical benefit for the treatment of the hospitalized patients with severe COVID-19; (4) a greater QT interval	[81–84]
HCQ + lopinavir + ritonavir	An alkalinizing lysosomatropic drug + anti-HIV drug	Minimal *in vitro* antiviral activity	[85]
HCQ + zinc supplements	An alkalinizing lysosomatropic drug + an essential micronutrient	No additive effect	[86]
MEDS433 + dipyridamole (DPY)	DHODH + the pyrimidine salvage pathway	Restored the anti-SARS-CoV-2 activity of MEDS433	[87]
Camostat mesylate + E-64d	TMPRSS2 + endosomal cysteine proteases cathepsin B and L	Complete inhibition of the SARS-CoV-2 cell entry	[2,88]
Camostat mesylate + HCQ	TMPRSS2 + an alkalinizing lysosomatropic drug	Relative clinical trials have been withdrawn or in an unknown status	NCT04355052, NCT04338906

hibitor of phosphoinositide 3-kinases and mammalian target of rapamycin, and is being developed for curing solid tumors [75]. Jang *et al.* [40] found three combination therapies (omipalisib/remdesivir, tipifarnib/omipalisib, and tipifarnib/remdesivir) demonstrating strong synergistic effects in curbing SARS-CoV-2, through virtual screening of 6, 218 drugs and cell-based assay. Calpeptin exhibited high antiviral activity against SARS-CoV-2 without apparent cytotoxicity via blocking extracellular vesicles (EVs) biogenesis/release as a cysteine proteinase inhibitor. Interestingly, a cocktail of calpeptin and remdesivir significantly enhanced anti-SARS-CoV-2 activity in comparison with monotherapy [76]. Lenvatinib, as a broad-spectrum host receptor tyrosine kinase (RTK) inhibitor, showed no inhibitory activity against Mpro *in vitro*, but exhibited remarkable synergistic effect with remdesivir to suppress SARS-CoV-2 replication, albeit selectively in Vero-CCL81 cells. Moreover, time-of-addition experiment revealed that lenvatinib/remdesivir combination remedy probably targeted SARS-CoV-2 replication process at a post-entry step [77]. Androgen was reported to function as a transcriptional regulator of ACE2 and TMPRSS2 in mouse and hu-

man cells. Notably, the combination of camostat (a TMPRSS2 serine protease inhibitor) with anti-androgen enzalutamide or androgen receptor degrader ARD-69 was more efficacious in blocking the entry of pseudotype SARS-CoV-2 into the host cells than the single drug [78]. Zhang *et al*. [79] have shown that heparin/heparan sulfate (HS) binds directly to S protein and promotes the binding of viral particles carrying S protein to the cell surface to assist viral entry. Tilorone used to be a pan-antiviral agent and also prevented SARS-CoV-2 infection *in vitro*. Raloxifene, a heparin/HS-binding drug, in combination with tilorone, was found to be synergistic against cytopathic issue induced by virus infection. Furthermore, no notable cytotoxicity emerged in the combination regimen even at highest concentrations [79]. Since it was established that SARS-CoV-2 infection could induce the activation of tissue factor-mediated coagulation via activation of acid sphingomyelinase [91], and blockage of acid sphingomyelinase prevented uptake of SARS-CoV-2 by epithelial cells [92], targeting the acid sphingomyelinase is a promising strategy to combat COVID-19. Fluoxetine and fluvoxamine are well studied inhibitors of acid sphingomyelinase and show great therapeutic potential for SARS-CoV-2 infection [93–95]. Remarkably, fluoxetine, in combination with GS-441524 (a plasma metabolite of remdesivir), exerted synergistic antiviral effects against different SARS-CoV-2 variants *in vitro* [80]. Despite its small sample size, an open-label non-randomized clinical trial showed that HCQ (an alkalinizing lysosomatropic drug) treatment was extremely efficient for virus clearance in COVID-19 patients and it is synergic when combined with azithromycin [81]. Similarly, a retrospective analysis of larger scale samples (3119 samples, 83.5% of 3737) suggested that early treatment with at least 3 days of HCQ/azithromycin led to a better clinical status and a quicker virus eradication than other treatments [82]. However, Molina *et al*. [83] pointed out that the combination of HCQ and azithromycin brought no clinical benefit for the severe COVID-19 patients. Notably, treatment with azithromycin combined with HCQ caused a greater QT interval prolongation than treatment with either drug alone [84]. Since the addition of azithromycin doesn't increase the activity of HCQ, the combination was no longer recommended as treatment or prophylaxis for COVID-19 by the WHO. Further, Kang *et al*. [85] reported that the *in vitro* antiviral activity of HCQ at concentrations with little concern of toxicity was minimal, no matter it was used alone or with lopinavir/ritonavir. A randomized, multicenter trial was conducted to probe whether Zinc supplements can enhance the clinical efficacy of HCQ. And no additive effect was observed when zinc supplements were added to HCQ as a combination therapy [86]. As to more combination therapies comprising chloroquine or HCQ and side-effects, mechanisms thereof, you may refer to another review [96].

