This is not for patient use but a proposal for the development of clinical trials. This information is not intended to diagnose, treat, mitigate or cure any disease or illness.

A Literature Review Supporting the Addition of Intravenous Methylene Blue, High-dose Intravenous Vitamin C, Intravenous Glutathione, and Nutraceuticals, to Standard Care for Hospitalized COVID-19 Patients

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ABSTRACT:

Background and Aim: COVID-19 (coronavirus 19 disease) due to SARS-CoV-2 has caused significant morbidity and mortality worldwide. There is currently no established cure and no vaccine is available. Critically ill patients with COVID-19 are dying of sepsis and multiorgan system failure. Currently proposed treatments have included hydroxychloroquine, azithromycin, and several antivirals. In this current article we discuss the addition of intravenous methylene blue, high-dose intravenous vitamin C, intravenous glutathione, and several nutraceuticals to standard care. The aim of this article is to review agents which have known activity against SARS-CoV-2, or show evidence of favorable immune regulation, or show direct antiviral activity. Goals would be to lead to clinical to prevent progression of illness in hospitalized, non-critically ill and critically ill patients, to reduce mortality, and to reduce the time patients require mechanical ventilation. Hydroxychloroquine, azithromycin, and antivirals are discussed briefly in this paper since they are already being studied in clinical trials. This discussion and clinical research to follow is meant to augment standard care and not necessarily replace it.

Methods: To collect information required to study and formulate this protocol, we conducted extensive searches of the PubMed electronic reference database (https://www.ncbi.nlm.nih.gov/pubmed/) and Google (www.google.com) with the following keywords: “COVID”, “COVID-19”, “coronavirus”, “SARS-CoV-2”, “SARS-CoV-1”, “SARS”, “high-dose vitamin C”, “glutathione”, “methylene blue”, “oxidative stress”, “zinc”, “melatonin”, “immune system” “hydroxychloroquine”, “chloroquine”, ”cytokine storm”, and “antiviral(s)”. The search terms were used alone and in combination. Both authors reviewed the articles, first by title and abstract, and then by reviewing the full text of the articles. Non-scientific sources and anecdotal information are identified as such and are included not only to provide useful

information on the characteristics of this disease from those who are currently treating it, but also to address some other topics in a scientific manner that have led to widespread speculation in the media and on social media platforms.

Results: Over 200 references were identified which contributed to this work. Publications reviewed were both original research articles and review or conceptual articles. There were in vitro studies, in vivo studies, literature reviews, meta-analyses, and epidemiologic works. Several basic science articles on the biochemistry of this group of compounds were also reviewed and referenced where applicable. We feel that this information will add substantially to the improvement in the course of illness for these patients once it has been proven safe and effective in an approved clinical trial.

Conclusion: Several novel treatment interventions were discovered which have either in vitro or in vivo antiviral activity including some with confirmed activity against SARS-CoV-2. We also identified agents which help correct oxidative stress, reduce inflammatory cytokines, and can be used to treat sepsis-induced distributive shock. These agents are inexpensive, readily available, and have a long track record of safety in humans. We look forward to clinical trials studying these therapeutics as adjuncts to current care.

Keywords: COVID-19, methylene blue, IV vitamin C, glutathione, zinc, melatonin

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Introduction:

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 virus) is highly transmissible among people. Due to the ability to pass from person to person easily, the death toll is substantial even if the percentage of mortality is not as high as other significant coronavirus outbreaks (SARS-CoV-1 and MERS-CoV). It has caused substantial morbidity, mortality, and fear around the world. There are currently more than 20 clinical trials underway to look for safe and effective treatments for this disease which has taken a heavy toll both in terms of human lives as impact to the worldwide economy.

Methods:

To collect information required to study and formulate this protocol, we conducted extensive searches of both the PubMed database (https://www.ncbi.nlm.nih.gov/pubmed/) and Google (www.google.com) with the following keywords: “COVID”, “COVID-19”, “coronavirus”, “SARS-CoV-2”, “SARS-CoV-1”, “SARS”, “high-dose vitamin C”, “glutathione”, “methylene blue”, “oxidative stress”, “zinc”, “melatonin”, “immune system” “hydroxychloroquine”, “chloroquine”, ”cytokine storm”, and “antiviral(s)”. The search terms were used alone and in combination. Both authors reviewed the articles, first by title and abstract, and then by reviewing the full text of the articles. Non-scientific sources and anecdotal information are identified as such and are included not only to provide useful information on the characteristics of this disease from those who are currently treating it, but also to address some other topics in a scientific manner that have led to widespread speculation in the media and on social media platforms.

