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Zinc Ions-Induced Immunology for SARS-CoV-2 Infectious Prevention and Severe Acute COVID-19 Defenses

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Abstract

Zinc (II) ions-immune antiviral activities for SARS-CoV-2 prevention and COVID-19 infectious defenses of severe bronchitis and acute pneumonia are argued, and the molecular mechanism has been partly clarified by zinc ions-centered tetrahedrally coordinated binding to catalytic triad of Ser, His, Asp residues. The 2019-nCoV RNA or SARS-CoV-2 is RNA virus with rapid mutation rate that the virus structure at least contains four viral proteins, the spike (S) protein, the membrane (M) protein, the envelope (E) protein, and nucleocapsid (N) protein. Zinc ions could inhibit virus entry and membrane fusion of S1 and S2 domains of spike protein with zinc ion-binding interaction. Zinc homeostatic status of zinc(II) has antiviral effects, improves immune responses and suppresses viral replication. The zinc-homeostatic immune concentration may provide a protective role against the COVID-19 pandemic, likely by improving the host's resistance against viral infection. Zn^{2+} ions can prevent in the early stage of SARS-CoV-2 infected patient with antiviral zinc homeostatic immunity and have important roles for respirarory and pulmonary process of COVID-19 disease. Zn^{2+} inhibits corona-virus and anterivirus RNA polymelase activity, and the combination of Zn^{2+} and pyrithione at low concentrations inhibits the replication of SARS-CoV and arterivirus RNA.

Zinc-finger antiviral protein (ZAP) controls viral entry, DNA/RNA replication, and spreading against viral infection. ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation that ZAP inhibits Retroviral RNA production and HIV-1 infection by promoting the degradation of specific viral mRNAs. The mutations of both protein and RNA at the RNA-ZAP interacting surface reduce the in vitro binding affinity and antiviral activity, in which ZAP coordination promotes downstream RNA degradation. Thus, ZAP could be found to restrict SARS-CoV-2 RNA virus replication. However, this ZAP's efficiency for COVID-19 RNA mutation remains yet unclear.

Zn^{2+} ions-induced prevention and antibody against SARS-CoV-2 infection are required with Zn homeostatic immune concentration 50 mg/day, Zn supplementation in combination with CQ/HCQ, and transient receptor potential vanilloid 1 (TRPV1) prevention. Lower Zn^{2+} concentration may be efficient for vaccine candidate and higher Zn^{2+} concentration may prevent respiratory ailment and acute pneumonia spreading against human coronaviruses (HCoVs).

Zn^{2+} ions-induced virucidal defenses from COVID-19 severe bronchitis and acute pneumonia are required that zinc ions can prevent in the early stage of 2019-nCoV infected patient, and the zinc ions have important roles for respirarory and pulmonary process of COVID-19 disease. Zn^{2+} ions-induced virucidal defenses from COVID-19 severe bronchitis and acute pneumonia are required that the transmembrane protease, serine 2 (TMPRSS2) inhibitors block the cellular entry of the SARS-CoV-2 virus through the downregulated priming of the SARS-CoV-2 spike protein.

In order to prevent that an outbreak of respiratory sickness caused by a novel coronavirus (Covid-19) has become a serious public threat and disrupted many lives, assessing the efficacy of Zn-ejector drugs such as disulfiram combined with interferon to treat Covid-19 infected patients has been proposed. SARS coronavirus envelope protein promotes virus fitness and pathogenesis, in which E protein ion channel activity represents a new determinant for SARS-CoV virulence.

For Zn^{2+} ions-induced virucidal defenses from COVID-19 acute pneumonia, the antiviral compounds including zinc N-ethyl-N-phenyldithio-carbamate (EPDTC) inhibit the viral protease, thus preventing humancoxsackievirus strain B3 (CVB3) genome replication. Transient zinc chelation (TPEN) induces endoplasmic reticulum (ER) stress and antiviral response by activating NF-κB leading to induction of interferon signaling and zinc plays divergent roles in rotavirus and dengue virus infections. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C and 3C-like proteases. Thus, zinc-ions complexes as SARS-CoV-2 3C-like protease inhibitors may play important role for this Zn^{2+}-centered coordination pattern that the zinc-coordinating inhibitor is tetrahedrally coordinated binding to such as the catalytic triad of Ser, His, Asp residues.

Keywords: *2019-nCoV RNA mutation, ZAP and ZBD, RNA degradation, Zinc chelation, Zinc-coordinated inhibitor*

Abbreviations

ADAR= adenosine deaminases acting on RNA, **ACE2=** angiotensin-converting enzyme 2, **APN=**Amino-peptidase N protein, **ARDS=**acute respiratory distress syndrome**, CoVs=**coronaviruses, **COVID-19=**coronavirus disease 2019, **CPGs=**3'-O-(2'-Deoxy-5'-guanylyl)-2-'-deoxy-5'-cytidylic acid, **CQ/HCQ=** chloroquine/hydroxychloroquine. **CVB3=** humancoxsackievirus strain B3, **EPDTC=**N-ethyl-N-phenyldithio-carbamate, **ER=**endoplasmic reticulum, **FDA=**food and drug administration, **HCoV=**human coronavirus, **HR1=**heptad repeat 1, **IFITMs=**Interferon induced transmembrane proteins, **INFs=**interferons, **ISGs=**Interferon-stimulated genes, **MERS-CoV=**Middle East respiratory syndrome coronavirus, **MLV=**murine leukemia virus, **NAC=**N-acetyl-cysteine, **ORFs=**open reading frames, **2019-nCoV=**novel coronavirus 2019, **PCR=**polymerase chain reaction, **PPI=**protein-protein interaction, **RCT=**randomized controlled trial, **RdRp=**RNA-dependent RNA-polymerase, **ROS=**reactive oxygen species, **RSV=** respiratory syncytial virus, **SARS-CoV=** severe acute respiratory syndrome coronavirus, **SARS-CoV-2=**severe acute respiratory syndrome coronavirus 2, **SIN=** Sindbis virus, **SNP=**single nucleotide polymor-phisms, **6-HB=**six-helical bundle, **TMPRSS2=** transmembrane protease, serine2, **TPEN=**N,N,N',N' -tetrakis(2-pyridinyl-methyl)-1,2-ethanediamine,**TPEN=**transient zinc chelation, **TRPV1=**transient receptor potential vanilloid 1, **ZAPs=**zinc finge r antivirus proteins, **ZNFs=**zinc-finger proteins, **UTR=**untranslated region.