MEDS433 is a new inhibitor of the human dihydroorotate dehydrogenase (*h*DHODH), a key enzyme of the de novo pyrimidine biosynthesis pathway. The pyrimidine salvage pathway may attenuate the antiviral efficacy of an *h*DHODH inhibitor through transporting nucleosides from extracellular medium [97]. Thus, a combination strategy was adopted with MEDS433 and dipyridamole (DPY), the latter inhibiting the pyrimidine salvage pathway. And this combination strategy restored the lost anti-SARS-CoV-2 activity of MEDS433 in the presence of exogenous uridine [87]. Camostat mesylate is a clinically efficient serine protease inhibitor and active against TMPRSS2 [88]. Complete inhibition of the SARS-CoV-2 cell entry was realized when camostat mesylate and E-64d, an inhibitor of endosomal cysteine proteases cathepsin B and L (CatB/L), were added [2]. However, two clinical trials (NCT04355052, NCT04338906) exploring the combination remedy of TMPRSS2 inhibitor camostat mesylate and HCQ have been withdrawn or in an unknown status (Table 2).

4.1.3 Antiviral Combined with Pharmacokinetic Enhancer

PF-07321332 (Nirmatrelvir) is an oral antiviral drug developed by Pfizer. Ritonavir is able to slow the metabolism of PF-07321332 by cytochrome enzymes, therefore maintaining higher concentrations of the primary drug. Thus PF-07321332 and ritonavir was used as a combination medication in Phase III studies and was marketed as Paxlovid for the treatment of COVID-19. Now Paxlovid has been utilized in market across the world since it was able to reduce the risk of hospitalization or death by 89% compared with placebo [98] (Table 3, Ref. [98–112]). WHO has strongly recommended the use of nirmatrelvir/ritonavir in patients with non-severe illness at the highest risk of hospitalization, however provided conditional recommendation against the use of them in patients with non-severe illness at a low risk of hospitalization (published 22 April 2022) (https://www.who.int/teams/health-care-readiness/covid-19/therapeutics). Cobicistat is an FDA-approved drug that can boost the activity of major drug via blocking the activity of cytochrome P450-3As (CYP3As) and P-glycoprotein (P-gp). Recently, it was reported to inhibit SARS-CoV-2 replication through suppressing the fusion of the viral S-glycoprotein to the cell membrane. In combination with remdesivir, cobicistat exhibited a synergistic antiviral effect *in vitro* and decreased viral titers and disease progression in Syrian hamsters [99]. Darunavir, a protease inhibitor and its pharmacokinetic enhancer, cobicistat, work as a whole to treat HIV infection. A pilot study was conducted at Shanghai Public Health Clinical Center (SPHCC) to preliminarily evaluate the efficacy and safety of darunavir/cobicistat in treating COVID-19 pneumonia. There was no any tendency of improvement observed in the darunavir/cobicistat group in comparison with the control group, although it was well tolerated (clinicaltrials.gov: NCT04252274) (Table 3) [100].

Table 3. Combination therapies with antiviral plus pharmacokinetic enhancer or complication-treating drug.

Drugs for Combination	Targets or action mechanisms	Results	Reference
PF-07321332 (Nirmatrelvir) + ritonavir	Mpro + CYP3A	Decrease the risk of hospitalization or death by 89%	[98]
Cobicistat + remdesivir	CYP3As + P-gp + S-glycoprotein + RdRp	Decreased viral titers and disease progression	[99]
Darunavir + cobicistat	A protease inhibitor + CYP3As+ P-gp	No any trend of improvement	[100]
Quercetin + Vitamin C (VC)	Anti-inflammatory + a broad spectrum antiviral agent	Synergistic antiviral, antioxidant and immunomodulatory effects	[101]
Plitidepsin + dexamethasone	host cell's eEF1A + anti-inflammation	A phase III trial is underway	[102]
Standard of care (SOC) + remdesivir + dexamethasone	RdRp + anti-inflammation	A reduction in 30-day mortality	[103]
Methylprednisolone + remdesivir	RdRp	(1) Prevented body weight loss and inflammation; (2) dampened viral protein expression and viral load	[104]
olfactory rehabilitation + palmitoylethanolamide + luteolin	NA*	Effective in improving recovery of olfactory function	[105]
Cannabidiol (CBD) + terpenes	An anti-inflammatory molecule + antimicrobials	Demonstrated mild to moderate antivirus effect	[106,107]
Pentoxifylline + oxypurinol	TNF-α production + xanthine oxidase	Just a suggestion, no experimental data	[108]
VD + DPP-4i	Anti-inflammatory + immunomodulatory	A perspective, no experimental data	[109–111]
Dapsone + doxycycline	Suppress production of various cytokines + anti-microbial	Just a suggestion, no experimental data	[112]

* NA, not available.