Results:

Characteristics of the virus

SARS-CoV-2 is a single stranded RNA virus which is optimized[1] for spike protein binding to the Angiotensin converting enzyme II (ACE-2) receptor[2] Binding affinity is ten times greater than that of SARS-CoV-1.[3] The virus binds to the host cell by a structural change of the spike protein. Cell membranes fuse and viral RNA is injected into the host and copied to make more of the virus. The viral load actually decreases during the second week of infection[4], but, similar to SARS-CoV-1, there may be acute worsening during the second week. In SARS-CoV-1, this was

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[1] http://virological.org/t/the-proximal-origin-of-sars-cov-2/398, accessed 8 April 2020

[2] Wan, Yushun, et al. "Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus." *Journal of virology* 94.7 (2020)

[3] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020 Feb 19. pii: eabb2507 doi: 10.1126/science.abb2507. [Epub ahead of print]. PMID:32075877

[4] To, Kelvin Kai-Wang, et al. "Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study." *The Lancet Infectious Diseases* (2020)

noted to be due to the host immunopathologic response rather than viral replication.[5] IL-6 (an inflammatory marker) is significantly elevated in patients who have adverse outcomes.[6] Some investigation of IL-6 inhibitors as a potential treatment option is currently being discussed.

Hospital course

Asymptomatic carriers can transmit the disease and it has been noted that disorders of taste and smell may occur.[7] In a very large study (>70,000 cases)[8], 81% of patients were noted to have mild disease while 14% had severe disease, defined as patients having dyspnea, hypoxia, or >50% lung involvement on imaging. Critical illness (sepsis, multi-organ system failure) was seen in 5%. Of the patients in this study, deaths only occurred in the critically ill group. Overall fatality in this study was 2.3% while fatality in the critical group was 49%.

The CDC reports that the average time from infection to the onset of dyspnea is 5-8 days. The average time from initial infection to development of acute respiratory distress syndrome (ARDS) is 8-12 days, and the average time to ICU admission is 10-12 days. It is also noted that "rapid deterioration 1 week after illness onset may occur."[9] Chinese studies published in the journal *Pediatrics* have shown that children seem to have less severe disease.[10] Advanced age and comorbid conditions increase the chance of poor outcomes, especially in patients with cardiovascular and pulmonary disease.

ACE inhibitors and ARBs were initially thought to possibly be a risk for worse outcomes since they upregulate ACE-2 receptors in the heart in animal models. This upregulation has not been seen in humans and no adverse outcomes in COVID-19 patients are known to be associated. The American College of Cardiology has recommended continuing these medications since they have actually been shown to be beneficial in some viral pneumonia cases.[11] The Council on Hypertension of the European Society of Cardiology published a similar

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[5] Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet*. 2003;361(9371):1767–1772 doi:10.1016/s0140-6736(03)13412-5

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[8] Wu Z, McGoogan JM. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. *Jama.* 2020

[9] https://www.cdc.gov/coronavirus/2019-ncov/hcp/clinical-guidance-management-patients.html (accessed 8 April, 2020)

[10] Dong Y, Mo X, Hu Y, et al. Epidemiological Characteristics of 2143 Pediatric Patients With 2019 Coronavirus Disease in China. *Pediatrics.* 2020

[11] ACC https://www.acc.org/latest-in-cardiology/articles/2020/03/17/08/59/hfsa-acc-aha-statement-addresses-concerns-re-using-raas-antagonists-in-covid-19

recommendation citing lack of evidence of harm.[12] A great review on this subject from the New England Journal of Medicine outlines current literature and recommends continuing ACE inhibitors and ARBs.[13]

Laboratory and Radiology findings

About 83% of patients have been noted to have lymphopenia. Elevated levels of AST, ALT, LDH, CRP, elevated D-dimer, and very high ferritin are associated with more severe disease.[14] Radiology studies may show bilateral infiltrates and ground-glass appearance on CT.[15,16,17]

Oxygen measurement discrepancy

In COVID-19 patients, a trend has been noted where oxygen saturation levels measured by pulse oximetry are very low (70-80% with normal being >93%) and patients do not seem to be in a degree of respiratory distress corresponding to these readings.[18,19] There is a "dissociation" between their relatively well-preserved lung mechanics and the severity of hypoxemia.[20] Several personal anecdotal reports given by ED physicians in Indianapolis[21] as well as at least 20 reports from physicians nationwide on a large ED physician email group[22], report saturations in the 70% to 80% range with disproportionately mild symptoms.[23,24]

Pulse oximetry measures oxygenated vs deoxygenated blood indirectly, while PaO_2 (the partial pressure of oxygen) is measured via an arterial blood gas. Dyshemoglobinemias such as

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[12] Position statement of the ESC council on hypertension on ACE-inhibitors and angiotensin receptor blockers. From European Society of Cardiology website. Accessed 2020 Mar 18. Available from https://www.escardio.org/Councils/Council-on-Hypertension-(CHT)/News/position-statement-of-the-esc-council-on-hypertension-on-ace-inhibitors-and-ang. (accessed 8 April 2020)
[13] Vaduganathan M, Vardeny O, Michel T, McMurray JJV, Pfeffer MA, Solomon SD. Renin-Angiotensin-Aldosterone System Inhibitors in Patients with Covid-19 [published online ahead of print, 2020 Mar 30]. *N Engl J Med*. 2020;NEJMsr2005760. doi:10.1056/NEJMsr2005760
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[15] Shi H, Han X, Jiang N, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. *The Lancet Infectious diseases.* 2020
[16] Ai T, Yang Z, Hou H, et al. Correlation of Chest CT and RT-PCR Testing in Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology.* 2020:200642
[17] Bernheim A, Mei X, Huang M, et al. Chest CT Findings in Coronavirus Disease-19 (COVID-19): Relationship to Duration of Infection. *Radiology.* 2020:200463
[18] https://www.webmd.com/lung/news/20200407/doctors-puzzle-over-covid19-lung-problems (accessed 8 April 2020)
[19] https://www.esicm.org/wp-content/uploads/2020/04/684_author-proof.pdf (accessed 8 April 2020)
[20] https://www.atsjournals.org/doi/pdf/10.1164/rccm.202003-0817LE (accessed 8 April 2020)
[21] Personal communication, April 2020
[22] Personal communication, April 2020
[23] https://emcrit.org/pulmcrit/cpap-covid/ (accessed 8 April 2020)
[24] http://pulmcast.com/covid19education (accessed 8 April 2020)