Introduction

Zinc promotes immunity for coronavirus, reduces allergic reactions and leads to anti-inflammation that zinc-induced ROS generation in COVID-19 respiratory ailment and pneumonia occurs **[1].** Zinc deficiency is a risk factor for the development of allergic disease or a secondary outcome of these diseases that zinc deficiency is supposed to play a role in the pathogenesis and severity of allergic skin and respiratory tract diseases. Therefore, maintaining normal zinc levels might lower the risk of development and progression of allergic diseases, anti-inflammatory and anti-allergic effects of Zinc are very important to prevent skin and airway diseases of the organism, subsequent to losing these beneficial effect **[2].**

Zinc is a fundamental trace element in human body that the recommended daily intake of zinc depends on several factors. Average values of recommended intake may be 7~11 mg/day for adults. Zinc is the second abundant trace metal with human body 2~3 g and a plasma concentration of 12-16 μM, 90% in muscle and bone, and 10% other organs include prostate, liver, the gastrointestinal tract, kidney, skin, lung brain, heart, and pancreas in humans that cellular zinc underlies an efficient homeostatic control that avoids accumulation of zinc in excess. Zinc status play an important role in antiviral immunity, mainly during the early stage of the infection that the most effective antiviral antibodies are neutralizing antibodies which bind to the viral envelope or capsid proteins, and regulate

the virus entering into host cell **[3].** In mammals, the plasma concentration of zinc ranges from 14 to 23 μmol/l under normal physiological conditions, and serum zinc accounts for only 0.1% of the body's total zinc pool, 80% loosely bound by albumin and 20% bound by macroglobulin. Thus, sufficient daily intake of zinc is required to achieve steady state levels. In order to meet the daily requirement, the World Health Organization recommends a daily zinc intake of 9.4~10 mg and 6.5~7.1 mg for men and women, respectively **[4].** A variety of effects of zinc on immune cells depend on the zinc concentration. In a concentration of 100 μmol/L, zinc suppresses natural killer cell killing and T-cell function whereas monocytes are activated direcly, and in a concentration of 500 μmol/L, zinc evokes a direct chemotactic activation of neutrophil granulocytes **[5].**

Zinc(II) ions play important role for these infectious diseases. Zinc is essential for highly growth and develop-ment of all organisms in the human body, especially the immune system that zinc has antiviral effects; it improves immune responses and suppresses viral replication. Zinc homeostatic status is a key factor in maintaining a healthy immune system. Zinc ions are involved in regulating intracellular signaling pathways in innate and adaptive immune cells that the influences of zinc status on the overall immune function are present in zinc defficiency as overproduction of pro-inflammatory cytokines and reactive mediators, zinc homeostasis as balanced immune cell functions, and zinc excess as suppression of T and B cell functions **[6].** Zn^{2+} ions have an important role for RNA viral destruction that the zinc-finger antiviral protein could regulate RNA virus degradation of SARS-CoV's and MERS-CoV's RNA virus. Zinc ions can lead to RNA virus degradation and to viral death by a receptor-destroying enzyme **[7].** Zinc ions become used as Zn^{2+}-coordinated inhibitors for viral regulation of virucidal activities **[8].**

Human coronaviuses (HCoVs) are recognised as coronaviruses (CoVs) associated with multiple respiratory diseases of varying severity, including common cold, pneumonia and bronchilitis that to date, seventh known HCoVs have been identified, namely HCoVs-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and recently new-typed 2019-nCoV or SARS-CoV-2, corona-virus disease 19 COVID-19, subsequent phylogenetic studies pointed to the bat origin of SARS-CoV based on sequences of SARS-like virus found in bats **[9].** After the first outbreak of coronavirus disease 19 COVID-19 infection, human-to-human transmission has accelerated the outbreak and case reports have started from other countries that coronaviruses are viruses whose genome structure is best known among all RNA viruses and prevention in this early stage of COVID-19 outbreak has been proposed that very recently, novel coronavirus (COVID-19) outbreak and quickly serious large spread worldwide caused by SARS-CoV-2 infection has posed a serious threat to global public health **[10].** This 2019-nCoV has single-stranded positive-sense RNA virus with 5'-cap and 3'-poly-A tail and the genome size of ~30kb which is the largest among all RNA viruses that caused a major outbreak of coronavirus disease 2019 COVID-19 and instigated a widespread fear and has threatened global health seculity **[11].** Factor of present outbreak of disease 19 COVID-19 may be considered to be due to RNA virus mutation that the RNA viruses have high mutation rate and these high rates are correlated with enhanced virulence and evolvability, traits considered beneficial for viruses **[12].**

Furthermore, zinc-finger proteins (ZNFs) for health and disease have play an important role with DNA, RNA, PAR (poly-ADP-ribose) and ZFNs are involved in the regulation of several cellular processes such as transcriptional regulation, ubiquitin-mediated protein degradation, signal transduction, DNA repair, cell migration, and other processes **[13].** Increasing the intracellular Zn^{2+} dose could capably damage the replication of a wide range of RNA viruses, such as poliovirus, influenza virus and SARS-CoV, and equine arteritis virus (EAV) **[14].** Thus, zinc ions can prevent in the early stage of COVID-19 coronavirus outbreak, and in the final stage could defend COVID-19 infection from severe respiratory and acute pulmonary disease.

In this review, firstly SARS-CoV-2 molecular structure with RNA virus mutation is described. Nextly. Zn^{2+} ions-induced homeostatic immune infectious activities for preventative and antibody SARS-CoV-2, and defending of severe respiratory and acute pulmonary COVID-19 patient are discussed under the concept that zinc-finger antiviral proteins (ZAPs) inhibit virus entry, RNA replication, and spread, promote RNA virus degradation, and generate ROS in respiratory and

pulmonary COVID-19, lastly, in which the molecular mechanisms of zinc ions-centered coordinated binding are clarified against SARS-CoV-2 infection.

Zinc Immunity in Infection

Zinc has a regulation of immunity that zinc homeostasis in immune system pathways is complex, since it participates both in pro-inflammatory and regulatory pathways, and it seems clear that deficient or excessive zinc levels can lead to malfunction of the adaptive and innate immune systems. Inflammation is a natural process required to protect the host from tissue damage and infections, which leads to the resolution of the inflammatory response and the restoration of homeostasis. Zinc regulates the proliferation, maturation and functioning of lymphocytes, and other leukocytes **[15].** The innate immune system represents the defense first line against a pathogen before the adaptive system can develop the appropriate response. Zinc is involved in inflammation, elevating inflammatory responses and inducing cell-mediated immunity, and is a key component of pathogen-eliminating transduction pathways that contribute to neutrophil extracellular traps**.** Many organs are affected by zinc deficiency, especially the immune system that is markedly susceptible to changes of zinc levels which the immune response involves in the regulation of the innate and adaptive immunity, and this zinc homeostasis is critical for sustaining proper immune function **[16].**

Reference Intakes recommended intake for the adult of 11 mg/day for males and 8 mg/day for females. However, besides of reduced zinc dietary intake, some age-related factors including intestinal absorption, drug interactions, subcellular processes, among others, may jeopardize his activity. Zinc supplementation must be assessed individually, considering cases of zinc deficiency, low dietary intake, and related diseases. Evaluated zinc supplementation with different doses and duration, 20~40 mg/day appears to be a safe and effective dosage **[17].**

Zinc binding to proteins in high zinc concentration can activate or inactivate thir activity, whiles, zinc homeostasis is primarily controlled via the expression, but also the transport of zinc into one of those organelles that zinc homeostasis during acute phase response is the temporal transfer of serum zinc to the tissues, causing transient serum hypozincemia, which is rebalanced during resolution of the inflammatory response that intracellularly increased zinc can intoxicate engulfed pathogens and acts cytoprotective by promotion of neutralizing reactive oxygen species (ROS) and nitrogen species (RNS) **[18].**

SARS-CoV-2 Spike, Membrane, Envelope, and Nucleocapsid Proteins, and 2019-nCoV RNA Mutation