4.1.4 One Antiviral and Another Drug Treating Complications

In plants, quercetin is a flavonoid compound, produced from the phenylpropanoid pathway and ultimately derived from phenylalanine. There is a tremendous amount of literature supporting its anti-inflammatory and antiviral properties, especially against several respiratory viruses in both *in vitro* and *in vivo* experiments (Table 3) [101]. Vitamin C (VC) is a broad spectrum antiviral agent and an inhibitor of aerobic glycolysis. Treatment with quercetin in combination with VC provided synergistic antiviral, antioxidant and immunomodulatory effects due to overlapping antiviral and immunomodulatory properties and the capacity of VC to regenerate quercetin (Table 3) [101]. Plitidepsin is a cyclic depsipeptide known for its anti-tumor and antiviral activity, mainly acting on isoforms of the host cell's eukaryotic-translation-elongation-factor-1-alpha (eEF1A). Through blocking eEF1A and therefore translation of essential viral proteins, it exhibits anti-SARS-CoV-2 potential. A phase III trial is underway to compare the plitidepsin/dexamethasone remedy with the standard of care in moderate hospitalized patients (ClinicalTrials.gov Identifier: NCT04784559) [102]. A comparative study of the effectiveness of remdesivir/dexamethasone plus standard of care (SOC) versus SOC alone was proceeded in a clinical trial, and the result showed a reduction in 30-day mortality with the combination treatment [103]. In the hamster model of SARS-CoV-2 infection, treatment with methylprednisolone suppressed viral induction of proinflammatory cytokines but enhanced RNA replication of SARS-CoV-2. Although weight reduction, along with nasal and pulmonary inflammation, was relieved with methylprednisolone monotherapy, both viral loads enhancement and antibody response weakening also accompanied. On the contrary, a combination therapy methylprednisolone/remdesivir not only restrained weight reduction and inflammation, but also dampened viral protein expression and viral loads. Furthermore, the suppression of methylprednisolone on antibody response was also attenuated in this combination therapy [104]. Approximately 30% of COVID-19 patients were reported to have obstinate smell or taste dysfunction as prolonged sequalae of infection. Treatment combining olfactory rehabilitation with oral supplementation with palmitoylethanolamide and luteolin was effective in improving recovery of olfactory function, especially in those patients with longstanding olfactory dysfunction [105]. Cannabidiol (CBD) is widely available as medicinal compounds with various applications, most involved in modulating the inflammation processes [106]. Santos *et al.* [107] have evaluated the anti-infection effect of the combination of CBD with terpenes, as an anti-inflammatory chemical and anti-microbial, re-

spectively. The virucide effectiveness of CBD and terpene-based six formulations were tested in different cell lines and the result demonstrated mild to moderate antivirus effect [107].

Pentoxifylline is an inhibitor of TNF-α production while oxypurinol an inhibitor of xanthine oxidase. Accordingly, pentoxifylline alone, or combined with oxypurinol, was reported to reduce the systemic inflammation caused by experimentally-induced pancreatitis in rats. Therefore, pentoxifylline in combination with oxypurinol was suggested as an early remedy for COVID-19 patients to prevent the fatal acute respiratory distress syndrome (ARDS) [108]. Pinheiro *et al*. [111] published a perspective article discussing the synergistic effect of joint application of vitamin D (VD) and dipeptidyl peptidase-4 inhibitors (DPP-4i). After analysis of the relative progress of biological activity of VD and DPP-4i and pathologic profile of COVID-19, they proposed that co-administration of VD and DPP-4i might exert anti-inflammatory and immunomodulatory activity to a greater extent than VD or DPP-4i alone. Besides, VD and DPP-4i might bring about beneficial effect on endothelial dysfunction [109,110], which was implicated in COVID-19 pathophysiology [113]. Overall, the authors provided us the plausibility for the combination therapy VD/DPP-4i as an immunomodulation strategy to dampen the virulence of SARS-CoV-2, inhibit disease deterioration and modulate the cytokine storm in COVID-19 [111]. Dapsone, belonging to a class of sulfone drugs, suppresses production of various cytokines including interleukin (IL)1α, IL8, IL1β, IL6, and IL8 and tumor necrosis factor-α [114]. Thus, it was suggested to combine with doxycycline to treat severe COVID-19 patients (Table 3) [112].

