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Review

COVID-19: The Potential Role of Copper and N-acetylcysteine (NAC) in a Combination of Candidate Antiviral Treatments Against SARS-CoV-2

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Abstract. *Background: On March 11, 2020, the World Health Organization (WHO) declared the outbreak of coronavirus disease (COVID-19) a pandemic. Since then, thousands of people have suffered and died, making the need for a treatment of severe acute respiratory syndrome-related coronavirus-2 (SARS-CoV-2) more crucial than ever. Materials and Methods: The authors carried out a search in PubMed, Clinical Trials.gov and New England Journal of Medicine (NEJM) for COVID-19 to provide information on the most promising treatments against SARS-CoV-2. Results: Possible COVID-19 agents with promising efficacy and favorable safety profile were identified. The results support the combination of copper, N-acetylcysteine (NAC), colchicine and nitric oxide (NO) with candidate antiviral agents, remdesivir or EIDD-2801, as a treatment for patients positive for SARS-CoV-2. Conclusion: The authors propose to study the effects of the combination of copper, NAC, colchicine, NO and currently used experimental antiviral agents, remdesivir or EIDD-2801, as a potential treatment scheme for SARS-COV-2.*

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Nowadays, the world is facing a pandemic of a newly discovered coronavirus disease, named COVID-19, with 4,037,574 confirmed cases and 279,236 deaths on May 10, 2020, worldwide (1). The most affected countries so far are: People's Republic of China, the United States of America (USA), Spain, Italy, Germany, France, Iran and the UK (1). CoVs are a group of enveloped viruses with a positive-sense single-stranded RNA genome that infect the pulmonary system, the intestines, the liver and the nerve cells of animals and humans (2). The taxonomy of these viruses includes the following genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus (3, 4). In each genus, several species that affect humans include: Human coronavirus 229E, Human coronavirus NL63, Betacoronavirus 1 (Human coronavirus OC43), Human coronavirus HKU1, Severe acute respiratory syndrome-related coronavirus (SARS-CoV, SARS-CoV-2), Middle East respiratory syndrome-related coronavirus (MERS-CoV) and Infectious bronchitis virus (3, 4).

Infection with CoVs begins when an envelope glycoprotein, the viral spike (S) protein, which attaches to the host cells' angiotensin converting enzyme 2 (ACE2) receptor (5). This allows the virus to enter the host cell (5) by endocytosis or by direct fusion with the host cell membranes (6). This is considered as the main function of this glycoprotein (6), which assembles into trimers on the surface of the virus in order to form the "corona", the crown appearance of the virus (7). The protein is organized into two domains: a N-terminal S1 domain responsible for receptor binding and a C-terminal S2 domain responsible for viral fusion (7). The S protein consists of a furin site (polybasic cleavage site- RRAR) at the boundary of its two domains (8). This allows effective cleavage by furin and other

proteases and has a role in determining viral infectivity and host range (8). Specifically, six amino acids of the receptor-binding domain (RBD) bind to ACE2 human receptors and infect a range of cells, that can act as hosts to the CoVs (8).

The molecular profile of COVID-19 disease is associated with haemophagocytic lymphohistiocytosis (sHLH), a severe systemic inflammatory syndrome characterised by uncontrolled, systemic activation of macrophages (9-11). The infection is characterised by an increase in inflammatory markers interleukin (IL)-2, IL-7, granulocyte-colony stimulating factor (GCSF), interferon-γ inducible protein 10 (IP10), interferon-γ (IFN-γ), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1-α (MIP-1α) and tumour necrosis factor-α (TNFα) (11). The activated macrophages cause acute respiratory distress syndrome (ARDS) by attacking vital organs such as the blood, liver and brain, and it is the leading cause of COVID-19 mortality (12).

Currently, there is no established treatment against SARS-CoV-2 and possible medications are administered in clinical trials, off-label or through compassionate use programs. In a recent press release, the European Medicines Agency (EMA) announced several potential treatments for COVID-19 used in clinical trials (13). These include: remdesivir (RDV) (as an investigational treatment), lopinavir/ritonavir (approved as anti-HIV treatment), chloroquine and hydroxychloroquine (approved nationally as treatments against malaria and other diseases, such as rheumatoid arthritis) and systemic interferons (particularly interferon Beta, a treatment for multiple sclerosis) (13). Another antiviral agent that has recently shown promising antiviral activity against SARS-CoV-2 is an NHC prodrug, EIDD-2801 (14). Colchicine and nitric oxide (NO) have also entered the COVID-19 treatment arena and are being investigated in clinical trials (15-22). Recently, lerolimab, a chemokine receptor type 5 (CCR5) antagonist is being investigated in COVID-19 patients (23). Other agents known as interleukin-6 inhibitors, such as sarilumab (Kevzara) and tocilizumab (Actemra), are also being tested in clinical trials (24, 25). In all clinical trials, the above medicinal products are administered as monotherapy. According to the International Severe Acute Respiratory and Emerging Infections Consortium (ISARIC), the main treatments administered during hospital stay include: antibiotics, oxygen therapy, antiviral agents, corticosteroids and invasive ventilation (26).

The main symptoms of SARS-CoV-2 reported upon admission of patients positive to COVID-19 include fever, shortness of breath, extreme tiredness/malaise and cough with no sputum (26). Symptoms' severity vary with acute pneumonia, cardiovascular complications such as acute myocarditis, sepsis and death reported in some cases (26 - 29). Clinical signs among admitted patients include: lymphocytopenia (83.2%) thrombocytopenia (36.2%), leukopenia (33.7%) and extremely high ferritin levels (30, 31). Increased levels of C-reactive protein (CRP) is the most common clinical sign and uncommon elevation is observed in alanine aminotransferase, aspartate aminotransferase, creatine kinase and D-dimer. (30). Other laboratory findings which characterizes severe cases includes Vitamin D insufficiency (VDI) (32). All signs and symptoms resemble those of the pathology of acute inflammation (33). Reported COVID-19 patients' pre-existing comorbidities include cerebrovascular diseases, hypertension, diabetes mellitus and coronary heart diseases (30, 34). The above reveal that amongst the most vulnerable groups to be diagnosed with COVID-19 disease are the elderly, immunocompromised patients, diabetics and patients with heart diseases (hypertension or coronary heart disease).

Often, we tend to forget that the human body, the physical substance of the living organism, is composed of living cells and possesses sophisticated self-healing mechanisms triggered when threatened. Since this virus attacks vital organs, such as the lungs, heart and kidneys, a combination of medicinal products, that act synergistically, may be the best approach to treat COVID-19.

There are mainly five drug mechanisms against SARS-CoV-2 that can: 1) Augment a physiological immune system response (boost immune system); 2) Kill the virus, act on the virus itself (pathogen-free); 3) Inhibit virus cell entry (block host cell docking proteins or virus binging proteins); 4) Inhibit virus replication (delay pathogen spread to enable effective immune system response); 5) Treat symptoms (protect vital organs).

The first option is considered to be the most beneficial to the patient, as it is the most "friendly", potentially long-lasting and can also have protective effects against future pathogen attacks. Information regarding the human body's complicated mechanisms is most of the times scattered across the literature with pieces of the puzzle not being linked to each other or just missing. In this review, we used current knowledge from the literature regarding virucidal agents, investigational medicinal products for COVID-19, acute inflammation's mechanism, immune response to inflammation, and COVID-19 clinical manifestation, and concluded with a potential therapeutic scheme for COVID-19.