Coronaviruses (CoVs) are viruses whose genome structure is best known among all RNA viruses that typical CoV genome contains at least six open reading frames (ORFs) which the first ORF(ORF1a/b) is about two-thirds of the whole genome length encodes 16 non-structural proteins, in which ORFs near 3' end of the genome encodes at least four main structural proteins including spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins **[19].** Most of the non-structural proteins have encodes viral polymerase, RNA-dependent-RNA-polymerase (RdRp)**.** CoVs are enveloped, spherical or pleiomophic viruses, with typical sizes ranging from 80 to 120 nm that they possess a 5' capped, single-strand positive sense RNA genome with a length between 26.2 and 31.7 kb. 2019-nCoV makes use of a densely glycosylated S protein to gain entry into host cells. The S protein is a trimeric class I fusion protein that exists in a metastable prefusion conformation that undergoes a substantial structural rearrangement to fuse the viral membrane with host cell membrane. Spike is cleaved into S1 and S2 by the host cell protease that the main function of S1 is to bind ewith the host cell surface receptors, and the S2 submit mediates virus-cell and cell-cell membrane fusion, Hence, the therapeutic stragies to block coronavirus from entering host cells by spike proteins or specific receptors on the host surface may be valuable for the antiviral development **[20].** The ionophore in this RNA virus makes the cell membrane porous to the zinc ion that Zn^{2+} ions could inhibit virus entry and membrane fusion of S1 and S2 domains of Spike Protein with zinc ion-binding interaction**.** This process is triggered when the S1 submit binds to a host cell receptor, and receptor binding destabilizes the prefusion trimer, resulting in shedding of the S1 subunit and transition of the S2 subunit to a stable postfusion conformation **[21].** The SARS-CoV-2 E protein is a small, integral membrane protein involved in several aspects of the virus' life cycle that the most progress has been made on SARS-CoV E, highlighting specific structural requirements for its functions in

the CoV life cycle as well as mechanism behind its pathogenesis**[22].** The typical CoV genome includes a 5'-cap, 5'-untranslated region (UTR), open reading frames, a 3'-UTR, and 3'-poly(A) tail. In addition, SARS-CoV-2 has a unique four amino acid insertion between S1 and S2 domains of the spike protein, which created a potential furin or TMPRSS2 cleavage site. 2019-nCoV may increase its infectivity through the receptor binding domain recombination and a cleavage site insertion **[23]**.

Mutations and adaptation in the S and N genes could affect virus stability and pathogenicity. As more genomes are made publicly available, analysis of the genome sequence diversity across samples has revealed the highest diversity occurring in the structural genes, especially the S protein, ORF3a, and ORF8 **[24].** Thus, the major mutations are in the critical proteins, including the S protein, RNA polymerase, RNA primase, and nucleoprotein [**25**]. This nove Yinl coronavirus (SARS-CoV-2) outbreak has caused a global pandemic resulting many infected persons and deaths worldwide that the RdRp catalyzed the synthesis of viral RNA, is a key component of coronaviral replication/transcription as a primary targeted antiviral drug **[26].** It is unclear whether zinc ions can suppress RNA mutation and outbreak by RNA mutation.

Zinc-Finger Antiviral Protein Inhibits Virus Entry, RNA Replication and Spread and Promotes RNA Degradation

The zinc-finger antiviral protein (ZAP) controls virus entry, DNA/RNA replication, and spreading against viral infection. The ZAP in first steps of HCV infection may be used as entry inhibitor [**27**]. Interferon induced transmembrane proteins (IFITMs) inhibit the cellular entry of a broad range of viruses that IFITM-mediated restriction requires recognition of viral RNA elements [**28**]. The interferon-stimulated genes serve as enhancers of antiviral innate immunity [**29**]. ZAP inhibits alphavirus replication that elucidation of the antiviral mechanism by which ZAP inhibits Sindbis virus (SINV) translation may lead to the development of agents with broad activity against alphaviruses [**30**]. The ZAP inhibits Influenza A virus (IAV) protein expression, in which suggests an important role of ZAP in the host effort to control IAV infection and the importance of the threat of ZAP to the virus [**31**]. The host cell restriction factors that limit IAV have been investigated [**32**]. ZAP also may regulate DNA and RNA virus replication. Inhibition of bacterial DNA replication during nitrosative stress is accompanied by zinc mobilization **[33].** Zn72D both 20 regulates adenosine deaminases acting on RNA (ADAR) protein levels and interacts with ADAR in an RNA-dependent fashion, and similar to ADAR, Zn72D is necessary to maintain proper neuromuscular junction architecture and motility in the fly **[34].** ZAP inhibits Retroviral RNA production **[35]** that ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation **[36].** The four zinc fingers of ZAP form extensive interactions with RNA, but mutations of both protein and RNA at the RNA-ZAP interacting surface reduce the *in vitro* binding affinity and antiviral activity, in which ZAP coordination promotes downstream RNA degradation **[37].** ZAP could inhibit SARS-CoV-2 RNA virus replication that SARS-CoV-2 is highly susceptible to interferons (IFNs), specifically targets of 3'-O-(2'-Deoxy-5'-guanylyl)-2'-deoxy-5'-cytidylic acid (CpG) dinucleotides in viral RNA sequences restricts SARS-CoV-2, and might motivate assessment of combination therapies including IFN for treatment of COVID-19, in which ZAP restricts SARS-CoV-2 and contributes to its inhibition by IFNs **[38].**

Furthermore, ZAP inhibits HIV-1 infection by promoting the degradation of specific viral mRNAs [**39**]. ZAP is a host antiviral factor that specifically inhibits the replication of Moloney murine leukemia virus (MLV) and Sindbis virus (SIN) by preventing accumulation of the viral mRNA in the cytoplasm. mRNA degradation, namely, the mRNA decay is largely determined by the cis-acting elements. ZAP directly interacted with the exosome component and that the binding region of ZAP was mapped to amino acids 224–254. Depletion of the exosome component with small interfering RNA significantly reduced ZAP's destabilizing activity which ZAP is a trans-acting factor that modulates mRNA stability **[40].** Thus, ZAP could inhibit SARS-CoV-2 RNA virus replication.

Zinc binding domain **(**ZBD) also could regulate 2019-nCoV RNA spike that zinc-binding status having Zn^{2+} ions-centered coordination structure could serve as the development of potential drugs for SARS therapies. A complex zinc finger ZBD modulates the enzymztic activities of coronaviridae-Nidovirus helicases, leading that the ZBD is critically involved in nidovirus replication and transcription [**41].**

ZAP regulates spread that ZAP' stress with antiviral activity and induced virus replication are regulated upon virus infection to inhibit virus spread [**42**]. ZAP-70 kinase regulates HIV cell-to-cell spread that HIV usurps components of the immunnological synapse machinery to ensure its own spread through cell-to-cell contacts [**43**]. An understanding of viral cell-to-cell transmission spreading will enhance our ability to intervene in the efficient spreading of viral infection [**44**].

Zn^{2+} Ions-Induced Prevention and Antibody Against SARS-CoV-2 Infection

Zinc is known to modulate antiviral and antibacterial immunity and regulate inflammatory response that the individual preventive and protective measures drive the personal risk of getting the disease. Zinc ions inhibit the RNA-dependent RNA polymerase, which crucially replicates copies of viral RNA in the host cells. Remdesivir inhibits coronavirus with the intact proofreading, thus renders its superior antiviral efficacy. Zn status inhibits respiratory syncytial virus (RSV) infection. Particularly, whole blood zinc was significantly lower in children with RSV pneumonia and Zn compounds were shown to inhibit respiratory syncytial virus replication and RSV plaque formation with a more than 1,000-fold reduction at 10 μm Zn preincubation. Thus, Zn may possess protective effect as preventive and adjuvant therapy of COVID-19 through reducing inflammation, improvement of mucociliary clearance, prevention of ventilator-induced lung injury, modulation of antiviral immunity **[45].** Enhancement of zinc immunity for preventing infection with the coronavirus SARS-CoV-2 that causes COVID-19 is urgently needed. Trials of prophylactic drugs or physical prophylaxis are often performed for infections, such as infection with the malaria pathogen Plasmodium falciparum or with influenza virus, respectively. Clinical trials are being set up at a rapid rate to test various approaches to preventing COVID-19 that **s**uch impaired antibody-mediated responses could be restored by zinc supplementation **[46].** Higher intracellular zinc concentration has shown to increase monocyte resistance to apoptosis via suppressing the activation of caspase, zinc 50 mg/day might provide an additional shield against the COVID-19 pandemic, possibly by increasing the host resistance to viral infection to minimize the burden of the disease. The potential beneficial role of zinc in COVID-19 infection needs further clinical validation, however, in this pandemic situation, using zinc to reduce disease burden would be a well-intentioned trial **[47].**