sulfhemoglobinemia, methemoglobinemia and carbon monoxide poisoning can cause a low oxygen saturation on pulse oximetry with a normal PaO_2. This is called a "saturation gap."[25] Acquired methemoglobinemia occurs due to medication or toxin exposures which cause oxidation of iron from the Fe2+ (ferrous) state to the Fe3+ (ferric) state. This abnormal methemoglobin (MetHb) is unable to carry oxygen. If the percentage of methemoglobinemia is significantly elevated, the patient becomes "functionally anemic" and experiences signs and symptoms such as dyspnea, cyanosis (including a bluish hue to the skin), and altered mental status. Significant methemoglobinemia does not respond to supplemental oxygen. Levels of MetHb over 70% may be fatal.

Cytochrome-b5 reductase utilizes NADH formed during glycolysis to reduce methemoglobin back to functional hemoglobin unless overwhelmed by oxidative stress.[26] The enzyme NADPH-MetHb reductase is an alternative pathway to restore methemoglobin (MetHb) to normal hemoglobin. It is usually not used in normal physiology.

Methylene blue in methemoglobinemia

The mainstay of therapy to treat methemoglobinemia is a medication called methylene blue (MB). MB is an electron donor[27] which is administered intravenously. It upregulates metabolism of MetHb through the NADPH-MetHb pathway. If methylene blue does not work or is not available, vitamin C may be given (including IV high dose vitamin C, up to 10 grams).[28,29,30,31] Patients with renal insufficiency can develop hyperoxaluria or renal failure with MB, so caution is advised. Oral vitamin C at this high dosage may not be tolerated as it may produce GI distress with diarrhea. Hyperbaric oxygen therapy (HBOT) has been used alone (if methylene blue is not available) and may be used in addition to MB if it is ineffective.[32] No studies measuring methemoglobinemia have been found in reference to COVID-19.

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SARS-CoV-2 and hemoglobin

Hemoglobin has 2 **α** subunits and 2 **β** subunits. Each subunit has a central porphyrin containing heme. Heme contains iron. Divalent ($Fe2^+$) iron can release CO_2 and capture oxygen in the lungs which converts it to trivalent iron ($Fe3^+$). It then gets to tissue and releases the oxygen and picks up CO_2, converting back to divalent iron. A recent paper[33] is appearing on the internet on social media sites which states that the coronavirus attacks the hemoglobin subunit **β**. The authors then postulate (with no further evidence provided) that this causes release of ferric ($Fe3^+$) iron which is actually what damages the lungs in this disease. In this highly technical study, the researchers report that they used "molecular docking"[34] technology. The authors report that the ORF-8 protein of the virus attacks the **β**-1 chain of hemoglobin, dissociating the iron from heme. Due to the technical nature of this report it is difficult to draw any conclusion as to its validity.

One of the references in the article, (the Feb 15, 2020 *Lancet* article[35] from Chen, et al.) provides an early description of 99 coronavirus patients. In this report, ferritin was noted to be significantly elevated in these patients. Lactate dehydrogenase (LDH), C-reactive protein, and D-dimer elevations were also noted. The authors of the molecular docking article[31] use this article by Chen, et al., to support their hypothesis. They state that since ferritin increases in conditions where free iron increases, this explains the elevated ferritin in patients with COVID-19. While it is true that free iron is toxic to cells and catalyzes the formation of reactive oxygen species via the Fenton reaction, oxidative stress (in the absence of free iron) and inflammation increase ferritin as well[36] since ferritin is an acute phase reactant. In addition, endotoxins from infections can cause upregulation of ferritin production.[37] Since ferritin may be elevated solely due to

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[33] Wenzhong, liu; Hualan, Li (2020): COVID-19: Attacks the 1-Beta Chain of Hemoglobin and Captures the Porphyrin to Inhibit Human Heme Metabolism. ChemRxiv. Preprint. https://doi.org/10.26434/chemrxiv.11938173.v4

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inflammation, measurement of other markers of oxidative stress (8-OHdG[38] and myeloperoxidase[39,40]) can help differentiate this.

The other finding reported by the authors of the molecular docking article[31] was that they studied chloroquine and found that it inhibits ORF-8 binding and several other viral proteins of SARS-CoV-2, which they state supports its therapeutic use in this disease. Once again, the highly technical nature of this paper and several unsubstantiated leaps that it takes make it difficult to assess its utility.