Materials and Methods

The authors performed a search in PubMed, Clinical Trials.gov, New England Journal of Medicine (NEJM) and EMA's website for COVID-19. The search was divided in two phases. The first phase included a general search using a combination of terms derived from current knowledge on COVID-19: "inflammation", "immune system response", "cardiovascular disease (CVD)", "virucidal", "antiviral therapies", "diabetes mellitus", "insulin resistance", "vitamin D insufficiency", "high ferritin" and "iron". The authors also studied the manifestation of this viral infection and contemplated it with other inflammatory diseases. During the

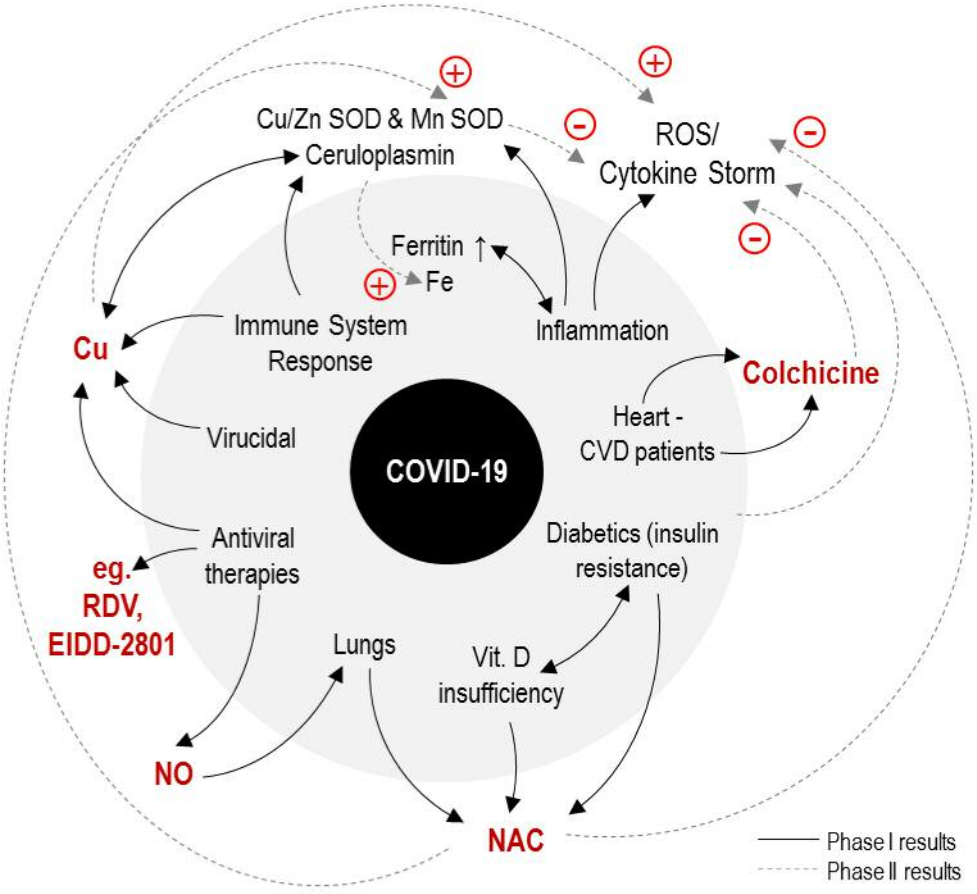

Figure 1. *Research method: Phase I and Phase II.*

second phase, the selection of candidate agents was performed using two criteria: 1) the candidate had to provide evidence of efficacy or benefit in at least two steps of the disease pathway and 2) the candidate had to possess a favorable safety profile.

In the second phase, a further literature search was performed for the short listed agents using the terms: "COVID-19", "SARS-CoV-2", "copper", "remdesivir", "N4-hydroxycytidine", "EIDD-2801", "N-acetycysteine", "colchicine", "nitric oxide", "chloroquine", "hydroxychloroquine", "sarilumab", "tocilizumab", "lerolimab" and "convalescent plasma". The search revealed more than 200 articles, 130 were used and 70 were rejected because they did not contain any relevant information to this review. Figure 1 presents the methodology of the two phases.

Results

Several treatments are being tested for SARS-CoV-2. In this article, we provide information regarding a potentially effective combination treatment against SARS-CoV-2: an antiviral medicinal product, such as RDV or EIDD-2801; copper as a compound with known virucidal effects with N-acetylcysteine (NAC); colchicine due to its strong anti-inflammatory properties and NO because of its supportive inhibitory activity in viral replication. Figure 2 provides an overview of the stages of COVID-19 progression and potential inhibition/blockage of these stages by the above-mentioned agents and is further analyzed in the Discussion section. The safety profile of the proposed treatments is presented in Table I. Cooper potential virucidal properties are summarized in Table II. Current clinical trials with RDV, colchicine and NO are shown in Tables III, IV and V.

Antiviral agents

Remdesivir (RDV, GS-5734). One of the antiviral treatments that are currently under investigation for the treatment of COVID-19 is RDV (13). RDV is a nucleotide analogue, originally developed for the treatment of Ebola virus disease, with antiviral activity against multiple filo-, pneumo-, paramyxo-, and corona-viruses, such as SARS-CoV and MERS-CoV (35, 36). Following a formal request by Estonia, Greece, Romania and the Netherlands, EMA's Committee for Medicinal Products for Human Use (CHMP) provided

