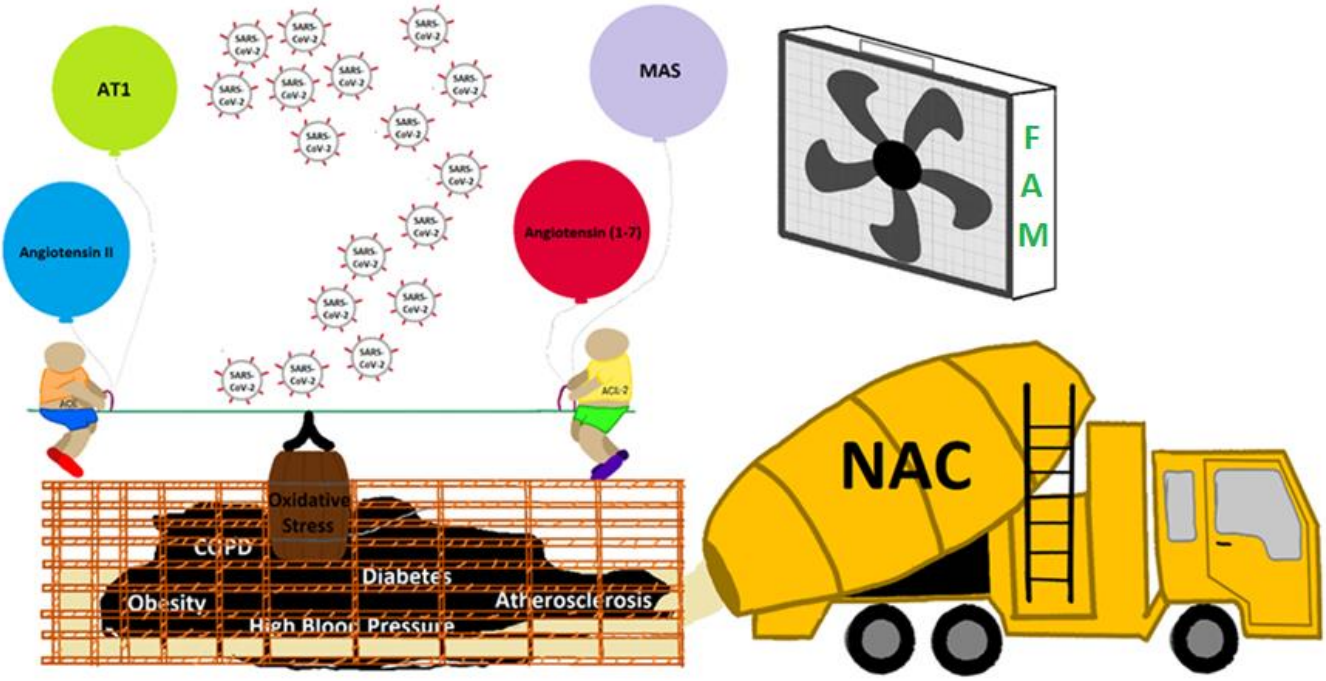
PI Name: John Joseph O'Connell, MD

Additional authors:
Larisa D Coldebella Perino, MD; Jennica Siddle, MD; Phillip Moschella, MD, PhD; Arwen BL Declan, MD, PhD; Angelo Perino, MD; Amanda Schnee, MD; Deborah Kunkel, PhD; Corey Andrew Kalbaugh, PhD; Lior Rennert, PhD; Alex Ewing, PhD; Prerana Roth, MD; Michael Cohen, PharmD; Halee Bryant Vaughn; David Y Pritchett; Benjamin R Hutto; Josiah N McDonald; Angela Orsky, DNP; Timothy F McTigue, MD; and W. Jeffery Edenfield, MD

Study Title: A Phase I Clinical Trial of the Combination of Famotidine (FAM) and Oral N-Acetyl Cysteine (NAC) Open Label for Outpatient Treatment of Subjects with Newly Diagnosed SARS-CoV-2 Infection

Protocol Date: 18 August 2020



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I) SPECIFIC AIMS

i) Aim 1

The primary outcome is to assess the safety and toxicity profile of the combination of famotidine and oral n-acetyl cysteine in adult outpatients with newly diagnosed SARS-CoV-2 infection.

ii) Hypothesis 1

We hypothesize that the combination of famotidine and n-acetyl cysteine are a safe combination of therapies in adult outpatients with newly diagnosed SARS-CoV-2 infection.

iii) Aim 2

The secondary outcome is to assess whether certain markers (e.g., IL-1β, IL-2, IL-6, Tryptase, Glutathione, F2-Isoprostane/Creatinine, N-methylhistamine, CAP-SYM 18, IPSS and CURB-65) are predictive of outcomes and/or treatment response.

iv) Hypothesis 2

We hypothesize that treatment with the combination of n-acetyl cysteine and famotidine in addition to the potential for reducing the primary endpoint of risk of hospitalization may also affect specific markers of infection which may prove useful surrogates for predicting the severity of the infection.