COVID-19 and high-altitude pulmonary edema (HAPE) similarities

Another recent open access publication[41] shared frequently online compares respiratory parameters of high-altitude pulmonary edema and COVID-19. The author suggests using Acetazolamide, Nifedipine, and phosphodiesterase inhibitors such as sildenafil therapeutically. We could not find any other supporting articles aside from this one opinion piece. There is also a video of a Critical Care Physician circulating online on several social media sites which echoes this hypothesis. One assertion of both the video and the publication mentioned above is that the lung pathology is not ARDS or pneumonia. These are questions best answered by the Pulmonology/Critical Care community caring for these patients at this time. It is unclear to us whether such distinctions would make any difference in the management of these patients.

Methylene Blue for COVID19

As mentioned above, methylene blue helps restore oxygen carrying capacity of blood when methemoglobinemia is present. If methemoglobinemia is present in sick patients with COVID-19, this should be measurable. If so, MB would be an obvious starting place. Contraindications to methylene blue are pregnancy (category X), renal insufficiency, and G6PD deficiency. Since multiorgan system failure would be expected to have some degree of renal insufficiency, earlier initiation of treatment would be best. It can also produce serotonin syndrome if patients are on certain psychiatric medications. A remaining question is, if COVID-19 produces

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[40] Chia-Chao Wu, Jin-Shuen Chen, Wen-Mein Wu, Tung-Nan Liao, Pauling Chu, Shih-Hua Lin, Chien-Huei Chuang, Yuh-Feng Lin, Myeloperoxidase serves as a marker of oxidative stress during single haemodialysis session using two different biocompatible dialysis membranes, *Nephrology Dialysis Transplantation*, Volume 20, Issue 6, June 2005, Pages 1134–1139

[41] Solaimanzadeh I. Acetazolamide, Nifedipine and Phosphodiesterase Inhibitors: Rationale for Their Utilization as Adjunctive Countermeasures in the Treatment of Coronavirus Disease 2019 (COVID-19). *Cureus*. 2020;12(3):e7343. Published 2020 Mar 20. doi:10.7759/cureus.7343

a condition similar to methemoglobinemia, could the saturation gap be present and significant even in the absence of measurable MetHb on co-oximetry measurement?

Even if there is no measurable component of methemoglobinemia, methylene blue is an excellent therapeutic choice in COVID-19 for several reasons. MB has been used since the late 1800s. It is cheap and relatively safe. It has been known that this compound is virucidal for more than 80 years.[42,43] It is a "photosensitizer". When administered in the early 20th century, patients were exposed to bright sunlight (photobiomodulation) immediately afterward. MB is known to kill coronavirus (specifically SARS-CoV-2) and HIV in the blood supply (when combined with photobiomodulation).[44,45,46] The last of these three articles is a preliminary report from China. MB and UV-C or visible light has also been shown to inactivate other serious viruses in platelet concentrates and plasma including SARS-CoV-1, Crimean-Congo hemorrhagic fever virus, Ebola virus, MERS-CoV, and Nipah virus.[47,48] An animal model study[49] (in vivo) also demonstrated the ability of methylene blue plus fluorescent light to inactivate West Nile Virus. Although this was a very small study (5 mice in each group), 100% of treated animals survived and 100% of the placebo group died. Treatment of the blood supply with methylene blue and photo treatment produces no change in red blood cell morphology or functions.[50] MB (orally) was the first malaria medication used and is now being used again, especially for patients who are resistant to other medications.[51] In addition, other malaria drugs were derived from MB.

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MB is a very potent anti-oxidant and inhibits nitric oxide synthase and production of guanylate cyclase. Nitric oxide synthase generates large amounts of reactive nitrogen species. If controlled, this reaction may help rid the body of viruses. If dysregulated (as in shock) the generation of these reactive nitrogen species can be very harmful. This is specifically seen in viral pneumonitis. During this process, oxyhemoglobin (HbO_2) is converted to methemoglobin (MetHb) as well. In higher doses (>5mg/kg), MB can have pro-oxidant features and can cause nausea and vomiting. Inhibition of nitric oxide synthase and guanylate cyclase both prevents and treats shock (post-operative, septic, and anaphylactic shock).[52,53,54,55,56,57,58,59,60] MB prevents and effectively treats vasoplegic shock after cardiac bypass.[61,62,63,64,65]

There is one case series report which describes a cohort of 2500 immunocompromised (i.e. high risk) French cancer patients who were on methylene blue previous to the outbreak and had no cases of COVID-19 as of March 27, 2020.[66] It would be interesting to see if this is still the

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case as infection rates have increased in Europe since then. Of note, the patients in this cohort also took alpha lipoic acid (the body's only fat- and water-soluble antioxidant), hydroxycitrate, and were on a "low carbohydrate diet." This same article contains a very detailed explanation of the actions of methylene blue, chloroquine, and hydroxychloroquine which is well worth reading. MB is actually an ancestor of chloroquine. There is another report that some Plasmodium parasites are infected themselves with an RNA virus called MaRNAV-1.[67]