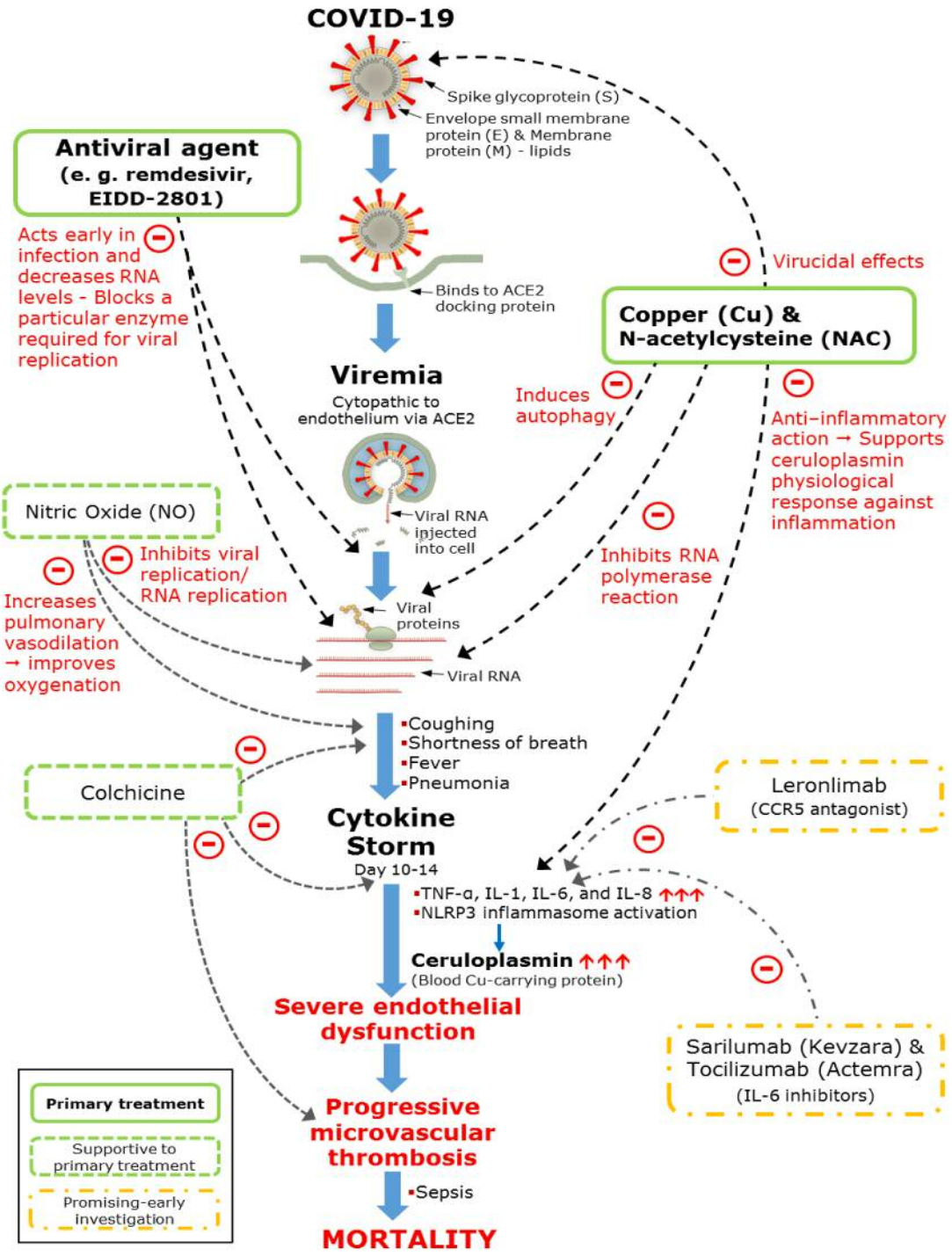

Figure 2. *Overview of stages of COVID-19 progression and potential inhibition/blockage by candidate treatment agents.*

recommendations on how RDV could be administered for the medical care of COVID-19 in compassionate use programmes in Europe (13, 36). This antiviral compound has shown efficacy against MERS-CoV, SARS-CoV and CoV strains from bats (35). The half-maximum effective concentrations (EC_{50}s) of the compound against SARS-Cov and MERS-CoV were 0.069 μM and 0.074 μM, respectively, in cell cultures (37). These studies revealed that RDV could

Table I. *Safety profile of the proposed treatments for COVID-19*.

Adverse reactions	RDV	Copper	NAC	Colcichine	Nitric oxide (NO)
Cardiac disorders					
Bradycardia (following abrupt discontinuation of therapy)					X
Other heart problems		X			
Hepatobiliary disorders					
Hepatotoxicity	X	X		X	
Transaminase increases					
Renal and urinary disorders					
Kidney failure		X			
Renal damage				X	
Vascular disorders					
Phebitis	X				
Hemorrhages		X	X		
Hypotension		X			X
Gastrointestinal disorders					
Nausea	X	X	X	X	
Vomiting		X	X	X	
Diarrhea		X	X	X	
Stomach pain		X			
Gastrointestinal haemorrhage				X	
Stomatitis			X		
Abdominal pain			X	X	
Constipation	X				
Dyspepsia			X		
Blood and lymphatic system disorders					
Bone marrow depression with agranulocytosis				X	
Aplastic anemia/Anemia		X		X	
Thrombocytopenia				X	X
Methaemoglobinaemia					X
General disorders and administration site conditions					
Fever		X	X		
Nervous system disorders					
Headache	X		X		X
Dizziness					X
Peripheral neuritis				X	
Neuropathy				X	
Musculoskeletal and connective tissue disorders					
Myopathy				X	
Rhabdomyolysis				X	
Pain in extremity	X				
Respiratory, thoracic and mediastinal disorders					
Atelectasis					X
Hypoxia					X
Dyspnea					X
Chest discomfort					X
Dry throat					X
Immune system disorder					
Hypersensitivity (bronchospasm, dyspnea, pruritus, urticaria, rash, angioedema and tachycardia)			X		
Anaphylactic shock			X		
Anaphylactic/anaphylactoid reactions			X		
Skin and subcutaneous tissue disorders					
Alopecia				X	
Rash				X	
Facial oedema			X		
Ecchymosis	X				
Reproductive system and breast disorders					
Amenorrhoea				X	
Dysmenorrhoea				X	
Oligospermia				X	
Azoospermia				X	
Ear and labyrinth disorders					
Tinnitus			X		
Investigations					
Low blood pressure			X		

Table II. *Copper (Cu) virucidal properties and mechanisms of action.*

Virus	Cu form	Mechanism of action	Reference
Junin virus (JV)	3.17 mg Cu (II) per litter	Virucidal effect of the copper salt.	Sagripanti, 1992
ΦX174, T7, Φ6 and herpes simplex virus (HSV)	Cu(II) 1,000-mg/ litter at 24˚C, PH 7.4	99% inactivation after 30 min. Enveloped viruses (Φ6, JV, and HSV) were more sensitive to copper(II) inactivation than the non-enveloped ones (ΦX174 and T7). The presence of RNA or lipid may render the virus particle more sensitive to inactivation by Cu(II).	Sagripanti *et al.*, 1993
Human immuno-deficiency virus (HIV)	6 mM Cu(II)	Completely inhibited the formation of syncytia and the synthesis of virus-specific p24 antigen when infected cells were treated for 30 min at 20˚C. The cells continued to preserve their viability.	Sagripanti & Lightfoote, 1996
HSV	100-200 mg Cu(II) per litre	Damaged HSV DNA 90% of the HSV was inactivated in 30	Sagripanti *et al.*, 1997
H9N2 virus	Cu^{2+}	Inhibited the infectivity of H9N2 virus in a time-dependent manner when MDCK infected cells were treated with 2.5-250 μM Cu^{2+}. Furthermore, H9N2 virus neuraminidase (NA) activity was drastically reduced by 25 mM Cu^{2+} and marginally reduced by 250 μM Cu2	Horie *et al.*, 2008
Virus feline calicivirus (FCV)	CuI nanoparticles	Exhibited extremely high antiviral activity against the non-enveloped virus FCV due to the Cu(+) effects and the generation of ROS which led to capsid protein oxidation.	Shionoiri *et al.*, 2012
Influenza A virus	CuI nanoparticles	Generated ROS. Exhibited antiviral activity against influenza A virus of swine origin. The virus titter dropped in a dose-dependent manner when treated with CuI nanoparticles with the 50% effective concentration being approximately 17 μg/ml after 60 minutes of exposure.	Fujimori *et al.*, 2012
Influenza A virus	Cells were treated with 50 μM $CuCl_2$	Resulted in significant viral Influenza A growth defects. Exogenously increasing copper concentration or chelating copper resulted in RNA interference (RNAi) knockdown of the high-affinity copper importer CTR1.	Rupp *et al.*, 2017