4.1.5 Multiple Drugs for Combination Use

The pathophysiology of SARS-Cov-2 relates to inflammation, immune dysregulation, coagulopathy, and endothelial dysfunction. No single therapeutic agent can manage all these pathophysiologic conditions. Hence, a randomized open-label trial was initiated to investigate a triple combination remedy (aspirin, atorvastatin, and nicorandil) with anti-inflammatory, antithrombotic, immunomodulatory, and vasodilator properties against COVID-19 in India [115] (Table 4, Ref. [115–132]). Procter *et al*. [116, 117] have evaluated the effects of multidrug combination therapy on high-risk patients. At least two anti-SARS-CoV-2 agents (zinc, HCQ, ivermectin) and one antibiotic (azithromycin, doxycycline, ceftriaxone) were used as well as inhaled budesonide and/or intramuscular dexamethasone. And it was concluded that early ambulatory multidrug therapy was associated with low rates of hospitalization and death (Table 4) [116,117]. The outcome of a triple therapy (zinc, azithromycin, and HCQ) was evaluated in COVID-19 patients. No adverse cardiac events and notably fewer hospitalizations were observed [118].

Umifenovir (also termed as arbidol), an antiviral agent with broad spectrum, functions primarily through inhibition of membrane fusion between the viral envelope and host cell membrane, therefore, suppressing viral entry and infection [119]. Deng *et al*. [120] conducted a retrospective cohort study to compare arbidol and lopinavir/ritonavir (LPV/r) combination treatment for COVID-19 patients with LPV/r alone. They found that viral load vanished after 14 days in 94% patients of the combination group, compared to 52.9% of the monotherapy group (LPV/r) [120]. Recently, another retrospective cohort study was carried out to understand the clinical effectiveness and safety of Shufeng Jiedu Capsules (a Chinese herbal compound composed of eight medicinal plants [133]) in combination with umifenovir (Arbidol) for common-type COVID-19. The subsidence of a fever was observed more rapidly and the chest CT scan also showed better resolution of pneumonia symptoms in the combination treatment group than that in the control group (treated with arbidol hydrochloride capsules alone) [121]. A Phase II interventional study testing whether treatment with HCQ, Vitamin C, Vitamin D, and Zinc can prevent symptoms of COVID-19 is ongoing (NCT04335084).

4.1.6 Other Antimicrobials for Combination Use against SARS-CoV-2

Clofazimine was discovered as an anti-tuberculosis drug and later used for the treatment of leprosy [134]. Clofazimine, in combination with remdesivir, exhibited synergistic antiviral activity *in vitro* and *in vivo*, and restricted viral shedding from the upper respiratory tract (Table 4) [122]. The antiviral activity of several anti-malarial artemisinin-based combination therapies (ACT), including mefloquine/artesunate, artesunate/amodiaquine, artemether/lumefantrine, artesunate/pyronaridine, or dihydroartemisinin/piperaquine, was tested *in vitro* against a SARS-CoV-2 strain (IHUMI-3) in Vero E6 cells. Mefloquine/artesunate demonstrated the strongest antiviral activity with % inhibition of 72.1 $\pm$ 18.3% at the relative concentration in malaria treatment. However all the other combinations showed anti-SARS-CoV-2 activity with % inhibition in the same ranges (27.1 to 34.1%) [123]. Mefloquine, an anti-malarial drug, exhibited stronger anti-SARS-CoV-2 activity than HCQ in VeroE6/TMPRSS2 and Calu-3 cells, through blocking viral entry after attachment to the host cell. Joint treatment with Mefloquine and Nelfinavir manifested synergistic antiviral activity in wide concentration ranges [124]. Itraconazole is a member of the triazole group of broad-spectrum antifungals [135]. The *in vitro* antiviral activities of itraconazole and its metabolite against SARS-CoV-2 were proved in low micromolar level [136]. Furthermore, itraconazole/remdesivir combination prohibited the production of SARS-CoV-2 particles >90% in a synergistic fashion [132]. However, the antiviral effect of itraconazole was lost *in vivo* [137]. Nitazoxanide, a commercial antiprotozoal agent with broad-spectrum antiviral ac-

Table 4. Combination therapies with multiple drugs or some specific antimicrobials.