Like chloroquine, MB can form a weak base by losing an electron (pK_a=9).[49] MB increases reduced glutathione (the body's most powerful antioxidant).[68] It is both an inhibitor and a substrate for production of reactive oxygen species. MB irreversibly consumes NADPH and molecular oxygen and leaves less glutathione for malarial parasites. It is concentrated only in red blood cells infected with malarial parasites. MB also increases ATP production while preventing the production of reactive oxygen species in the electron transport chain.[69] MB has also been shown to inhibit[70] the NLRP3 inflammasome which is upregulated in many pulmonary diseases including SARS-COV-1.[71]

Molecular mechanisms of action of Chloroquine and Hydroxychloroquine

Hydroxychloroquine is less toxic than chloroquine and studies suggest that it may be more potent.[72] Proposed mechanisms of action are noted in this source.[73] It is a weak base (like methylene blue) which increases pH in the lysosomal and Trans-Golgi Network (TGN) vesicles to make an unfavorable condition for viral replication. Alkalinization also inhibits the post-translational modification of newly synthesized envelope proteins in those same locations (which is required by coronavirus).[60,74] Chloroquine decreases free iron concentration in the cells which

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impairs some enzymes involved in genetic expression.[60] Chloroquine lowers inflammatory substances like TNFα in vitro.[75,76] Both Chloroquine and hydroxychloroquine inhibit cytokine production, specifically IL-6 which is implicated in the cytokine storm in COVID-19 patients in vitro.[77,78] The most serious complication of the use of chloroquine and hydroxychloroquine is macular retinopathy (typically with prolonged use).[60]

The SARS-CoV-1 virus and the SARS-CoV-2 virus both bind to the ACE-2 receptor. In studies with SARS-Co-V-1, chloroquine was found to glycosylate the ACE-2 receptor which inhibited attachment and spread of the SARS-CoV-1 virus in primate cells.[79] Chloroquine has been shown to have antiviral activity for many diverse RNA viruses including rabies virus, poliovirus, HIV, hepatitis A virus, hepatitis C virus, influenza A and B viruses, influenza A H5N1 virus, Chikungunya virus, Dengue virus, Zika virus, Lassa virus, Hendra and Nipah viruses, Crimean–Congo hemorrhagic fever virus, and Ebola virus.[62,66]

Evidence for hydroxychloroquine and chloroquine and COVID-19

An article from the *Journal of Critical Care* from 10 March 2020 concluded that "there is rational, pre-clinical evidence of effectiveness and evidence of safety from long-time clinical use for other indications to justify clinical research on chloroquine in patients with COVID-19."[80] One reference[81] in this paper described an in vitro study using Vero E6 cells. In the study, five drugs including ribavirin, penciclovir, nitazoxanide, nafamostat, chloroquine and two antiviral drugs remdesivir and favipiravir were tested for activity against a clinical isolate of SARS-CoV-2 in vitro. In the study, remdesivir and chloroquine were shown to be effective at controlling the virus. Hydroxychloroquine also inhibited SARS-CoV-2 in an in vitro study.[82]

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The first patient study[83] using the combination of hydroxychloroquine and azithromycin came from France on 20 March 2020. It was an open label non-randomized trial. It was a small trial with 36 patients, 20 in the treatment arm and 16 controls. Hydroxychloroquine caused viral clearance (by PCR test) statistically sooner than placebo. Adding azithromycin augmented this result. In a randomized, Chinese study[84] of 62 patients (31 in each treatment arm), hydroxychloroquine significantly shortened time to clinical recovery compared to the control group. Both groups received "antivirals, antimicrobials, steroids, and standard care." Another French study found benefit to the combination of hydroxychloroquine and azithromycin. This was a case series of 80 patients.[85]

There are at least 23 clinical trials currently underway or proposed using hydroxychloroquine and azithromycin. The FDA recently released an emergency use authorization[86] for the drugs for clinical trials or for patients without an available clinical trial. The data for these medications is not robust at this time and most of the literature comes from centers where they are using antivirals and other medications concomitantly. However, as patient progression with this disease can be rapid and unpredictable, medications with a favorable safety profile, plausible mechanism of action, and long track record in humans are being used as part of a "no holds barred" approach.

Azithromycin

Since hydroxychloroquine and azithromycin both prolong the QT interval, caution is advised, especially with older patients and those with cardiovascular conditions and those on other medications which may prolong the QT as well. Azithromycin has been shown to have antiviral effects in-vitro[87] Proposed mechanisms of action included preventing internalization of the virus into host cells during the early phase of infection, targeting newly budded progeny virus,

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and inactivation of endocytic activity. It has been shown in vitro to inhibit Zika virus infection.[88,89,90] One prospective study[91] was done with IV azithromycin in patients with severe ARDS and demonstrated a statistically significant decrease in 90-day mortality and decreased time to discontinuation of mechanical ventilation. In an in vitro study,[92] azithromycin suppressed inflammatory chemokines. In a prospective, randomized, open label study[93] of hospitalized patients with influenza, azithromycin plus oseltamivir therapy was compared with oseltamivir alone and addition of azithromycin provided statistically significant reduction of inflammatory cytokines (including IL-6) and C-reactive protein. Studies in SARS-CoV-2 are limited to combination therapy with hydroxychloroquine. The limitations of these studies are discussed above.