be highly active against the human CoVs OC43 and 229E, exhibiting significant activity against several other CoVs (37). Preventive and early administration of RDV resulted in a significant reduction of the viral load in the lungs in mouse models infected with SARS-CoV virus (37). Furthermore, this agent produced better results in terms of disease clinical signs and pulmonary function in comparison with control animals that did not receive treatment (35). RDV demonstrated higher efficacy *in vivo* and *in vitro* in MERS-CoV compared to the combination of lopinavir/ritonavir/ interferon beta (38). Moreover, another study revealed that RDV was highly active against certain mutant variants of MERS-CoV virus (F476L and V553L) (39). Several clinical trials and expanded access programs testing RDV are underway worldwide (40-47). More information can be found in Table III. On April 10, 2020, new data from a compassionate use program showed that clinical signs were improved in 36 out of 53 patients (68%), however, it was noted that for the establishment of RDV efficacy it is necessary to perform randomized, placebo-controlled trials (48). RDV preliminary results from the Adaptive COVID-19 Treatment Trial have shown that RDV treated patients with advanced COVID-19 and lung involvement had a 31% faster time to recovery than those who received placebo, with a median time to recovery of 15 days for RDV *versus* 11 days for placebo (49).

Prodrug of β-D-N4-hydroxycytidine (EIDD-2801). EIDD-2801 is an orally bioavailable prodrug of β-D-N4-hydroxycytidine (NHC, EIDD-1931), a ribonucleotide, whose chemical structure resembles that of RDV (14, 50). It has been shown to inhibit the viral activity of various influenza strains and the Venezuelan equine encephalitis virus (VEEV) (51). NHC has also shown broad spectrum antiviral activity towards several CoVs (MERS-CoV, SARS-CoV and SARS-CoV-2) and other zoonotic bat-CoVs (14). Agostini *et al.* in 2019 showed that EIDD-1931 inhibited MERS-CoV's and murine hepatitis virus's (MHV) activity,

Table III. *Clinical trials with remdesivir (GS-5734TM).*

	Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
Remdesivir (GS-5734™)										
1	Study to evaluate the safety and antiviral activity of remdesivir (GS-5734™) in participants with severe coronavirus disease (COVID-19) (to evaluate the efficacy of 2 RDV regimens with respect to clinical status assessed by a 7-point ordinal scale on Day 14.)	NCT04292899	Phase III	Part A: RDV, 5 Days (Not Mechanically Ventilated) D1 200 mg, D2-5 100 mg Part A: RDV, 10 Days (Not Mechanically Ventilated) D1 200 mg, D2-10 100 mg Part B: RDV, 5 or 10 Days (Extension) after Part A is complete. continued SOC + RDV D1 200 mg, D2-10 100 mg. Part B: RDV 10 days (Mechanically Ventilated) continued standard of care therapy + RDV D1 200 mg, D2-10 100 mg	6000	6 March, 2020	May 2020	May 2020	Gilead Sciences	Recruiting
2	Study to evaluate the safety and antiviral activity of remdesivir (GS-5734™) in participants with moderate coronavirus disease (COVID-19) compared to standard of care treatment (to evaluate the efficacy of 2 RDV regimens compared to SOC, with respect to clinical status assessed by a 7-point ordinal scale on Day 11)	NCT04292730	Phase III	Part A: RDV, 5 Days continued SOC + RDV D1 200 mg, D2-5 100 mg Part A: RDV, 10 Days continued SOC + RDV D1 200 mg, D2-10 100 mg Part A: Continued SOC Therapy Part B: RDV, 5 or 10 Days (Extension) continued SOC + RDV D1 200 mg, D2-10 100 mg.	1600	15 March, 2020	May 2020	May 2020	Gilead Sciences	Recruiting
3	Trial of treatments for COVID-19 in hospitalized adults (DisCoVeRy) (to evaluate the clinical efficacy	NCT04315948	Phase III	Experimental: RDV for the duration of the hospitalization up to D=10. D1 200 mg, D2-10 100 mg Experimental:	3100 (620/ drug)	20 March, 2020	March 2023	March 2023	Institut National de la Santé Et de la Recherche Médicale, France	Recruiting

Table III. *Continued*

Table III. *Continued*

	Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
	and safety of different investigational therapeutics relative to the control arm in patients hospitalized with COVID-19, the primary endpoint is the subject clinical status (on a 7-point ordinal scale) at day 15.)			Lopinavir/ritonavir (400 mg/100 mg) - 1 tab. bd, po, D=14 - unable to take medications by mouth, 5 ml susp bd, *via* a nasogastric tube, D=14 Experimental: Lopinavir/ritonavir + Interferon β-1a Lopinavir/ritonavir (400 mg/100 mg) - 1 tab. bd po, D=14. - unable to take medications by mouth, 5 ml susp bd, *via* a nasogastric tube, D=14. Interferon β1a 44 μg SC., 3 doses (D1, D3, D6). Experimental: Hydroxychloroquine D1: 400 mg bd, po. D2-9: 400 mg od, po For nasogastric tube: D1: 600 mg bd D2-9: 400 mg od Active Comparator: SOC						
4	Adaptive COVID-19 treatment Trial (ACTT) (to evaluate the clinica l efficacy of different investigational therapeutics relative to the control arm in adults hospitalized with COVID-19)	NCT04280705	Phase III	Experimental: Remdesivir D1: 200 mg IV D2-10: 100 mg IV for the duration of the hospitalization up to D=10. Placebo D1: 200 mg IV D2-10: 100 mg for the duration of the hospitalization up to D=10.	440 (220/ drug)	21 February, 2020	1 April, 2023	1 April, 2023	National Institute of Allergy and Infectious Diseases (NIAID)	Recruiting
5	Mild/Moderate 2019-nCoV remdesivir RCT (randomized, controlled, double blind trial will evaluate the efficacy and safety of RDV in patients hospitalized with mild or moderate 2019-nCoV respiratory disease)	NCT04252664	Phase III	Drug: Remdesivir D1: 200 mg od IV D2-9: 100 mg od IV Drug: Remdesivir placebo D1: 200 mg od IV D2-9: 100 mg od IV	308	12 February, 2020	10 April, 2020	27 April, 2020	Capital Medical University	Recruiting