Drugs for Combination	Targets or action mechanisms	Results	Reference
Aspirin + atorvastatin + nicorandil	Anti-inflammatory, antithrombotic, immunomodulatory and vasodilator properties	NA*	[115]
Two antivirals of (zinc, HCQ, ivermectin) + one antibiotic of (azithromycin, doxycycline, ceftriaxone) + budesonide + dexamethasone	Multiple targets	Low rates of hospitalization and death	[116,117]
Zinc + azithromycin + HCQ	Multiple targets	No adverse cardiac events and notably fewer hospitalizations	[118]
Umifenovir (arbidol) + LPV/r	Inhibition of membrane fusion + anti-HIV	Viral load vanished in 94% patients of the combination group, compared to 52.9% of the monotherapy group (LPV/r)	[119,120]
Shufeng Jiedu Capsules + umifenovir	Multiple targets	The more rapid subsidence of a fever and better resolution of pneumonia symptoms	[121]
HCQ + VC + VD + Zinc	An alkalinizing lysosomatropic drug + broad-spectrum antiviral	Ongoing	NCT04335084
Clofazimine + remdesivir	An anti-tuberculosis drug + RdRp	Synergistic antiviral activity	[122]
Mefloquine + artesunate, artesunate + amodiaquine, artemether + lumefantrine, artesunate + pyronaridine, or dihydroartemisinin + piperaquine	Antimalarial drug	Mefloquine/artesunate demonstrated the strongest antiviral activity with % inhibition of 72.1 $\pm$ 18.3%, others 27.1 to 34.1%	[123]
Mefloquine + Nelfinavir	Blocking viral entry + Mpro inhibitor	Synergistic antiviral activity	[124]
Nitazoxanide + favipiravir	A commercial antiprotozoal agent + RdRp	Underway	NCT04918927
ATV+ RTV	An HIV-1 protease inhibitor + Cytochrome P450 3A	More potent	[125]
LPV/r+azithromycin	Anti-HIV + antibiotic	The most effective combination group among 8 groups of combination drugs	[126]
LPV/r	Anti-HIV drug	No significant benefit and more gastrointestinal adverse events	[127]
DOXY + HCQ	Antibiotic + an alkalinizing lysosomatropic drug	Reduction in recovery time and mortality and lower rate of transfer to hospital	[128]
DOXY+ ivermectin	Antibiotic	Recovered earlier	[129]
DOXY+ VC	Antibiotic + broad-spectrum antiviral	Suggestion	[130]
Minocycline + HCQ	Antibiotic + an alkalinizing lysosomatropic drug	Just an appeal for further clinical studies	[131]
Itraconazole + remdesivir	Antifungals + RdRp	Synergistically prohibited the production of SARS-CoV-2 particles *in vitro*	[132]

* NA, not available.

tivity against various viruses including human and animal coronaviruses, was reported to inhibit the SARS-CoV-2 at a low-micromolar concentration *in vitro* [138]. A proof-of-principle placebo-controlled clinical trial is underway to investigate the effect of early antiviral treatment with nitazoxanide plus or minus favipiravir (RdRp inhibitor) in preventing progression to the later phase of the disease (ClinicalTrials.gov Identifier: NCT04918927). Atazanavir (ATV) is an HIV-1 protease inhibitor currently suggested as a first-line treatment for naive HIV-infected patients. ATV in combination with ritonavir (RTV) was more potent than ATV alone in different cell assays against SARS-CoV-2 replication [125]. Lopinavir/ritonavir (LPV/r) is a combinational antiviral drug commonly used in the treatment of HIV, the causative agent for acquired immunodeficiency syndrome (AIDS) [139]. More and more evidence proved that lopinavir/ritonavir could be considered an efficient redemy for CoVs-induced infections [140]. Pur-

wati *et al*. [126] have evaluated 8 groups of combination drugs for the anti-SARS-CoV-2 activity *in vitro*, and found LPV/r/azithromycin is the most effective combination group among them. However, a trial of LPV/r treatment in severe COVID-19 adult patients showed that LPV/r didn't bring about significant benefit [127]. So was it in another similar randomized trial [141]. Besides, there were more gastrointestinal adverse events including nausea, vomiting, and diarrhea in the LPV/r group than in the standard-care group [127]. Apart from the gastrointestinal side effects, LPV/r use can also be associated with skin rash, hepatitis, metabolic derangements (hypercholesterolemia, hyperglycemia), neutropenia, thrombocytopenia, and QT prolongation [142]. Consequently, due to lack of efficacy, WHO withdrew LPV/r from their solidarity trial and provided strong recommendation against LPV/r in COVID-19 patients of any severity (published 17 December 2020) (https://www.who.int/teams/health-care-readiness/covid-19/therapeutics).