Zinc induced preventative antibody that neutralizes SARS-CoV-2 binds a conserved epitope on the spike receptor binding domain explaining its ability to cross-neutralize SARS-CoV and SARS-CoV-2, using a mechanism that is independent of receptor binding inhibition. This antibody will be useful for development of antigen detection tests and serological assays targeting SARS-CoV-2. Neutralizing antibodies can alter the course of infection in the infected host supporting virus clearance or protect an uninfected host that is exposed to the virus. Hence, this antibody offers the potential to prevent and/or treat COVID-19, and possibly also other future emerging diseases in humans caused by viruses from the Sarbecovirus subgenus **[48].**

In order to prevent that an outbreak of respiratory sickness caused by a novel coronavirus (Covid-19) has become a serious public threat and disrupted many lives, assessing the efficacy of FDA-approved Zn-ejector drugs such as disulfiram combined with interferon to treat COVID-19 infected patients has been proposed, in which based on evolutionary and physical principles of the key factors controlling the reactivity of Zn-bound Cys, having identified putative labile Zn-sites in COVID-19 that can be targeted by Zn-ejector drugs, leading to Zn^{2+} release and viral structure/function disruption [**49].**

Zinc ions anti-inflammatory transient receptor potential vanilloid 1 (TRPV1) prevention against COVID-19 would lead one to look for therapeutic agents to down regulate the inflammatory response due to TRPV1 activation **[50].**

Amino-peptidase N (APN) protein is a zinc-dependent aminopeptidase that cleaves one residue from the N-terminus of many physiological peptides and plays multifunctional roles such as in pain regulation, blood pressure regulation, and tumor cell angiogenesis. Sugars decorate many proteins and fats on cell surfaces and function in many biological processes such as immunity and cell-cell communication. How these cell-surface molecules are selected by viruses as their entry receptors have been a major puzzle in virology **[51].**

Evidence for vitamins C, D and zinc and their roles in preventing pneumonia and respiratory infections (vitamins C and D) and reinforcing immunity (zinc) appears to look particularly promising. Tolerable upper intake levels (ULs) are intake levels which should not be surpassed as toxicity problems could appear. For vitamin D a UL of 50 μg/day is advised and for zinc a UL of 25 mg/day is recommended. supplemental daily doses of up to about 1 g, in addition to normal dietary intake, are not associated with adverse gastrointestinal effects **[52].**

Zn^{2+} Ions-Induced Virucidal Activities for COVID-19 Defenses of Severe Bronchitis and Acute Pneumonia

Zn^{2+} inhibits coronavirus and anterivirus RNA polymelase activity, and zinc ionophores block the virus replication that Zn^{2+} and pyrithione at low concentrations inhibit the replication of SARS-CoV and arterivirus RNA which high zinc ion concentration and the addition of compounds that stimulate cellular zinc ions were found to inhibit the replication of various RNA virus, influenza viruses, respiratory syncytial virus and coronaviruses **[53]**.

The defense on the severe bronchitis patients infected with SARS-CoV, MERS-CoV and SARS-CoV-2 has clinical features range from mild respiratory illness to severe acute respiratory disease. Both MERS and SARS patients in later stages develop respiratory distress and renal failure. The pneumonia appears to be the most frequent manifestation of SARS-CoV-2 infection, characterized primarily by fever, cough, dyspnea, and bilateral infiltrates on chest imaging that the period from infection to appearance of symptoms varies **[54].** SARS-CoV-2 enters the target cells through the angiotensin-converting enzyme 2 (ACE2) receptor and the transmembrane protease, serine 2 (TMPRSS2). The TMPRSS2 inhibitors block the cellular entry of the SARS-CoV-2 virus through the downregulated priming of the SARS-CoV-2 spike protein **[55]**. The other, zinc used as anti-inflammatory agent inhibits transient receptor potential vanilloid 1 **(**TRPV1) to alleviate neuropathic pain **[56]** that TRPV1 might decrease the severity of the acute respiratory distress syndrome present in COVID-19 patients **[57].**

For defenses from severe acute COVID-19 disease, parenteral zinc **+** chloroquine/hydroxychloroquine (CQ/HCQ) in the treatment of hospitalized COVID-19 patients may help to improve clinical outcomes and to limit the COVID-19 fatality rates. Therefore, whether zinc supplementation in combination with CQ/HCQ should be recommended for high risk or also younger patients outside of clinical trials as a prevention or treatment approach during SARS-CoV-2 pandemic, should be considered only on a case-by-case basis [**58, 59].** SARS coronavirus envelope protein ion channel activity promotes virus fitness and pathogenesis that inflammasome-activated IL-1β levels were reduced in the lung airways of the animals infected with viruses lacking E protein ion channel activity and acute respiratory distress syndrome (ARDS) leading to death, in which E protein ion channel activity represents a new determinant for SARS-CoV virulence [**60].**

On the case of preventing lung and pulmonia, firstly, 2019-nCoV nucleic acid detection is carried out that accurate RNA detection of 2019-nCoV is with diagnostic value (Strong recommendation). The RNA of 2019-nCoV positive in the throat swab sampling or other respiratory tract sampling by fluorescence quantitative polymerase chain reaction (PCR) method, especially that from multiple samples and detection kits, excluding sample quality, sample collection time, contaminatory and technical problems, is of great support for etiological diagnosis. Drug treatment;(1) At present, there is no evidence from randomized controlled trial (RCT) to support specific drug treatment against the new coronavirus in suspected or confirmed cases. (2)The α-interferon atomization inhalation can be considered (5 million U per time for adults in sterile injection water, twice a day) (Weak recommendation) **[61].**

The key strategies for preventing lung damages include avoiding direct lung infection, altering host-virus interactions, promoting immune responses, diluting virus concentrations in lung tissues by promoting viral migration to the rest of the body, maintaining waste removal balance, protecting heart function and renal function, avoiding other infections, reducing allergic reactions and anti-inflammatory. The first strategy is avoiding exposures that could result in widespread damages to lungs and taking post exposure mitigating measures that would reduce disease severity. The second strategy is reducing death rate and disability rate from the current levels to one tenth for infected patients by using multiple factors health optimization method. The double reduction strategies are expected

to generate a series of chain reactions that favor mitigating or ending the pandemic [**62].**

Improve lung micro circulation to prevent damages to the lungs, Vitamins and essential nutrients for the immune system (but not for the virus) may shorten the phase lag by one to two days and thus make a difference; deep breaching can improve energy metabolism by as much as 30% (for experienced, it may improve more); and avoiding exercise may save MET values by up to 70%; relaxation exercise can reduce blood circulation by 10% to 30%; avoiding a secondary infection can reduce burden on the immune system, reduce viral burden on lungs, kidneys and heart, and help maintain the waste balance in the lungs [**63].** However, Zn supplementation did not yield a statistically significant reduction in symptoms in children with severe pneumonia. Zinc supplements given during an acute episode are not beneficial in short-term clinical recovery from severe pneumonia in hospitalized children [**64, 65].**