Table III. *Continued*

Table III. *Continued*

	Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
6	Severe 2019-nCoV remdesivir RCT (randomized, controlled, double blind trial will evaluate the efficacy and safety of RDV in patients hospitalized with severe 2019-nCoV respiratory disease.)	NCT04257656	Phase III	Drug: Remdesivir D1: 200 mg od IV D2-9: 100 mg od IV Drug: Remdesivir placebo D1: 200 mg od IV D2-9: 100 mg od IV	453	6 February, 2020	3 April, 2020	1 May, 2020	Capital Medical University	Recruiting
7	Expanded access treatment protocol: RDV (GS-5734) for the treatment of SARS-CoV2 (CoV) infection (to provide expanded access of RDV for the treatment of severe acute respiratory syndrome coronavirus (SARS-CoV2) infection.)	NCT04323761	Expanded Access	Drug: Remdesivir IV for 30 to 120 minute period		27 March, 2020			Gilead Sciences	Available
8	Expanded access RDV (GS-5734™)	NCT04302766	Expanded Access	Drug: Remdesivir Remdesivir (RDV,GS-5734)		10 March, 2020			Gilead Sciences	Available

with MHV inhibition occurring only when given at the early stages of infection (52). Because it is known that RDV and 5FU ineffectively inhibit WT CoVs due to the presence of EXoN proofreading activity, the sensitivity of ExoN (–) MHV to NHC inhibition was tested (52). NHC inhibits MHV, irrespective of the presence of ExoN proofreading activity (52). Evidence suggests that the NHC antiviral effects are mediated by the selective introduction of mutations in viral RNA but not in the RNA of the host, indicating a high genetic barrier to NHC resistance (14, 52). NHC exhibited a favorable safety profile, as minimal cytotoxicity was observed (52). EIDD-2801 enhanced the respiratory function and reduced viral titter in SARS-CoV and MERS-CoV infected mice (14). It is important to note that this agent showed extensive potency against RDV resistant CoV mutations (14). The small ring size of NHC blocks virus cell entry (50). Furthermore, its resemblance with potent antivirals, such as cytidine, supports its antiviral effect (50). Given its pharmacologic profile, its privilege of oral administration and its antiviral activity, this active substance could be a treatment against COVID-19.

Copper. Copper is an essential trace dietary mineral present almost in all living organisms (53). Copper can boost the host's immune system response against pathogens, exhibiting strong antibacterial, antifungal, antiviral and anti-inflammatory effects (53-74). A recent study from the US National Institutes of Health (NIH) showed that SARS-CoV-2 virus survives no more than 4 h on copper surfaces compared to up to 24 h on cardboard, ≈48 h on stainless steel and ≈72 h on plastic (54). The same study showed that SARS-CoV-1 survives no more than 8 h on copper surfaces compared to up to 24 h on cardboard, ≈48 h on stainless steel and ≈72 h on plastic (54). The pathogenic human coronavirus 229E (HuCoV-229E) was rapidly inactivated on a range of copper alloys within <40 min on Cu brasses and within 120

Table IV. *Clinical trials with colchicine*.

	Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
Colchicine										
1	The Greek Study in the effects of colchicine in Covid-19 complications prevention (GRECCO-19) (whether relatively low dose colchicine, could potentially have an effect the patients' clinical course by limiting the myocardial necrosis and pneumonia development in the context of COVID-19.)	NCT04326790		Drug: Colchicine, 0.5 mg bid + standard treatment Active Comparator: SOC, including all medications recommended by the National Public Health Organization	180	6 April, 2020	31 August, 2020	30 September, 2020	National and Kapodistrian University of Athens	Not yet recruiting
2	Colchicine efficacy in COVID-19 pneumonia	NCT04322565	Phase II	Active Comparator: Colchicine 1 mg (or 0.5 mg in CKD)/ day + SOC for COVID 19 pneumonia Standard of care SOC for COVID 19 pneumonia Intervention: Drug: Colchicine	100	1 April, 2020	30 May, 2020	30 June, 2020	Lucio Manenti, Azienda Ospedaliero-Universitaria di Parma	Not yet recruiting
3	The ECLA PHRI COLCOVID trial (COLCOVID) (to test the effects of colchicine on severe hospitalized COVID-19 cases with the aim of reducing mortality.)	NCT04328480	Phase III	Drug: Colchicine 1. Lopinavir/Ritonavir o D1: Loading dose of 1.5 mg followed by 0.5 mg after 2 hrs o D2-14: or until discharge 0.5 mg 2. Lopinavir/Ritonavir o D1: Loading dose 0.5 mg o After 72 hrs from the loading dose, 0.5 mg/ 72 hrs for D=14 or until discharge. 3. Patients under treatment with Colchicine that are starting with Lopinavir/Ritonavir o 0.5 mg 72 hrs after starting Lopinavir/ Ritonavir. o Continue with 0.5 mg/ 72 hrs for D=14 or until discharge. Other: Local standard of care	2500	March, 2020	30 May, 2020	30 June, 2020		Not yet recruiting

Table IV. *Continued*

Table IV. *Continued*

	Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
4	Colchicine coronavirus SARS-CoV2 trial (COLCORONA) (COVID-19) (to determine whether short-term treatment with colchicine reduces the rate of death and lung complications related to COVID-19 and is to determine the safety of treatment with colchicine in this patient population.)	NCT04322682	Phase III	Drug: Colchicine D1-3: 0.5 mg bd po D4-30: 0.5 mg od po If a dose is missed, not to be replaced 0.5 mg bd per os D1-3: 0.5 mg bd po D4-30: 0.5 mg od po If a dose is missed, not to be replaced	6000	23 March, 2020	September 2020	September 2020	Montreal Heart Institute	Recruiting

min on Cu/Zn brasses, suggesting a concentration-response relationship (55). Exposure to copper resulted in unreversed damage in virus morphology (*i.e.* envelope and surface spikes) and destruction of the viral genomes (55). Another study revealed that the application of the metal catalyst Cu/Al2O3 to surfaces for 5-20 min can destroy the replication and propagation abilities of SARS-CoV (56).

Copper's potential mechanism of action against viruses has been described in the literature (53-66). These effects are usually concentration and time-dependent (57, 58). There are mainly three mechanisms by which copper acts: (A) it damages virus membranes and "envelopes" and can destroy the DNA or RNA of the viruses (59-65), (B) it generates reactive oxygen species (ROS) that can kill the virus (58, 65) and, (C) it interferes with proteins that operate important functions for the virus (57, 66). Sagripanti *et al*. in 1993 demonstrated that Cu^{2+} resulted in 99% inactivation of viruses *in vitro* after 30 min (60). Enveloped viruses were more sensitive to Cu^{2+} inactivation than the non-enveloped ones (60). The presence of RNA or lipid may render the virus particle more sensitive to inactivation by Cu^{2+} (60). It has been shown that Cu^{2+} can inhibit RNA polymerase activity by more than 60%, with copper exhibiting the strongest effect compared to other metal ions (62). The biological activity of these metallo-peptide drugs appears to be higher when copper is bound to a chelating amino acid or acetylacetonate (64). Table II presents an overview of the viral mechanisms sensitive to copper virucidal effects. These results demonstrate that different biochemical processes may be inactivated by Cu^{2+}, documenting the broad spectrum of virucidal properties of Cu^{2+}.