Prescription antivirals

Detailed discussion of the literature concerning antivirals will not be undertaken at this time in an effort to address other agents. Currently both single and combination antivirals are being used which have shown in vitro activity against SARS-CoV-1 and SARS-CoV-2.

Zinc

Zinc is an essential mineral for the body and must be obtained from the diet or supplementation since the body has no storage system for zinc.[94] Zinc deficiencies have been documented[95] in 30% of diabetic patients and in a large number[96] of "healthy" elderly patients. At

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least 35-45% of adults over 60 are zinc deficient.[97] It supports over 300 enzymatic functions in the body and is vital for normal growth and development.[98,99]

Zinc is essential to immune function and even mild deficiencies in zinc can cause impairment of the immune system. It is particularly important for T-cell immune function.[84] Zinc deficiency decreases natural killer cell lytic activity and the percentage of precursors of cytolytic T cells.[100,101] Deficiency also leads to defects in innate immunity and decreases the ability to deal with oxidative stress.[102] Zinc supplementation decreases oxidative stress.[103] Prolonged intake of high doses of zinc can cause low copper levels which can, in unusual cases, result in myoneuropathy and anemia.[104] Zinc is required for a proper sense of smell[87] although intranasal use of some zinc containing products has been associated with anosmia.[105]

A randomized, prospective study of over 1600 infants under age 2 who received zinc supplementation showed decreased rates of pneumonia.[106] An observational study[107] of nursing home residents showed that, compared with subjects with low zinc concentrations, subjects with normal serum zinc concentrations had a lower incidence of pneumonia, fewer (by almost 50%) new antibiotic prescriptions, a shorter duration of pneumonia, and fewer days of antibiotic use. Normal baseline serum zinc concentrations were associated with a reduction in all-cause mortality. A Cochrane database review[108] and a trial in the *Annals of Internal Medicine*[109] showed

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that zinc supplementation reduced the duration of the common cold. In vitro studies[110] show zinc inhibits RNA replication in SARS-CoV-1 virus as well as Dengue virus.[111] An article[112] published in 2014, reported that chloroquine is a "zinc ionophore" and it increases zinc concentrations in lysosomes in vitro. This led to speculation[113] that this combination may lead to a role against SARS-CoV-2. We were unable to find direct studies of monotherapy with zinc in vivo or in vitro in reference to SARS-CoV-2.

Melatonin

Melatonin is a hormone produced by the pineal gland. It is traditionally known to initiate sleep and is released in low light conditions at night.[114] It is also produced in the GI tract, the retina, and in leukocytes both in blood and the bone marrow.[115] In an animal model of respiratory syncytial virus (RSV) infection, treatment with melatonin resulted in a marked reduction of reactive oxygen and nitrogen species with an increase in antioxidants including glutathione and superoxide dismutase. In the same study, levels of the inflammatory compound TNFα decreased.[116] TNFα is a prooxidant cytokine which amplifies the host inflammatory response. Melatonin is associated with increased survival of newborns with sepsis with reduction of levels of lipid peroxidation (malondialdehyde).[117] Positive results have also been seen in preterm infants with respiratory distress syndrome.[118] It reduced levels of proinflammatory cytokines, including IL-6, IL-8, and TNFα. Melatonin also reduced these same cytokines in another study in newborns with respiratory distress and improved clinical outcomes.[119]

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The role of melatonin in sepsis due to bacterial and viral infections has been extensively discussed.[120,121] A large meta-analysis of 22 datasets of 749 adult patients also showed significant reduction of TNFα and IL-6.[122] IL-6 has been implicated in the "cytokine storm" in severe COVID-19 cases.[123] Recent articles[124,125] support the theory that melatonin may help with SARS-CoV-2, since it regulates the NLRP-3 inflammasome and reduces neutrophil and macrophage entry in acute lung injury, ARDS, oxygen-induced lung injury, and radiation injury to the lungs. Several publications have suggested melatonin as a treatment for Ebola virus.[126,127,128] Melatonin levels decrease with age[129,130] which may be one of the reasons that elderly patients with COVID-19 have a worse prognosis. We were unable to find any in vitro or in vivo studies with melatonin use and SARS-CoV-2.

Glutathione

As previously mentioned, glutathione is the body's most potent antioxidant and plays a very important role in reducing reactive oxygen species.[131] Glutathione depletion is associated

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with ARDS.[132] Serum GGT is sometimes elevated when there is a need for glutathione.[133] Although oral glutathione does not raise red cell levels, liposomal preparations of glutathione were shown in one small but well performed study[134] to raise glutathione concentrations 40% in whole blood, 25% in erythrocytes, 28% in plasma and 100% in mononuclear cells after 2 weeks. Glutathione is also available as an intravenous preparation.