II) BACKGROUND AND SIGNIFICANCE

i) Background

N-Acetyl Cysteine (NAC) is a prodrug to L-cysteine which is a precursor to the biologic antioxidant glutathione. Administration is FDA approved for acetaminophen poisoning for which it is nearly 100% effective if given with 8 hours[1] and works through replenishing glutathione stores.[2]

Famotidine is a histamine-2 (H_2) receptor antagonist. Administration is approved in adult and pediatric patients 40 kg and above for the treatment of active duodenal ulcer, active gastric ulcer, symptomatic non-erosive gastroesophageal reflux disease (GERD), and erosive esophagitis due to GERD if diagnosed by biopsy. Administration is also approved in adults for the treatment of pathological hypersecretory conditions (e.g., Zollinger-Ellison Syndrome, multiple endocrine neoplasias) and for the reduction of the risk of duodenal ulcer recurrence.

There have been no studies on the use of the combination of FAM and NAC in the treatment of outpatients who test positive for SARS-CoV-2 infection.

a) Biochemical rationale

1. ***Angiotensin Converting Enzyme 2 (ACE2) is SARS-CoV-2 Virus Binding Site***

ACE2 is also one of the primary receptors for SARS-CoV invasion into the human body.[3,4] The recent outbreak causing novel coronavirus pneumonia (COVID-19) is caused by the virus (2019-nCoV, SARS-CoV-2) which has also been shown to invade human alveolar epithelial cells through mainly ACE2.[5]

2. ***The type II Transmembrane Serine Protease (TMPRSS2) is Critical in SARS-CoV-2 Infection and its Expression is Upregulated by ROS and Decreased by NAC***

It has recently been demonstrated that the SARS-CoV-2 virus binds to the ACE2 receptor and the serine protease TMPRSS2 is critical for Spike protein priming. [6] In SARS-CoV it had been previously demonstrated that TMPRSS2 can cleave and activate the SARS-CoV Spike protein which then allows the viral membrane to fuse with the cell membrane.[7]

In normal porcine epithelial cells it has been demonstrated that oxidative stress enhanced paracellular permeability of the cell layer which was accompanied by predominantly cytoplasmic occurrence of TMPRSS2 embedded in cell membrane under physiological conditions. These results support that ROS can influence paracellular gate opening via multifaceted mode of action and that an altered distribution pattern of TMPRSS2 and relocalized transmembrane serine protease activity may contribute to weakening of epithelial barrier integrity under acute oxidative stress.[8] Thus, it would be reasonable to hypothesize that cells exposed to oxidative stress may be more susceptible to SARS-CoV-2 infection through its interaction with TMPRSS2.

In fact, it has been demonstrated in-vitro that oxidation inhibition reduces the expression of TMPRSS2. Furthermore, ROS was demonstrated to significantly increase the mRNA

expression of TMPRSS2 and the addition of n-acetyl cysteine was demonstrated to significantly reduce the expression of mRNA level of TMPRSS2.[9]

3. ***Intra-Epithelial Lymphocytes are a Critical 1st Line of Defense to Coronaviruses***

The defense of a mucosal surface against viral infection is dependent in part on the leukocyte population resident at that site. It has been demonstrated that intraepithelial leukocytes (IEL) taken from the bovine intestinal epithelium were able to inhibit bovine coronavirus (BCV) replication, and this activity was markedly enhanced by interleukin-2 and tumor necrosis factor.[10] It was further demonstrated that the IEL mediated higher levels of IL-2-activated, antibody-dependent, and lectin-dependent cytotoxicity than did lymphocytes from mesenteric lymph nodes, Peyer's patches, or the spleen.

Further evidence of the role of IELs in the defense against coronaviruses has been demonstrated in mice when exposed to the enteric murine coronavirus (MHV-Y). A potent in-vitro cytotoxic activity was demonstrated by mucosal leukocytes, especially IEL, and spleen cells for MHV-Y-infected syngenic and allogeneic target cells.[11] Targets infected[12] with Pichinde virus, an enveloped nonenterotrophic virus, were not lysed by IELs.

4. ***Famotidine inhibits Histamine Suppression of Intra-Epithelial Lymphocytes***

Histamine has been demonstrated to significantly decrease IL-2 production in IELs as well as splenocytes. It has been further demonstrated that famotidine, but not histamine 1 or 3 receptor antagonists, competes with the inhibitory effect of histamine on the production of IL-2 and other cytokines in IELs.[13]

Therefore, since IEL activity against the bovine coronavirus was markedly enhanced by IL-2, and famotidine competes with the inhibition of IEL production of IL-2 by histamine, a mechanism of action by which famotidine may be beneficial in patients infected with the SARS-CoV-2 is suggested.