Copper may also have a role in the immune system response to inflammation (67-74). In inflammatory conditions, subjects exhibited higher mean serum copper concentrations related to disease activity (67, 68). Furthermore, liver copper levels were increased in adjuvant-induced arthritis in rats during the inflammatory process (68). Another study demonstrated that elevated IL-6 levels resulted in increased levels of ceruloplasmin, the major copper-carrying protein in the blood (69).Therefore, the increase in copper levels could be related to the body's physiological reaction to fight inflammation (68). In addition, the inflammatory disease may be the result of insufficient hepatic copper repositories that cannot support an anti-inflammatory response (70). Ceruloplasmin null ($Cp^{-/-}$) and wild-type (WT) mice with induced experimental colitis survived for 14 days and 30 days respectively (71). $Cp^{-/-}$ mice TNFα, KC and MCP-1 levels were significantly elevated compared to those in the MT type, suggesting that ceruloplasmin expression defects may influence inflammation onset or progression (71). In vitro studies have shown that ceruloplasmin may have a pathophysiological role in inflammatory diseases, acting as a physiologic inhibitor of myeloperoxidase (MPO) (72). Another study in a crescentic glomerulonephritis (Crgn) animal model showed the down-regulation of ceruloplasmin by RNA interference (RNAi), decreased markers of glomerular proinflammatory macrophage activation and suppressed a physiological response (73). Exogenous copper decreased the formation of systemic lupus erythematosus (SLE) cells in rats with a hydralazine induced collagen disease (74). Differences in inflammatory response were observed in rats fed with copper supplemented diet compared to standard diet (74). The

Table V. *Clinical trials with nitric oxide (NO).*

	Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
Nitric Oxide (NO)										
1	Nitric oxide gas inhalation in severe acute respiratory syndrome in COVID-19 (NOSARSCOVID) (is to determine whether inhaled NO improves oxygenation in patients with hypoxic SARS-CoV2.)	NCT04306393	Phase II	Drug: Nitric Oxide Gas 80 ppm of inhaled NO for 48 hrs, followed by 40 ppm, followed by weaning before stop. Weaning criteria: maintenance of a PaO_2/FiO_2 ratio >/= 300 for at least 24 hrs consecutively.	200	21 March, 2020	21 March, 2021	21 March, 2022	Massachusetts General Hospital	Recruiting
2	Nitric oxide gas inhalation therapy for mild/moderate COVID-19 (NoCovid) (to reduce the incidence of patients requiring intubation and mechanical ventilation)	NCT04305457	Phase II	Drug: Nitric Oxide NO + SOC for 20-30 min. D1-14: bd, from time of enrolment. Targeted NO inhaled concentration: 140-180 ppm. The gas will be delivered through a CPAP circuit ensuring an end-expiratory pressure 2-10 cm H_2O or through a non-rebreathing mask without positive end-expiratory pressure, depending on the clinical needs of the patient.	240	21 March, 2020	1 April, 2021	1 April, 2022	Massachusetts General Hospital	Recruiting
3	NO prevention of COVID-19 for healthcare providers (NOpreventCOVID) (to test the percentage of positive SARS-CoV-2 RT-PCR test if fever or/and COVID-19)	NCT04312243	Phase II	Experimental: Treatment Group Inhaled NO (160 ppm) before and after the work shift. Daily monitoring of body temperature and symptoms. SARS-CoV-2 RT-PCR test if fever or COVID-19 symptoms No Intervention: Control Group Daily monitoring of body temperature and symptoms. SARS-CoV-2 RT-PCR test if fever or COVID-19 symptoms.	470	2 April, 2020	20 March, 2021	20 March, 2022	Massachusetts General Hospital	Not yet recruiting
4	Inhaled gaseous nitric oxide (gNO) antimicrobial treatment of difficult bacterial and viral lung (COVID-19) infections (NONTM)	NCT03331445	Phase II	Drug: Nitric Oxide 0.5%/Nitrogen 99.5% Gas for Inhalation Inhaled NO 160 ppm balance air	20	24 October, 2017	31 December, 2020	31 March, 2021	University of British Columbia	Active, not recruiting

Table V. *Continued*

Table V. *Continued*

Study title	ClinicalTrials.gov Identifier:	Study stage	Intervention/ Treatment	Sample size	Actual start date	Estimated primary completion date	Estimated study completion date	Sponsor	Status
(to see if NO therapy can reduce the bacterial load in the lungs, help the patients breath better; and in the case of COVID-19 act as an anti-viral agent resulting in the reduction of incidence of oxygen therapy, mechanical assistance of BIPAP, CPAP, intubation and mechanical ventilation during the study period.)									

SOC: standard of care; od: once daily; bd: twice daily; po: per os (orally); IV: intravenously; hrs: hours; D: day(s).

latter presented significantly lower SOD anti-oxidative activity (74). In addition to ceruloplasmin activity, copper chelation has been found to affect proteins involved in Fe metabolism, at the mRNA level, and in inflammatory diseases (69). Since inflammatory diseases and viral infections share the same inflammation signalling pathways, it could be inferred that exogenously administered copper may have anti-inflammatory effects in human viral infections, including COVID-19.

In acute inflammatory or infectious events as well as in inflammatory diseases, such as chronic cardiac disease, chronic kidney disease and inflammatory bowel disease, patients present high serum ferritin levels and iron deficiency with adverse clinical consequences (75). Ceruloplasmin is responsible for the reoxidation of Fe(II) to Fe(III), which is followed by loading of Fe(III) onto transferrin for systemic distribution to other sites (75). During inflammation, hepcidin levels increase in response to an IL-6 increase, causing degradation of the iron transporter ferroportin and reducing iron efflux from hepatocytes, enterocytes and splenic macrophages (75). This leads to a disruption in iron homeostasis, excess of iron stores (*i.e.* ferritin) and reduction in iron availability. *In vitro* and *in vivo* evidence have suggested that ceruloplasmin may have a role in iron trafficking across the enterocyte during inflammation, participating in host defence and balancing of ferritin levels (76, 77). In severe COVID-19 cases, high serum ferritin levels have been reported (31).

In viral infection, autophagy has been demonstrated to have an antiviral response to viral oxidative stress (78, 79). Autophagy enables cells to survive stress from an external environment attack, like a viral infection. Induction of autophagy, marked by autophagic vacuoles formation that degrade the viral invading proteins, limits the viral infection. It has been demonstrated that copper induces autophagy and apoptosis and is correlated with the formation of autophagic vacuoles maintaining the cell's anti-viral defence (80, 81). These findings, linking copper with autophagy and vacuoles formation, support further studies of copper as a candidate for the treatment of viral infections. The copper/autophagy interconnection opens potential therapeutic application studies and clinical development of copper to target COVID-19 infection.

N-acetylcysteine (NAC). NAC is the precursor of L-cysteine (82). It acts as a direct scavenger of ROS to regulate redox status, modulate inflammatory response and exhibit indirect antioxidant properties (82). In addition, a study revealed that NAC decreases airway inflammation and responsiveness in asthma, by modulating the tight junctional protein claudin 18 expression present in airway epithelial cells (82). Ozcelic *et al*., have shown that the administration of NAC in rats modulated redox system's antioxidant effects and reduced brain oxidative stress mediated by copper (83). Furthermore, hydroxyl radicals and radical production generated by CuI nanoparticles (or in the $CuSO_4 \cdot 5H_2O$ solution) in aqueous solution were blocked by NAC (58). This is also supported by Sagripanti *et al*., who found that inactivation of HSV by copper was enhanced by cysteine (59). This highlights the potential to use copper in the treatment of viral infections in combination with NAC, in order to reduce the redox properties of copper and avoid cellular damage.

Recently, a potential role of vitamin D deficiency in the development of insulin resistance and type II diabetes has been suggested (84, 85). Also, a study showed that the administration of NAC in vitamin D-deficient mice restored insulin resistance and suggested that oxidative stress could be the primary cause of insulin resistance by vitamin D deficiency (86). All the above, together with the current knowledge that diabetics are vulnerable to COVID-19 and the possible link between VDI and severe COVID-19 disease (32), reveal that NAC could have a potential benefit against COVID-19. Furthermore, NAC exhibits some indirect antioxidant effects by increasing manganese superoxide dismutase (MnSOD) activity and preventing sepsis-induced diaphragmatic dysfunction and hyperoxic lung injury in animal models (87, 88). Copper/zinc superoxide dismutase (Cu/ZnSOD) and MnSOD play a key role in protecting cells from oxidative stress-mediated toxicity, however data have shown that in high concentrations they effectively cleave RNA (89).