Doxycycline (DOXY) is a semisynthetic, second-generation class of tetracycline with a wide spectrum of antimicrobial activity. The activity of DOXY/HCQ combination therapy was studied in a series of fifty-four high-risk COVID-19 patients. And the clinical experience of this case series indicated a reduction in recovery time and death rate, and lower rate of transfer to hospital after treatment with DOXY/HCQ [128]. A double-blind and randomized interventional trial of a combination of DOXY and ivermectin was carried out with 400 participants. Patients with mild-to-moderate COVID-19 infection treated with ivermectin plus DOXY recovered earlier than those with placebo, were less possible to deteriorate, and were more inclined to be SARS-CoV-2 negative by RT-PCR at the end of the treatment (NCT04523831) [129]. It is suggested that co-administration of doxycycline and vitamin C shows more benefit against COVID-19 [130]. Minocycline is another semisynthetic, second-generation derivative of tetracycline with an activity against lots of microorganisms [143]. It also inhibits many proinflammatory cytokines, which are common in severe and complicated COVID-19 cases [144]. Gautam *et al*. [131] analyzed the pros and cons of the cocktail HCQ/minocycline in treating moderate to severe COVID-19 patients and called upon public and private healthcare bodies to implement large well-designed clinical studies for generating more convincing suggestions.

4.2 Combination Therapy with Biomacromolecule and Small Molecule

Interferons (IFNs) are glycoproteins with potential immunomodulatory and hormone-like functions [145]. Both IFNα and IFNβ have been considered as a potential therapy against COVID-19, especially combined with ribavirin [146]. In a non-controlled trial, the combination use of IFN-β-1a with HCQ and LPV/r in the management of COVID-19 offered positive results including virus eradication rates, fever subsidence, recovery time and safety characteristics [147] (Table 5, Ref. [147–168]). Retrospective data also revealed oxygenation increase, survival advantage and discharging of sever COVID-19 inpatients by the combination therapy of IFN-β-1a with LPV/r (Table 5) [148]. However, in a phase III trial, the combination regimen of LPV/r and IFN-β-1a demonstrated neither clinical improvement at day 15 nor reduction of SARS-CoV-2 load in respiratory specimens [149]. Aerosol IFN-α2b alone or in combination with arbidol exhibited superior performance in decreasing inflammatory markers in the blood of COVID-19 patients and accelerating viral clearance compared to arbidol treatment alone [150]. As for subcutaneous administration of IFN-α2b, an observational study of its use in combination with LPV/r showed a decline in the length of hospital stay and acceleration of viral eradication in COVID-19 patients [151]. An open-label, randomised, phase II trial was conducted to assess the efficacy and safety of triple combination of interferon beta-1b, LPV/r, and ribavirin in the treatment of hospitalized COVID-19 patients. And the outcome manifested the triple antiviral therapy was safe and superior to LPV/r alone in shortening virus shedding, relieving symptoms, and facilitating discharge of patients with mild to moderate COVID-19 (NCT04276688) [152]. It was known that IFNα-induced serpin E1 is a risk factor for thrombosis. Thus, a combination therapy was designed with IFNα and nafamostat, for the anticoagulative properties of nafamostat could compensate for the adverse effects of thrombosis by IFNα administration. And the combination suppressed SARS-CoV-2 infection in an additive fasion by cooperatively targeting host TMPRSS2 [153]. Besides, combinations of IFN-α2a with known SARS-CoV-2 inhibitors remdesivir, EIDD-2801, camostat or cycloheximide, showed a strong synergy in inhibiting SARS-CoV-2 infection [154].

COVID-19 may developed as a chronic disease in patients with some types of immunodeficiency. In this condition, remdesivir monotherapy is frequently ineffective, but the combination of remdesivir with antibody-based therapeutics holds promise. Remdesivir, combined with tocilizumab (an anti–interleukin-6 monoclonal antibody) and dexamethasone, was administered at the early stage of the disease, resulting in timely resolution of the cytokine storm and subsequent improvement in ARDS symptoms and eventual recovery [155]. Combination of remdesivir with convalescent plasma or anti-SARS-CoV-2 monoclonal antibodies (mAbs) achieved high viral clearance [156,157]. Successful outcomes with this combination therapy have also been demonstrated in other similar patients, such as with B-cell depleted [158], chronic lymphocytic leukemia [159] and X-linked agammaglobulinemia [160]. Considering that ruxolitinib, a Janus kinase (JAK) 1/2 inhibitor and eculizumab, an anti-C5a complement monoclonal antibody, function by acting on different but correlative pathological pathways, a combination therapy containing both

Table 5. Combination therapy with biomacromolecular and small molecular.