Transient zinc chelation N, N, N', N'-tetrakis(2-pyridinylmethyl)-1,2-ethanediamine (TPEN) led to induction of an antiviral state that in cells via induction of heat shock proteins and activation of NF-κB and upregulation of downstream effectors which inhibit DENV replication. Interferon-stimulated genes (ISGs) are a large group of genes which have diverse effects on viral infections and mostly act at early stages of virus life-cycle. Therefore, cellular or tissue zinc homeostasis may also determine the effciency with which pathogens replicate and disseminate in vivo. In the case of acute viral infections, strategies to transiently block zinc redistribution during viremic stages may inhibit viruses that depend on cellular zinc pools for replication. This would provide a window for the immune system to gain an upper hand and control viral infection. Zinc chelation abrogated dengue virus RNA replication and zinc chelation abrogated dengue virus RNA replication. Transient zinc chelation induces endoplasmic reticulum (ER) stress and antiviral response by activating NF-kappaB leading to induction of interferon signaling and zinc plays divergent roles in rotavirus and dengue virus infections in epithelial cells **[66].**

The antiviral compounds including zinc N-ethyl-N-phenyldithiocarbamate (EPDTC) inhibit the viral protease, thus preventing humancoxsackievirus strain B3 (CVB3) genome replication. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination(Zinc-coordination pattern) of the inhibitors against 3C and 3C-like proteases coordinated to such as catalytic triad of Serine, Histidine and Aspartate hydrogen residues of CVB3 $3C^{pro}$ **[67].**

In addition to their viral receptor functions, the receptors for coronaviruses have their own physiological functions angiotensin-converting enzyme 2 (ACE2) is a zinc-dependent carboxypeptidase that cleaves one residue from the C terminus of angiotensin peptides and functions in blood pressure regulation. ACE2 also protects against severe acute lung failure, and SARS-CoV-induced downregulation of ACE2 promotes lung injury.

Zinc Induced ROS Generation in Respiratory and Pulmonary COVID-19 Infected Cells

Zinc induced ROS generation in respiratory and pulmonary COVID-19 infected cells is that the univalent reduction of oxygen generates superoxide ($\bullet O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet OH$), all of which are reactive oxygen species (ROS). The production of ROS and its elimination by the anti-oxidant defense system in cells is a highly modulated process for maintaining normal physiological function in the body, in which the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases are a group of plasma membrane-associated enzymes which catalyze the production of superoxide $\bullet O_2^-$ from oxygen by using NADPH as the electron donor **[68].**

Zinc induced ROS generation in pulmonary COVID-19 infected cells is that alterations of ROS-producing and scavenging pathways that are caused by respiratory viral infections are implicated in inflammation, lung epithelial disruption, and tissue damage, and, in some cases, even pulmonary fibrosis. These events are at least partially interregulated: inflammation can contribute to lung damage and epithelial dysfunction and vice versa. The observation that ROS are implicated in the pathology of these viruses is mainly based on experimental infection models. Such inflammatory processes, especially sustained chronic conditions of inflammation, along with inflammation-induced oxidative stress from dead or injured cells, could lead to irreversible cell ularortissue damage with the passage oftime, which further contributes to the development of chronic degenerative diseases **[69].** The role of excessive immune activation as the

cause of lung destruction by SARS-CoV-2 supposes the causative virus of the pandemic coronavirus disease 19 (COVID-19). The oxidative stress by virally induced ROS production spirals cytokine release and immune cell infiltration in the lung as a result. The administration route should also be evaluated prior a trial start, as both oral infusion as well as inhalation administration routes are available. N-acetyl-cysteine (NAC) has also been show to interact and inhibit proteasome inhibitors, it remains to be shown if NAC or other pharmacological agents can affect virally released proteasome binding proteins and thereby aid proteasome function and by this, prevent tissue damage **[70].**

As mentioned above, Zn^{2+} ions-induced antiviral activities for prevention and antibody of SARS-CoV-2 and defenses from severe respiratory ailment and acute pulmonary disease against COVID-19 infection are represented in ***Table 1***. However, COVID-19 degradation or destruction by zinc-finger antiviral proteins remains yet unclear and further COVID-19 pulmonary care by zinc ion solutions may become of importance.

Table1. *Zn^{2+} ions-induced virucidal activities for SARS-CoV-2 prevention and antibody, and antiviral defenses from severe respiratory and acute pulmonary COVID-19 infection*

Zn^{2+} ions	**Zn^{2+} ions-induced antiviral activites for the prevention of SARS-CoV-2 and the infectious defenses of severe repiratory and acute pulmonary COVID-19**		
	Prevention and antibody	**Respiratory Bronchitis**	**Inflammatory Pneumonia**
Zn^{2+}	**→ Zn^{2+}** • Zn homeostatic immune concentration 50 mg/day • Zinc supplementation in combination with CQ/ HCQ • Zinc (15~30 mg/d) supplement prevents pneumonia in children • Lower Zn^{2+} conc may be efficient for vaccine candidate and higher Zn^{2+} conc may prevent respiratory ailment and acute pneumonia spreading against HCoVs • TRPV1 prevention	**→ Zn^{2+}, $\bullet O_2-$, H_2O_2, $\bullet OH$** • 2μM Zn^{2+} + 2μM Pyrithione (PT) inhibit RNA replication • Higher Zn^{2+}conc + HK inhibit virus entry against DV • Zinc chelation inhibits RNA replication • TMPRSS2 blocks cellular entry • FDA-approved Zn-ejector drugs such as disulfiram • ADAR-mediated RNA editing • 2019-nCoV RNA degradation by Zn^{2+} ions ? • ZnOTs inhibit HSV-1 entry and spread • 2,500mg/kg diet ZnO has antiviral activity of SARS-CoV • ZnONPs inhibit H1N1 influenza virus entry • Zn-TRPV1 respiratory drug	**→ Zn^{2+}, $\bullet O_2-$, H_2O_2, $\bullet OH$** • CQ/HCQ plus zinc inhibit RNA replication • Zinc-coordinated inhibitor • Zinc + chloroquine • Zn-ejectors + disulfiram • ADAR-mediated RNA editing targets • RNA degradation by zinc ions • Zinc-binding ACE2 • ZnONPs regulate microRNA in Ovarian granulosa cells • ZnONPs + DMN inhibit the production of mRNA of inflammatory cytokines • ZAP degrades SARS-CoV's and MERS-CoV's RNA • Complex zinc-finger inhibits nidovirus replication

Conclusions

2019-nCoV RNA virus structure at least contains four viral proteins, the spike (S) protein, the membrane (M) protein, the envelope (E) protein, and nucleocapsid (N) protein. The coronavirus spike glycoprotein S is a multifunctional molecular machine that mediates coronavirus entry into host cells. It first binds to a receptor on the host cell surface through its spike protein S1 subunit and then fuses viral and host membranes through its spike protein S2 subunit. Two domains in S1 from different corona viruses recognize a variety of host receptors, leading to viral attachment. The spike protein exists in two structurally distinct conformations, prefusion and post fusion. The transition from prefusion to postfusion conformation of the spike protein must be triggered, leading to membrane fusion.