Colchicine. Due to its anti-inflammatory effects, colchicine could be used to limit the cytokine storm (90). Colchicine primarily acts *via* tubulin disruption, causing modulation of innate immunity, followed by down-regulation of several inflammatory pathways (90). Recent data have shown that colchicine inhibits macrophage pathways through three mechanisms: the inhibition of NACHT-LRRPYD-containing protein 3 (NALP3) inflammasome, the inhibition of pore formation triggered by purinergic receptors and the stimulation of the maturation of dendritic cells and antigen presentation (90). It also presents anti-fibrotic properties and high endothelial activity (90). Because of its anti-inflammatory mechanism, colchicine could be included among the drugs chosen for the symptomatic treatment of COVID-19. Colchicine's potentially beneficial effects could reduce severe COVID-19 inflammatory symptoms, especially cardiovascular complications and sepsis (91, 92). The results of a recent trial assessing the safety and efficacy of the use of low-dose colchicine (0.5 mg daily) in patients who survived from a recent myocardial infarction, showed a statistically significant reduction of cardiovascular complications compared to placebo (92). Currently, there are four ongoing clinical trials regarding the use of colchicine, presented in Table IV (15-18).

Nitric oxide (NO). Another active substance currently tested in clinical trials for SARS-CoV-2 is NO gas (19-22). NO is an important signalling molecule and when inhaled, it produces pulmonary vasodilation (93, 94). It is produced by three main enzymes in mammalian cells, neuronal (n-NOS), endothelial (s-NOS) and inducible nitric oxide synthase (i-NOS). These enzymes catalyse the conversion of L-arginine to NO and L-citrulline (95). The inducible NO increases during virus infection and this can initiate either inhibition or stimulation of the viral infection (93, 96-102). Akerstrom *et al*. in 2009 showed that NO interferes with the replication of SARS-CoV in at least two ways: an effect in the production of the RNA of the virus in the early steps of replication and a depletion in the palmitoylation of the S protein towards the end of the replication cycle (102). Another study by Akerstrom *et al*. in 2005 concluded that NO prevented the replication cycle of SARS-CoV mainly during the early steps of infection and confirmed that the production of NO by i-NOS has an antiviral effect. However, the production of NO should be optimised in order to have an antiviral rather than a damaging effect (103). Keyaerts *et al*. have demonstrated that S-nitroso-N-acetylpenicillamine (SNAP), a NO donor agent, reduced the activity of SARS-CoV replication at non-toxic levels (222 μM) (104). The amount of NO that was released by 222 μM SNAP was approximately 30-55 μM (104). There are currently two ongoing phase II trials of NO that are recruiting and two trials that are listed but not active (19-22). More information regarding NO clinical trials is available in Table V.

Discussion

The need for a safe and effective treatment is becoming more and more pressing due to the high rates of COVID-19-related mortality observed across the globe. The selection of agents for a therapeutic scheme for SARS-CoV-2 was based on efficacy and safety data, their mechanism of action and potential interactions. The ability of the agents to boost the human body's physiological response to inflammation, their potential to assist in the homeostasis of clinical markers of inflammation as well as to act at early stages of the disease, were also considered. Figure 2 presents the synergistic actions of an antiviral agent (*e.g*. RDV or EIDD-2801), copper, NAC, colchicine and NO against COVID-19 and its clinical manifestations. Copper may increase ceruloplasmin levels and therefore boost the human body's response to inflammation. Evidence has shown that ceruloplasmin can balance the high levels of ferritin and participate in host defence (75-77). In addition, copper may induce autophagy and apoptosis, maintaining cell anti-viral defence (80, 81). It has been suggested that NAC contributes to immune response *via* increasing MnSOD activity with high levels of MnSOD causing RNA cleavage (89). NAC may also alleviate insulin resistance by vitamin D deficiency, revealing NAC's potential benefit for diabetics with COVID-19 disease (86). RDV, copper and NO can act synergistically in the early stages of COVID-19 infection inhibiting RNA replication (35, 36, 59-65, 102). Copper's virucidal activity is also related to ROS-mediated virus capsid protein oxidation (58, 65). NAC may protect host cells against copper-induced oxidative stress (82). It has been suggested

that cysteine (NAC's natural form) and glutathione, as stronger ligands, may remove copper from DNA *in vivo* (105) protecting the host cells from copper toxicity. This is in line with Sagripanti's *et al*. observation that cupric ascorbate irreversibly stops HSV replication, while cell recover (59). These support the hypothesis that the potential advantage of copper virucidal effects could be attributed to the capacity (*i.e*. cysteine and glutathione) of the host cell to repair the extensive copper-mediated molecular damage much faster for itself, than for the virus (59, 105). Colchicine, as a very potent anti-inflammatory medicinal product, can block several inflammatory pathways, including NALP3 inflammasome (90), protecting vital organs such as the heart, lungs and kidneys from severe inflammatory symptoms including sepsis and death (26, 91, 92). NO also acts on the lungs causing pulmonary vasodilation, which can improve oxygenation (106). The above presented mechanisms provide a basis for a potentially effective combination treatment against COVID-19.

Literature data suggests a favourable safety profile for the proposed treatments. In general, RDV was found to be adequately tolerated with typical antiviral drug side effects including anorexia, nausea and vomiting (107). Hepatotoxicity and transaminases increase are the main adverse reactions that have been reported with RDV use (108). Copper's related chronic or acute toxicity to humans is very rare as serum levels are regulated by an effective homeostatic mechanism that reduces absorption and increases excretion in case of excess copper intake (109). Therefore, copper supplementation adverse events may be reported in doses >10 mg/day, taken for more than 12 weeks (109). Chronic exposure to high levels of copper (30 mg/day) can result in gastrointestinal symptoms (*e.g*. abdominal pain, cramps, nausea, diarrhoea and vomiting), kidney and liver toxicity, resulting in coma (109-111). It is also known that the lowest acutely fatal dose in man is about 10 g of copper (110). Copper and ceruloplasmin normal serum concentrations are 10-25 mcmol/l (63.5-158.9 mcg/dl) and 180-400 mg/l respectively (111). NAC's reported adverse reactions are uncommon and include hypersensitivity reactions (bronchospasm, dyspnea, pruritus, urticaria, rash, angioedema and tachycardia), headache, tinnitus, stomatitis, abdominal pain, nausea, vomiting, diarrhea, pyrexia and low blood pressure (112). Colchicine is potentially toxic with a narrow therapeutic window, and common adverse reactions that include abdominal pain, nausea, vomiting and diarrhea (113). It may also cause severe bone marrow depression; therefore, periodic blood checks are essential (113). Hepatotoxicity, renal damage, myopathy and rhabdomyolysis have also been reported with colchicine (113). The most common side effects of NO are thrombocytopenia, hypokalaemia, hypotension, atelectasis and hyperbilirubinaemia (106). Hepatotoxicity, thrombocytopenia, gastrointestinal, cardiovascular disorders and renal function should be closely monitored as they have been reported in more than one agent and the co-administration of these medicines may augment these adverse events.