Drugs for Combination	Targets or action mechanisms	Results	Reference
IFN-β-1a + HCQ + LPV/r	Immunomodulatory + antiviral	Viral eradication rates, fever subsidence, recovery time and safety characteristics were improved	[147]
IFN-β-1a + LPV/r	Immunomodulatory + antiviral	Oxygenation increase, survival advantage and discharging of sever COVID-19	[148]
IFN-β-1a + LPV/r	Immunomodulatory + antiviral	Neither clinical improvement nor reduction of SARS-CoV-2 load	[149]
IFN-α2b + arbidol	Immunomodulatory + antiviral	Exhibited superior performance	[150]
IFN-α2b + LPV/r	Immunomodulatory + anti-HIV drug	A decline in the hospital time and acceleration of viral eradication	[151]
IFN-β-1b + LPV/r + ribavirin	Immunomodulatory + antivirals	Safe and superior to LPV/r alone	[152]
IFNα + nafamostat	Immunomodulatory + anticoagulant	Suppressed SARS-CoV-2 infection in an additive fasion	[153]
IFN-α2a + remdesivir; IFN-α2a + EIDD-2801; IFN-α2a + camostat; or IFN-α2a + cycloheximide	Immunomodulatory + SARS-CoV-2 inhibitors	A strong synergy in inhibiting SARS-CoV-2 infection	[154]
Remdesivir + tocilizumab + dexamethasone	Antiviral (small molecular and antibody) + anti-inflammation	Timely resolution of the cytokine storm and subsequent improvement in ARDS symptoms and eventual recovery	[155]
Remdesivir + convalescent plasma or mAbs	Antiviral (small molecular and antibody)	High viral clearance	[156–160]
Ruxolitinib + eculizumab	A Janus kinase (JAK) 1/2 inhibitor + an anti-C5a complement monoclonal antibody	Significant improvements in respiratory symptoms and radiographic pulmonary lesions and reduction of circulating D-dimer	[161]
Favilavir + tocilizumab	Antiviral + an anti–interleukin-6 monoclonal antibody	Its status has not been updated for 2 years	NCT04310228
Immunoglobulin + steroid pulses	Immunoglobulin + anti-inflammation	Useful in single-kidney transplanted patient with COVID-19	[162]
Sarilumab + standard of care (including corticosteroids)	IL6 alfa-receptor antibody + anti-inflammation	Not more effective	[163]
Dexamethasone + anti- SARS-CoV-2 mAbs	Anti-inflammatory + antiviral antibody	Synergistic	[164]
GRFT + EK1	An antiviral + spike S2 subunit	Strong synergistic effect	[165]
Bromelain + acetylcysteine	Virus glycoproteins	Synergistically weakened the infectivity	[166,167]
Bromelain + curcumin	Prevent entry of SARS-CoV-2 into cells and interfere with viral replication	A proposal	[168]

of them was conceived to test its effect on SARS-CoV-2-related ARDS. And the results showed that patients treated with the combination obtained significant improvement in respiratory symptoms and radiographic pulmonary lesions and reduction in circulating D-dimer concentrations compared to the best available therapy group. Since the number of participants was small, only 7 in this study, the authors suggested further clinical studies with larger populations [161]. NCT04310228 is a clinical trial assessing the curative effect and security of favilavir in combination with tocilizumab. But its status has not been updated for 2 years. It was proved useful that high-dose intravenous immunoglobulin plus steroid pulses in treating a case of COVID-19 pneumonia patient with a single-kidney transplanted requiring mechanical ventilation and hemodialysis [162]. Sarilumab is a recombinant human immunoglobulin monoclonal antibody of IL6 alfa-receptor. In hospitalized patients with COVID-19 pneumonia, an early treatment with sarilumab combined with standard of care (including corticosteroids) was not more efficatious than current standard of care alone [163]. We have known that from previous studies glucocorticoid treatment brought beneficial anti-inflammatory effects, with virus replication being rather strengthened. This outcome inspired us to apply glu-

cocorticoid together with virus-neutralizing mAbs. Based on histopathology and bulk and single-cell transcriptomic analysis, Wyler *et al*. [164] demonstrated the useful therapeutic effects of them and their potential as synergistic combination therapy in hamster models of moderate and severe COVID-19.

griffithsin (GRFT), a lectin isolated from the red alga *Griffithsia* sp, can recognize mannose with high specifity and has a broad-spectrum antiviral activity [169]. Combining GRFT and EK1, a pan-CoV fusion inhibitor targeting SARS-CoV-2 spike S2 subunit, exhibited strong synergistic effect against pseudotyped and live SARS-CoV-2 infection [165].