Zn^{2+} ions could inhibit virus entry and membrane fusion of S1 and S2 domains of Spike Protein with zinc ion-binding interaction. Whereas, the M and E proteins are involved in virus assembly, the spike glycoprotein is the leading mediator of viral entry that the spike protein is also the principal player in determining host range, and the spike protein plays a dual role in entry by mediating receptor binding and membrain fusion that the fusion process involves large conformational changes of the spike protein. 2019-nCoV may have similar membrane fusion mechanism as that of SARS-CoV.

Zinc homeostatic status of zinc(II) has antiviral effects, improves immune responses and suppresses viral replication. The zinc-homeostatic immune concentration may provide a protective role against the COVID-19 pandemic, likely by improving the host's resistance against viral infection. Zn^{2+} ions can prevent in the early stage of SARS-CoV-2 infected patient with antiviral zinc homeostatic immunity and have important roles for respirarory and pulmonary process of COVID-19 disease. Factor of present outbreak of disease 19 COVID-19 may be considered to be due to RNA virus mutation that the RNA viruses have high mutation rate and these high rates are correlated with enhanced virulence and evolvability, traits considered beneficial for viruses. However, it is unclear whether Zn^{2+} ions reduce the high mutation rate of RNA genome structure. Zn^{2+} inhibits corona-virus and anterivirus RNA polymelase activity, and the combination of Zn^{2+} and pyrithione at low concentrations inhibits the replication of SARS-CoV and arterivirus RNA.

Zinc-finger antiviral protein (ZAP) controls virus entry, DNA/RNA replication, and spreading against viral infection. ZAP specifically inhibits the replication of certain viruses and promotes viral RNA degradation that ZAP inhibits Retroviral RNA production and HIV-1 infection by promoting the degradation of specific viral mRNAs. The four zinc fingers of ZAP form extensive interactions with RNA, but mutations of both protein and RNA at the RNA-ZAP interacting surface reduce the *in vitro* binding affinity and antiviral activity, in which ZAP coordination promotes downstream RNA degradation. ZAP directly interacted with the exosome component and that the binding region of ZAP was mapped to amino acids 224–254. Depletion of the exosome component with small interfering RNA significantly reduced ZAP's destabilizing activity which ZAP is a trans-acting factor that modulates mRNA stability. ZAP also inhibits the replication of Moloney murine leukemia virus (MLV) and Sindbis virus (SIN) by preventing accumulation of the viral mRNA. The mRNA decay is largely determined by the cis-acting elements. Thus, ZAP could inhibit SARS-CoV-2 RNA virus replication. However, this ZAP's efficiency for COVID-19 remains yet unclear.

Zn^{2+} ions-induced prevention and antibody against SARS-CoV-2 infection are required Zn homeostatic immune concentration 50 mg/day, Zn supplementation in combination with CQ/HCQ, Zinc supplement (15~30 mg/d) preventing pneumonia in children, and transient receptor potential vanilloid 1 (TRPV1) prevention. Lower Zn^{2+} concentration may be efficient for vaccine candidate and higher Zn^{2+} concentration may prevent respiratory ailment and acute pneumonia spreading against HCoVs. In order to prevent that an outbreak of respiratory sickness caused by a novel coronavirus (Covid-19) has become a serious public threat and disrupted many lives, assessing the efficacy of Zn-ejector drugs such as disulfiram combined with interferon to treat Covid-19 infected patients has been proposed. SARS coronavirus envelope protein promotes virus fitness and pathogenesis, in which E protein ion channel activity represents a new determinant for SARS-CoV virulence.

In order to prevent that an outbreak of respiratory sickness caused by a novel coronavirus (Covid-19) has become a serious public threat and disrupted many

lives, assessing the efficacy of FDA-approved Zn-ejector drugs such as disulfiram combined with interferon to treat Covid-19 infected patients has been proposed. Zinc ions anti-inflammatory TRPV1 prevention against COVID-19 would lead one to look for therapeutic agents to down regulate the inflammatory response. APN protein is a zinc-dependent amino-peptidase that cleaves one residue from the N-terminus of many physiological peptides and plays multifunctional roles such as in pain regulation, blood pressure regulation, and tumor cell angiogenesis.

On the other hand, Zn^{2+} ions-induced virucidal defenses from COVID-19 severe bronchitis and acute pneumonia are required that zinc ions can prevent in the early stage of 2019-nCoV infected patient, and the zinc ions have important roles for respirarory and pulmonary process of COVID-19 disease. Zn^{2+} inhibits coronavirus and anterivirus RNA polymelase activity, and zinc ionophores block the virus replication that Zn^{2+} ions + pyrithione at low concentrations inhibits the replication of SARS-CoV and arterivirus RNA.

For COVID-19 defense from severe bronchitis, zinc supplementation plus CQ/HCQ also should be recommended for high risk or also younger patients outside of clinical trials as a prevention or treatment approach during SARS-CoV-2 pandemic. Zinc supplementation + CQ/HCQ should be recommended for high risk or also younger patients outside of clinical trials as a prevention or treatment approach during SARS-CoV-2 pandemic. TMPRSS2 inhibitors block the cellular entry of the SARS-CoV-2 virus through the downregulated priming of the SARS-CoV-2 spike protein. Transient zinc chelation TPEN induces ER stress and antiviral response by activating NF-κB leading to induction of interferon signaling and zinc plays divergent roles in rotavirus and dengue virus infections in epithelial cells.

For Zn^{2+} ions-induced virucidal defenses from COVID-19 acute pneumonia, the antiviral compounds including zinc N-ethyl-N-phenyldithio-carbamate (EPDTC) inhibit the viral protease, thus preventing humancoxsackie-virus strain B3 (CVB3) genome replication. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C and 3C-like proteases. Zinc-ions complexes as 2019-nCoV 3C-like protease inhibitors may play important role for this Zn^{2+}-centered coordination pattern that the zinc-coordinating inhibitor is tetrahedrally coordinated to such as the catalytic dyad of CVB3 $3C^{pro}$. Zinc-ions complexes as 2019-nCoV 3C-like protease inhibitors may play important role for this Zn^{2+}-centered coordination pattern that the zinc-coordinating inhibitor is tetrahedrally coordinated to the His^{40}-Cys^{147} catalytic dyad of CVB3 $3C^{pro}$.

The antiviral compounds including zinc N-ethyl-N-phenyldithio-carbamate (EPDTC) inhibit the viral protease, thus preventing humancoxsackie-virus strain B3 (CVB3) genome replication. The interactions had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C and 3C-like proteases. ACE2 also protects against severe acute lung failure, and SARS-CoV-induced downregulation of ACE2 promotes lung injury.

Thus, the interaction had been found on the binding specificity by Zn^{2+} ions-centered tetrahedral geometric coordination of the inhibitors against 3C proteases. Zinc ions complexes as SARS-CoV-2 3C-like protease inhibitors may play important role for this Zn^{2+}-centered coordination pattern that the zinc-coordinating inhibitor of tetrahedral zinc sites is tetrahedrally coordinated binding to such as the catalytic triad (Serine, Histidine and Aspartate hydrogen residues) of CVB3 $3C^{pro}$.

References

[1] T. Ishida; Zn^{2+} Ions-Immune Virucidal activities for children and adults with preventions against 2019-nCoV and COVID-19 infection. Journal of Child, Adult Vaccines and Immunology. 2020;6-15.

[2] Oner Ozdemir; Zinc and Allergy Relation MOJ Immunol 2014; 1, Issue 1: 1-5.

[3] Hong Gao, Wei Dai, Lu Zhao, Junxia Min, et al; The Role of Zinc and Zinc Homeostasis in Macrophage Function. *Journal of Immunology Research* 2018; Volume 2018, Article ID 6872621, 11 pages https://doi.org/10.1155/2018/6872621

[4] K-H. Ibs and L. Rink; Immunity enhanced by trace elements. *J. Nutr.* 2003; 133: 1452S-1456S.