Intravenous N-acetyl cysteine (the precursor of glutathione) is available and used to raise glutathione levels in acetaminophen overdose.[135,136] We were unable to find reports of adverse effects with IV glutathione. IV NAC has been shown in some patients with asthma to pose a risk for anaphylaxis. Glutathione and NAC inhibit the production and expression of TNFα, IL-6, and IL-8 by lipopolysaccharide-stimulated macrophages. The same review[137] also indicated that there is evidence that glutathione has direct antiviral effects and regulates part of the innate immune system. This has been echoed in other articles on glutathione and infection.[138,139] Oral liposomal glutathione was shown to increase NK cell cytotoxicity 400% and lymphocyte proliferation 60% after two weeks in one study.[132] There are several articles showing glutathione's role in combating various viral illnesses in vitro.[140,141,142] To our knowledge, there have been no direct studies either in vitro or in vivo showing effects of glutathione or NAC against coronavirus, specifically SARS-CoV-1 or SARS-CoV-2.

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Vitamin C

Vitamin C is a water-soluble antioxidant that protects the body from free radical damage.[143] The reduced form is known as ascorbate.[140] It is an essential nutrient from the diet because man cannot synthesize it in the body due to absence of an enzyme to do so.[144] Because vitamin C is a water soluble (and not fat soluble) antioxidant, toxicity is rare, even at high doses.[140] High oral doses of vitamin C downregulate vitamin C transport molecules, limiting the amount that can be absorbed orally.[145,146] The maximal dose of vitamin C orally is about 3000mg. If more is given, patients may have GI upset and diarrhea. Intravenous vitamin C at similar doses produces 6 fold greater blood levels and high doses (up to 50g) have been given without significant side effects.

Vitamin C has been shown to have anti-cancer activity.[147,148,149] Phase 1 trials in cancer have shown no adverse effects with doses up to 100g IV.[150] There is an excellent summary of the favorable research for IV vitamin C use for cancer from the National Cancer Institute.[151] It contains links to other studies which discuss the evidence.[152,153]

A Cochrane review showed that vitamin C produced a reduction in the number of symptom days with the common cold but did not prevent illness if taken prophylactically.[154] Higher doses up to 8 grams were noted to work better. Vitamin C stimulates or maintains T-cell proliferation to

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attack infections.[155,156] Vitamin C also enhances neutrophil activity and increases lysozyme-mediated killing.[157] High dose IV vitamin C reduces pro-inflammatory cytokines IL-1a, IL-2, IL-8, TNF-a, chemokine, and CRP in cancer patients.[158] Concentrations of vitamin C in the plasma and leukocytes rapidly decline during infections and stress. Supplementation of vitamin C in humans has been shown to improve antimicrobial and NK cell activities, lymphocyte proliferation, and chemotaxis.[161] In vivo animal studies show activity of vitamin C against influenza virus.[159] In vitro studies show that vitamin C reduces infectivity against a coronavirus.[160] We were unable to find any in vivo or in vitro studies of either oral or IV vitamin C in SARS-CoV-1 or SARS-CoV-2.

In addition, adequate Vitamin C intake significantly lowers LDL cholesterol and increases HDL cholesterol leading in animal and human models[161,162,163] and reduces cardiovascular mortality[164,165](a known increased risk factor in COVID-19 disease) and reduces all-cause mortality.[166]

Vitamin D

Multiple studies have shown that significant proportions of adults are deficient in vitamin D (25-OH vitamin D level < 20ng/mL).[167,168] Another study found that elderly people make about

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25% of the vitamin D compared to young adults when exposed to the same light.[169] In a study[170] performed with 290 patients in a general medical ward, 57% were found to have vitamin D levels < 15ng/mL, and 22% of patients were found to have severe deficiency (< 8 ng/mL). Serum 25-OH Vitamin D has been shown to be a good marker for tissue vitamin D.[171]

Many people assume that vitamin and mineral needs are met in the standard American diet. Food, however, is a poor source of vitamin D.[172] Even though much of the milk in the United States is fortified with vitamin D, it typically contains 100 IU or less per serving. A 3-year study[173] in Finland showed that vitamin D fortification of milk did not increase vitamin D levels significantly in a group of men who had vitamin D deficiency, including those who drank 4 or more glasses of fortified milk daily. Vitamin D supplement users (who were later excluded from the study) were found to have 25-OH vitamin D levels 52% higher than non-users.

Sun exposure alone in latitudes above 42° will not allow for adequate vitamin D production in humans since most UV-B is absorbed by the atmosphere.[174] However, deficiency has also been demonstrated in sunny locations like Brazil[175] and South Florida.[176]

Although often thought about only in the context of bone health, vitamin D has many extra-skeletal benefits. A 12,000 patient study[177] in Finland showed that vitamin D supplementation in infants (2000 IU daily) reduced the risk of type 1 diabetes by over 80%. Vitamin D supplementation to pregnant mothers reduces the risk of childhood asthma by 40%.[178] Vitamin D deficiency increases the risk of multiple sclerosis and levels over 41 mg/dL show reduction in risk in a study

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of 7 million military members.[179] Supplementation of vitamin D has been shown to reduce the risk of multiple sclerosis.[180] In addition to autoimmune disease, in one very large study there was an inverse relationship of vitamin D levels to several adverse health outcomes.[181]

The vitamin D receptor is ubiquitously expressed on immune cells (B cells, T cells and antigen presenting cells) and these immunologic cells modulate the innate and adaptive immune responses. Deficiency of vitamin D is associated not only with increased autoimmunity but also with an increased susceptibility to infection.[182,183]

Vitamin D and seasonal influenza has been studied.[184] In a well done, prospective, randomized study[185] by Urashima et al., 167 Japanese school children were given 1200 IU of vitamin D and experienced a 42% reduction of the incidence of influenza A. In addition, among children with asthma in the study, there was an 83% reduction of asthma attacks.