Concerning drug interactions between the proposed medications, no interactions have been reported between colchicine, NAC and copper (114). Concerning RDV's interaction with other drugs, no drug-drug interaction studies have been conducted with RDV (115). The only recommendation that is provided concerning RDV use with other medicinal products is that RDV should not be used with other drugs that have significant hepatotoxicity (115). Copper absorption can be increased by natural polybasic amino acids, high protein diet, and decreased by fiber (*i.e*. hemicellulose), fructose, ascorbic acid, cysteine and divalent metals, such as molybdenum, iron and zinc (116). Therefore, NAC may reduce copper's absorption. No drug interaction studies have been performed for NO (106). The only known interaction is with nitric oxide donor compounds, such as prilocaine, sodium nitroprusside and nitroglycerin due to the risk of developing methemoglobinemia (106). Macrolides, such as azithromycin, an antibiotic used for the treatment of COVID-19, increase the level or effect of colchicine therefore, co-administration should be avoided or the dose of colchicine should be adjusted (114). HIV-protease inhibitors, such as lopinavir and ritonavir, are also reported to increase the exposure to colchicine and concurrent administration should be avoided (114).

RDV is administered intravenously for a period of ten days at a dose of 200 mg the first day and 100 mg for the next nine days in all COVID-19 clinical trials (40-45). Based on preliminary results from a recent study, EMA's Committee for Medicinal Products for Human Use (CHMP) has recommended a treatment duration of 5 days of RDV alongside the longer 10-day course, suggesting that for patients not requiring mechanical ventilation or extracorporeal membrane oxygenation (ECMO), the treatment course may be shortened from 10 to 5 days without any loss in efficacy (117). Copper absorption studies suggest that doses of approximately 2.5 mg and 5 mg result in the same amount of copper absorbed by the body (118). Therefore, since there is no difference in the amount of copper absorbed, the lower dose of 2.5 mg could be preferable in order to balance possible hepatotoxicity. NAC is indicated for use in adults as a mucolytic in respiratory disorders such as bronchitis, emphysema, mucoviscidoses and bronchiectasis at a dose of 600 mg/day (112). However, a lower dose than that of 600 mg may be more appropriate as cysteine may have an inhibitory role in copper utilization (116). The four ongoing clinical trials with colchicine use a low dose of 0.5 mg twice daily or 1 mg once daily in order to limit myocardial necrosis and pneumonia development in patients with COVID-19 (15-18). The administration of NO for 3 days at 30 ppm (part per million) improved

oxygenation and reduced the time of ventilator support in SARS-CoV outbreak in 2004 (20). According to the NO's summary of product characteristics (SmPC), the maximum recommended dose is 20 ppm in pulmonary hypertension for adults associated with heart surgery, however, the dose may be increased up to 40 ppm if no sufficient clinical effects are observed (106). The two currently ongoing phase II clinical trials for COVID-19 use a different posology. In NCT04305457 trial, NO is used at 140-180 ppm for a period of 20-30 min, twice daily for 14 days and in NCT04306393 trial 80 ppm of NO are given for 48 h, followed by 40 ppm, followed by weaning with PaO2/FiO2 ratio ≥300 for 24 h before stop (20, 21).

Chloroquine and hydroxycholoquine, currently authorised for treating malaria and certain autoimmune diseases, are also being tested against SARS-CoV-2 (119). Despite the rationale of them being antimalarial medicinal products, their effectiveness in SARS-CoV-2, as well as the pre-clinical evidence of chloroquine's and hydroxycholoquine's shown efficacy, their safety profile is rather concerning (120-125). It has been shown that several patients may experience rare but potentially fatal side effects including: serious cutaneous adverse reactions (SCARS), liver failure and ventricular arrhythmias (especially when combined with azithromycin) (120-125). Although other antimalarial medicinal products with a more favourable safety profile are available, no evidence was found regarding their potential use in COVID-19 patients. Other treatments such as interleukin-6 inhibitors sarilumab (Kevzara) and tocilizumab (Actemra), CCR5 (chemokine receptor 5) inhibitors such as lerolimab and convalescent plasma are being tested in clinical trials (23-25). Efficacy and safety data on the use of these agents against SARS-CoV-2 are yet limited. Plasma transfusion has been associated with various transfusion-related risks such as lung injury, circulatory overload and allergic/anaphylactic reactions (23). Plasma also contains ferritin, which may further increase COVID-19 inflammation-mediated ferritin levels with potentially serious adverse reactions.

According to a recent worldwide phylogenetic network analysis of SARS-CoV-2 genomes, there are three central variants of SARS-CoV-2 named A, B and C distinguished by amino acid changes (126). It is speculated that A type is the ancestral, B is derived from A and C from B. All three types of genomes appear to be present (126). According to Forster *et al*., this is attributed to parallel evolution events as expected in an ongoing outbreak (126). A question though arises: Why do only three central variants exist? Arumugam *et al*. identified three robust clusters, later named enterotypes (Bacteroids, Prevotella and Ruminococcus), of the human gut microbiome, which similar to COVID-19 variants are neither nation nor continent-specific (127). Accumulating evidence suggests that the gut microbiota plays a key role in the absorption, metabolism and storage of nutrients (128, 129). Therefore, the absorption and metabolism of nutrients and medicinal products may be influenced by the gut microbiota. This may also somehow explain the presence of the three COVID-19 variants. Preliminary evidence in animal models suggests a negative correlation between copper concentration and Ruminococcus bacteria (130). However, the possible underlying mechanisms require further investigation and this is out of the scope of this review.

Since copper exhibits strong virucidal effects, acting on the virus itself (53-66), it could be combined with NAC at the early stages of the infection to decrease viral RNA levels (58, 59, 89). Therefore, in combination with the blocking of RNA replication by antivirals (such as RDV or EIDD-2801), copper, NAC and NO could potentially contain or even stop the infection at early stages.

Conclusion

Early in March 2020, the WHO declared coronavirus disease (COVID-19) as a pandemic. Since then, thousands of people have suffered and died, making the need for a treatment of SARS-CoV-2 more crucial than ever. While there are similarities between H1N1 and SAR-CoV-2, COVID-19 is nothing like the "flu". COVID-19's inflammatory response is much more difficult to turn off, causing vital organ damage and in some cases death. Therefore, a multi-treatment approach with agents that can block the cascade of viral infection and inflammation at different steps is considered the most appropriate. Based on the efficacy and safety data presented, the authors propose the combination of these five agents (RDV, copper, NAC, NO and colchicine) as a potentially effective treatment against SARS-CoV-2. Further studies such as randomized, double blind, placebo-controlled trials with combination of treatments are required to establish efficacy and safety against COVID-19.

Conflicts of Interest

The Authors have no conflicts of interest regarding this review paper.

Disclaimer

The opinions expressed in this manuscript are solely those of the authors and should not be understood or quoted as being made on behalf of or reflecting the position of the authors' affiliated organizations.

Authors' Contributions

Andreou A and Trantza S: Main authors of this article with experience in clinical safety assessment. Filippou D, Sipsas N and Tsiodras S: Revised the article during the whole process.

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