[5] S. A. Read, S. Obeid, C. Ahlenatiel, G. Ahlenstiel; The role of zinc in antiviral immunity. *Adv Nutr.* 2019; 0:1-15.

[6] I. Wessels, M. Maywald and L. Rink; Zinc as gatekeeper of immune function. *Nutrients.* 2017; 9(12): 1-44 pages.

[7] T. Ishida; Zinc Immune Anti-Infectious Activities of Bacteriolysis by Zn^{2+}-induced Bacterial PGN Autolysins and ZAP's Viral Destruction by Cell Surface Receptors. *Biomedical Research and Reviews.* 2020; 3(2):1-8.

[8] T. Ishida; Virucidal Activities of Zinc-Finger Antiviral Proteins and Zinc-Binding Domains for Virus Entry, DNA/RNA

[9] Y. X.Lim, Y. L.Ng, J. P. Tam and D.X. Liu; Human coronaviruses: A review of virus-host interactions. *Diseases. 2016;* 4: 1-28.

[10] A. Erdogan, P. M. Agaoglu, et al; 2019 novel coronavirus (COVID-19) outbreak: A review of the current literature. *EJMO* 2020;4(1):1-7.

[11] M. U. Mirza and M. Froeyen; Structural elucidation of SARS-CoV-2 vital proteins: computational methods reveal potential drug candidates against Main protease, Nsp12 RNA-dependent RNA polymerase and Nsp13 helicase. *Preprint.* 2020; 5 March: 2-23.

[12] S. Duffy; Why are virus mutation rates so damn high. *PLOS Biology.* 2018; 16(8): 1-6.

[13] M. Cassandri, A. Smirnov, F. Novelli,et al; Zinc-finger protein in health and disease. Cell Death Discovery. 2017; 3: 1-12.

[14] A. J. W. te Velthuis, S. H. E. van den Worm, A. C. Sims, et al; Zn^{2+} inhibits coronavirus and arterivirus RNA polymerase activity *in vitro* and zinc ionophores block the replication of these viruses in cell culture. *PLOS Pathogen.* 2010; 6, Issue 11: 1-10.

[15] Ander Mayor-Ibarguren1, Carmen Busca-Arenzana and Ángel Robles-Marhuenda: A hypothesis for the possible role of zinc in the immunological pathways related to the COVID-19 infection *frontiers in Immunology* 2020; 11, Article 1736:1-8.

[16] N. Z. Gammoh and Lothar Rink (2017); Zinc in infection and inflammation, *Nutrient,* 9: 1-25.

[17] Poliana Guiomar de Almeida Brasiel; The key role of zinc in elderly immunity: A possible approach in the COVID-19 crisis *Clinical Nutrition ESPEN* 2020; 38: 65-66.

[18] M. Maywald, Inga Wessels and L. Rink (2017); Zinc signals and immunity, *J. Mol. Sci.* 18:1-34.

[19] S. Belouzard, J. K. Millet, B. N. Licitra and G. R. Whittaker; Mechanisms of coronavirus cell entry mediated by the viral spike protein. *Viruses.* 2012; 4: 1011-1033.

[20] C. Wu, Y. Liu, Y. Yang, P. Zhang, et al; Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B.* 2020; 20: S2211-

[21] D. Wrapp, N. Wang, K. S. Corbett, et al; Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* 2020; 367: 1260-1263.

[22] D. Schoeman and B. Fielding; Coronavirus envelope protein: current knowledge. *Viology Journal.* 2019; 69:1-22.

[23] A. Wu, P. Niu, L. Wang, H. Zhou, et al; Mutation, recombination and insertion in the evolution of 2019-nCoV. *bioRxiv preprint.* 2020; 29 February: 1-38.

[24] JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH; SARS-CoV-2 Genetics. 2020; April 16 : 1-2.

[25] Changechuan Yin; Genotyping coronavirus SARS-CoV-2: methods and implications. *ArXiv preprint.* 2020; March 25: 1-12.

[26] Y. Gao, L. Yan, Y. Huang, F. Liu, et al; Structure of RNA-depndent RNA polymerase from 2019-nCoV, major antiviral drug target. *BioRxiv preprint.* 2020; 6: 1-24.

[27] THE ROCKEFELLER UNIVERSITY; Virus entry and virus-host interactions. *Laboratory of Virology and Infectious Disease.* 2019;December 15: 1 page.

[28] W-Y. J. Lee, R. Menhua, C. Liang & R. D. Sloan (2018); IFITM proteins inhibit HIV-1 protein synthesis. *Scientific Reports.* 8:1-15 pages.

[29] K. M. Crosse, E. Monson, M.R. Beard, K. J. Helbig (2018); Interferon-simulated genes as enhancers of antiviral innate immune signaling. *Journal of Innate Immunity.* 10: 85-93.

[30] M. J. Bick, J-W. N. Carroll, et al, (2003); Expression the zinc-finger antiviral protein inhibits Alphavirus replication. *Journal of Virology.* 77, No.21: 11555-11562.

[31] Q. Tang, X. Wang, G. Gao (2017); The short form of the zinc finger antiviral protein inhibits influenza A virus porotein expression and is antagonized by the virus-encoded NS1. *Journal of Virology.* 91,Issue 2: 1-14 pages

[32] F. Villalon-Letelier, A. G. Brooks, P. M. Saunders, et al (2017); Host cell restriction factors that limit influenza A infection. *Viruses.* 9(12):1-18pages.

[33] J. M. Schapiro, S. J. Libby, and F. Fang (2003); Inhibition of bacterial DNA replication by zinc mobilization during nitrosative stress. *PNAS.* 2003; 100, No.14: 8496-8501.

[34] Anne L. Sapiro, Emily C. Freund, Lucas Restrepo, Huan-Huan Qiao, et al; Zinc finger RNA binding protein Zn72D regulates ADAR-mediated RNA editing in neurons. *BioRxiv reprint.* 2020; 8 May:1-43. doi: https://doi.org/10.1101/631986

[35] G. Gao, X. Guo, S. P. Goff (2002); Inhibition of Retroviral RNA production by ZAP, a CCCH-type zinc finger protein. *SCIENCE,* 297: 1703-1706.

[36] X. Wang, F. Lv and G. Gao (2010); Mutagenesis analysis of the zinc-finger antiviral protein. *Retrovirology.* 7: 1-9 pages.

[37] X. Luo, X. Wang, Y. Gao, et al; Molecular mechanism of RNA recognition ny zinc-finger antiviral protein. *Cell Reports.* 2019; 30: 46-52.

[38] Rayhane Nchioua, Dorota Kmiec, Janis Müller, Carina Conzelmann, Rüdiger Groß, et al; The Zinc Finger Antiviral Protein restricts SARS-CoV-2 *bioRxiv preprint* 2020; June 8; 1-40. https://doi.org/10.1101/2020.06.04.134379. this version posted June 8.

[39] Anne L. Sapiro, Emily C. Freund, Lucas Restrepo, Huan-Huan Qiao, et al; Zinc finger RNA binding protein Zn72D regulates ADAR-mediated RNA editing in neurons. *BioRxiv* reprint. doi: https://doi.org/10.1101/631986 2019; BioRxiv reprint:1-43.