Vitamin D actually increases the antiviral activity of bronchial epithelial cells in vitro.[186] Vitamin D is integral to the production of defensins and cathelicidins which are antimicrobial peptides that provide a natural immune defense against microbiological pathogens. Vitamin D supplementation increases cathelicidin production and helps lung infection.[187] We were unable to find any studies investigating the role of vitamin D in coronavirus infection either alone or in combination with other therapies although it has been shown to have antiviral activity against enveloped viruses.[188]

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Optimal levels, dosage, and safety of vitamin D

The Institute of Medicine has listed the RDA for vitamin D as 400 IU-800 IU.[189] However, in 2017, several reviews[190,191,192] of the IOM data discovered a statistical error in the calculations used to estimate RDA. It was subsequently found, upon reanalysis of the data sets, that between 6000 IU and 9000 IU would be required for 97% of people to reach the most beneficial level, which may be, according to the authors, 40 mg/dL and not the usual lower limit of 20 mg/dL listed on most labs.

Vitamin D deficiency is often accompanied by elevation of parathyroid hormone. Elevated parathyroid hormone (PTH) has been associated with metabolic syndrome, increased cardiac risk and increased vascular calcification. Several small studies have defined the ideal serum 25-OH vitamin D level as the level of vitamin D at which the serum PTH reaches is lowest related value (the asymptote). Various 25-OH vitamin D levels have been suggested to accomplish this goal (12-44 ng/mL). A study[193] of 312,962 patients from 2012 using paired PTH and 25-OH vitamin D levels found that PTH continued to decrease even at vitamin D levels above 60 ng/mL.

In a study[194] of 61 adult patients who were randomized to a vitamin D supplement at either 1000 IU or 4000 IU dose, both doses increased the serum vitamin D level, and the higher dosage only produced an average level of 39 ng/mL. Even though the lower limit of “normal” lab values for 25-OH vitamin D are often listed as 20 ng/mL, “optimal” level for the favorable extra-skeletal

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benefits of vitamin D is likely around 60 ng/mL. [195,196,197] Even though most complications from vitamin D toxicity occur at 25-OH vitamin D levels of >200 ng/mL, much of the current literature defines the safe upper limit to be 100-120 ng/mL.[198]

Upper limits intake of vitamin D intake per day (including all sources) are commonly listed as 10,000 IU per day. Intake below 10,000 IU per day is unlikely to increase the risk of toxicity[199], especially if serum levels are followed. In a study[200] in the journal Neurology, higher intake of vitamin D (10,000 IU/day) was found to be safe and was associated with an immunomodulatory effect and less relapse risk for multiple sclerosis patients. Another article[201] from 2010 in the Annals of Neurology, suggested *raising* the vitamin D level by 20 ng/mL could halve the rate of MS relapse.

Published cases[202] of hypercalcemia which have been attributed to vitamin D are often associated with high calcium supplementation or when levels of vitamin D are not followed regularly. Repletion of deficiency is routinely[203] corrected by giving 50,000 IU once weekly for 8-12 weeks. This is equivalent to about 7143 IU daily. In fact, experts have concluded that we are likely significantly underdosing vitamin D and have noted that doses of up to 10,000 IU daily have not caused toxicity.[204] One exception is patients with granulomatous disease who are more likely to develop hypercalcemia and hyperphosphatemia if serum levels rise above 30 ng/mL.[205] There

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has been at least one study[206,207] which gave 200,000 IU and 600,000 IU (once) either orally or intramuscularly to patients with vitamin D deficiency. Levels were tested a few weeks later. If repletion with these doses is contemplated, patients should be screened for hypercalcemia and hypercalciuria prior to therapy, and urinary and serum calcium levels should be monitored during vitamin D treatment so as to avoid toxicity.

Several recent studies have been done in the past which have reported that vitamin D was "not found to be helpful" when given as a supplement in certain conditions. Unfortunately, close examination of the methods section of these studies most often reveals that either dosages were used that were much too low (400 IU) and/or blood levels were never checked. There is some genetic and gastrointestinal variability in the absorption of vitamin D. Patient therapy must be individualized and levels must be checked to guide therapy. Vitamin D3 is better absorbed than Vitamin D2.[208]

Results:

After careful analysis of the available literature, we were able to formulate a proposed protocol for use in clinical trials in hospitalized patients with COVID-19. The medications and nutraceuticals used in this protocol are relatively inexpensive and have a proven track record of safety over many years in patients. The combination of in vitro and in vivo evidence and the molecular mechanisms of action suggest that these agents may be effective for SARS-CoV-2. The agents are: IV methylene blue, IV vitamin C, zinc, melatonin, vitamin D, and IV glutathione.

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