Bromelain exists mainly in the stem of the pineapple plant (*Ananas comosus*) and contains a number of enzymes that enable it to hydrolyze glycosidic bonds. Experimental studies have shown that bromelain exhibits unique immunomodulatory activity through various pathways [168]. Acetylcysteine is a powerful antioxidant and able to reduce disulfide bonds. Bromelain and acetylcysteine combination regimen (BromAc) showed synergistic action against glycoproteins by breakage of glycosidic linkages and disulfide bonds. Thus the combination therapy, BromAc, synergistically weakened the infectivity of SARS-CoV-2 cultured on Vero cells [166]. Further study indicated strong mucolytic and anti-inflammatory effect of BromAc ex vivo in tracheal aspirates from severe patients with COVID-19 [167]. Curcumin (diferuloylmethane) is a natural phenol found in turmeric (*Curcuma longa)*, a member of the ginger family of plants [170]. Interestingly, curcumin has been shown *in silico* studies to prevent entry of SARS-CoV-2 into cells and interfere with viral replication, while bromelain may also prohibit viral entry demonstrated by a recent experimental study [171]. Notably, bromelain can markedly increase the absorption of curcumin, thereby compensating for its poor absorption drawback. Based on the analysis, Kritis *et al*. [168] reported to highlight the potential value of the synergistic effect of bromelain and curcumin in the prevention of severe COVID-19.

5. Discussion

The outbreak of COVID-19 has caused devastating economic and medical burden worldwide and seriously affects the living styles of most people. It has been more than two years since the first emergence of SARS-CoV-2 infected cases in Wuhan, China, during which time many scientists are trying to develop effective vaccines and therapies including antibodies and oral small molecules, at an unprecedented scale and pace. And fortunately, there have been several vaccines, antibodies and oral small molecules prove effective as prophylaxis or remedy in combating COVID-19. However, since its outbreak, SARS-CoV-2 has developed a variety of mutations, and different mutants have been identified in all four structural proteins and other viral proteins [172]. Mutations in the SARS-CoV-2 RBD or N-terminal domain (NTD) may endow these strains with enhanced replication and/or transmission ability, which lead to escape from antibody recognition and attenuated neutralizing activity of mAbs [173]. Besides, some new SARS-CoV-2 variants can also infect those people who have been vaccinated once or more. In theory, combination therapy may have additive or synergistic activity in preventing infection by escape mutants compared to monotherapy, thus is highly emphasized. However, the present studies on combination therapy against SARS-CoV-2 mutant strains focus on mAbs, the small molecule drugs scarcely being involved.

In a combination therapy, one drug may endow another more power to counter the disease. Ideally, the pharmacokinetic profiles as well as the pharmacodynamics characteristics of individual drug are improved when combined used. As aforementioned above, effective concentration of each drug would be reduced below the maximal plasma concentration, which therefore attenuates toxicity resulted by high concentration. However, in many cases, there were limited data regarding the pharmacokinetic profiles of individual drug in the combination therapies. We call for more attention on the drug interaction and the resulting pharmacokinetic profiles in the future research.

The virus invades the organ systems across the body, which triggers a variety of complications (concomitant symptoms), ranging from fever to multiple organ dysfunction syndrome, etc. Sometimes, the severe complications are fatal, needing to be tackled as a matter of urgency. Thus it is not enough for a combination therapy to counter just the disease-causing agent. An effective treatment strategy for COVID-19 should take comprehensive consideration of both the host symptom and the pathogenic microorganism. So we are a bit more optimistic about the therapeutic effect of those combination therapies targeting both the virus life cycle and host complications.

This review updates the progress of dual and multiple drug combination therapies (containing at least one small molecule drug) against COVID-19. The antiviral mechanism of each combination, especially the target of each component, and the outcome is highlighted. We can see that these combinations target the same viral enzyme with different action mechanisms, different viral enzymes playing key roles in virus life cycle, different enzymes from virus and host cell facilitating virus survival, or host complication-relating pathways. Also we find that some combination therapies were proposed as medical hypotheses or perspective based on previous knowledge about the pharmacology of individual drug, most of which deserve further exploration by experiment. Others were validated by clinical trials, animal experiments, cell or enzyme assays. Many combination therapies exhibited positive (additive or synergistic) outcome awaiting further efficiency clarification in SARS-CoV-2-relating animal model or clinical trial. Though some combinations turned out to be no

effect, even toxic, they are still of guiding value in clinical practice, especially for those combinations of repurposing drugs. In summary, this review is hopeful for facilitating us to select a proper anti-SARS-CoV-2 combination therapy for further research or clinical treatment.

Author Contributions

QL conceived the topic and wrote the manuscript. YZ assisted in writing and organizing the draft. JZ supervised the work and approved the final draft. All authors read and approved the final draft.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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