[40] Xuemin Guo, Jing Ma, Jing Sun, and Guangxia Gao; The zinc-finger antiviral protein recruits the RNA processing exosome to degrade the target mRNA. *PNAS.* 2007; 104, no. 1: 151–156

[41] A. Seybert, C. C. Posthuma, L. C. van Dinten, et al; A complex zinc finger controls the enzymatic activities of nidovirus helicases. *Journal of Virology.* 2005; 79, No.2: 696-704.

[42] L. M. John Law, B. S. Razooky, M. M. H. Li, et al (2019); ZAP's stress granule localization is correlated with its antiviral activity and induced by virus replication. *PLOS Pathogens.* 15(5): 1-22.

[43] Nathalie Sol-Foulon, Marion Sourisseau1, Franc¸oise Porrot1, Maria-Isabel Thoulouze, et al (2007); ZAP-70 kinase regulate HIV cell-to-cell spread and virological synapse formation. *The EMBO Journal.* 26: 516-526.

[44] W. Mothes, N. M. Sherer, J. Jin, P. Zhong (2010); Virus cell-to-cell transmission. *Journal of Virology.* 84, No.17: 8360-8368.

[45] ANATOLY V. SKALNY, LOTHAR RINK, OLGA P. AJSUVAKOVA, MICHAEL ASCHNER, et al; Zinc and respiratory tract infections:Perspectives for COVID-19 Review). *International Journal of Molecular Medicine.* 2020;13April: 1-10.

[46] Marc Lipsitch, Rebecca Kahn and Michael J. Mina; Antibody testing will enhance the power and accuracy of COVID-19-prevention trials. *Nature Medicine.* 2020;April 27:1-2. doi.org/10.1038/s41591-020-0887-3

[47] Mohammed S. Razzaque; COVID-19 pandemic: can maintaining optimal zinc balance enhance host resistance? Preprint 2020; 29 March:1-11.

[48] Chunyan Wanga, Wentao Lia, Dubravka Drabekb, et al;A human monoclonal antibody blocking SARS-CoV-2 infection. BioRxiv 2020; March 12; 1-24. https://doi.org/10.1101/2020.03.11.987958

[49] Karen Sargsyan, Ting Chen, Cédric Grauffel, and Carmay Lim; Identifying COVID-19 Drug-Sites Susceptible To Clinically Safe Zn-ejector Drugs Using Evolutionary */Physical Principles.* 2020; Print:1-10.

[50] Kim D. Janda and Michael J. Iadarola; Standing out from the crowd in treating COVID-19. *Medicine in Drug Discovery.* 2020; 6: 1. http://dx.doi.

org/20.1016/ j. medidd.2020.100034 Published by Elsevier B.V.

[51] Fang Li; Receptor Recognition Mechanisms of Coronaviruses: a Decade of Structural Studies. *Journal of Virology.* 2015; 89 No.4: 1954-1964.

[52] Emma Derbyshire, Joanne Delange; COVID-19: is there a role for immunonutrition, particularly in the over 65s? *BMJ Nutritioh Prevention & Health.* 2020; 0: 1- 6. doi. org/ 10. 1136/ bmjnph- 2020-000071.

[53] A. J. W. te Velthuis, S. H. E. van den Worm, A. C. Sims, et al; Zn^{2+} inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PLOS Pathogen.* 2010; 6, Issue 11: 1-10.

[54] Manoj Kumar Gupta, Sarojamma Vemula, Ravindra Donde, Gayatri Gouda, Lambodar Behera & Ramakrishna Vadde; In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. *Journal of Biomolecular Structure and Dynamics.* 2020; April,2020: 739-1102

[55] Markus Hoffmann,HannahKleine-Weber, Simon Schroeder, et al; SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell.* 2020; 181: 271-280.

[56] XJialie Luo, Alexis Bavencoffe, Pu Yang, XJing Feng, Shijin Yin, Aihua Qian, Weihua Yu,et al; Zinc Inhibits TRPV1 to Alleviate Chemotherapy-Induced Neuropathic Pain *The Journal of Neuroscience,* 2018; January 10, 38(2): 474–483.

[57] A. Nahama, R. Ramachandran, A. F. Cisternas, H. Ji; The role of afferent pulmonary innervation in ARDS associated with COVID-19 and potential use of resiniferatoxin to improve prognosis: A review. *Medicine in Drug Discovery* 2020; 5: 1-5.

[58] Derwand R and Scholz M; Does zinc supplementation enhance the clinical efficacy of chloroquine/hydroxychloroquine to win todays battle against COVID-19? Preprint doi:10.20944/preprints202004.0124.v1 2020; 8, April:1-10.

[59] Amir Noeparast, Gil Verschelden; Can Zinc correction in SARS-CoV-2 patients improve treatment outcomes? preprint, 2020; 7 April:1-14 doi:10.20944/preprints202004.0094.v1.

[60] J. L. Nieto-Torres, M. L. DeDiego, C. Verdia-Baguena, et al; Severe acute respiratotry syndrome coronavirus envelope ion channel activity poromotes virus fitness and pathogenesis. *PLOS pathogens.* 2014; 10, Issue 5: 1-19.

[61] Ying-Hui Jin1, Lin Cai, Zhen-Shun Cheng, Hong Cheng, Tong Deng, Yi-Pin Fan, et al; A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Military Medical Research* 2020; 7(4):1-23.

[62] Jianqing Wu and Ping Zha; Preventive, Mitigating and Treatment Strategies for Containing or Ending The COVID-19 Pandemic. *Preprint for comments.* 2020; Preprint for comment: 1-38.

[63] Jianqing Wu1, and Ping Zha; Treatment Strategies for Reducing Damages to Lungs In Patients with Coronavirus and Other Infections. *Preprint* 2020; 9 February: 1-31.

[64] Ghulam Shabbir Laghari, Zahid Hussain, Muhammad Taimur, Nasir Jamil; Therapeutic Role of Zinc Supplementation in Children Hospitalized with Pneumonia. *Cureus* 2019; 11(4): 1-6. Open Access Original Article DOI: 10.7759/cureus. 4475. 1-6.

[65] UIS E. CUEVAS & AI KOYANAGI; Zinc and infection: a review. *Annals of Tropical Paediatrics.* 2005; 25: 149–160.

[66] Meenakshi Kar, Naseem Ahmed Khan, Aleksha Panwar, Sachendra S. Bais, et al; Zinc Chelation Specifically Inhibits Early Stages of Dengue Virus Replication by Activation of NF-κB and Induction of Antiviral Response in Epithelial Cells. *Frontiers in Immunity.* 2019; 10: 1-15.

[67] Cheng-Chung Lee, Chih-JungKuo, Tzu-Ping, et al; Structural Basis of Inhibition Specificities of 3C and 3C-like Proteases by Zinc-Coordinating and Peptidomimetic Compounds. *Journal of Biological Chemistry.* 2009; 284, No. 12: 7646–7655.

[68] Ananda S. Prasad and Bin Bao; Molecular Mechanisms of Zinc as a Pro-Antioxidant

Mediator: Clinical Therapeutic Implications. *Antioxidants.* 2019; 8:1-22.

[69] Olga A. Khomich, Sergey N. Kochetkov 1, Birke Bartosch and Alexander V. Ivanov; Redox Biology of Respiratory Viral Infections. *Viruses.* 2018; 10:1-27. doi:10.3390/v10080392

[70] Aikaterini Nasi, Stephanie McArdle, Gustav Gaudernack, et al; Proteasome and reactive oxygen species dysfunction as risk factors for SARS-CoV-2 infection; consider N-acetylcystein as therapeutic intervention. *FULLTEXT02.* 2020;4-01:1-5.

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