5. ***Contribution of Mast Cells to SARS-CoV-2 Induced Inflammation***

Mast cells (MCs) express TLR which can be quickly recognized by pathogens including viruses. Viral activated MCs release early inflammatory chemical compounds including histamine, tryptases and chimase; while late activation provoke the generation of pro-inflammatory IL-1 family members including IL-1, IL-6 and IL-33.[12] While there is substantial evidence for mast cell mobilization and activation of effector cells following viral challenge, mast cells have also been implicated in inappropriate inflammatory responses, long term fibrosis and vascular leakage associated with viral infections.[14] Therefore, strategies to modulate the activity of mast cells but not entirely suppress them may take advantage of the positive effects of mast cells in helping the immune system to fight a SARS-CoV-2 infection while potentially mitigating the negative effects caused by the release of chemical mediators of inflammation and the secretion of pro-inflammatory cytokines that can substantially aggravate the pathological state of the patient.[12]

6. ***ACE2 Depletion Results in Oxidative Stress, Tissue Fibrosis and Thrombosis***

Distribution of ACE2 throughout the Body

In an non-disease status the ratio of ACE to angiotensin converting enzyme 2 (ACE2) appears to be relatively stable with ACE far more abundant.[15] In fact, ACE2 has been found in rats to co-regionalize with ACE throughout a body's tissues.[16] Thus, as would be expected, the endothelium of human lungs express a not insignificant amounts of ACE2 but remarkably surface expression of ACE2 is also found in lung alveolar epithelial cells.[17] In fact, lung tissue has high RAS activity and is the leading site of Angiotensin II synthesis.[3] Other locations which express ACE2 include enterocytes of the small intestine; and arterial smooth muscle cells, arterial, and venous endothelial cells, within the stomach, small intestine, colon, spleen, liver, kidney and brain.[17]

Intra-organ Renin-Angiotensin System (RAS)

Apart from the circulating RAS, the existence of local or intra-organ RASs have been described in a number of organs, including the heart, brain, kidney, lung, pancreas and liver.[18–22] These local systems have been shown to be responsive to various stimuli of physiological and pathophysiological importance. Moreover, the locally generated angiotensin peptides fragments have a plethora of actions and have been implicated in cell growth, anti-proliferation, apoptosis, reactive oxygen species (ROS) generation, hormonal secretion, pro-inflammatory and pro-fibrogenic actions (see figure 1).[18,23]

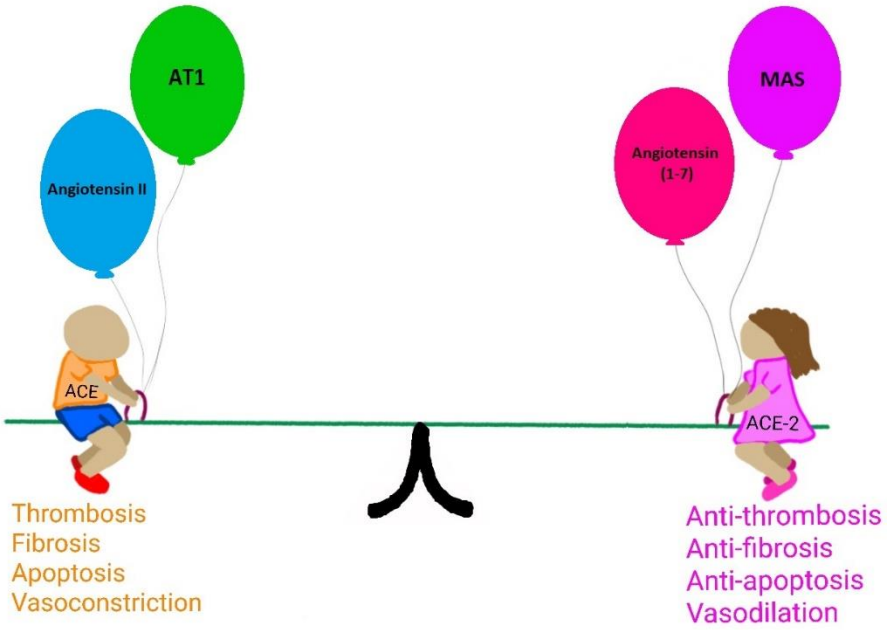

Figure 1: A cartoon drawing[23] of the putative ACE2-angiotensin-(1-7)-Mas axis, a counter-regulatory arm of the RAS that produce effects that oppose those of the ACE-angiotensin II-AT I receptor axis.

Mechanism of action of ACE2 – Serves a counter balance to ACE and reduces ROS

The renin angiotensin system (RAS) plays an important role in inflammation and fibrosis. The classical axis of the RAS, formed by angiotensin converting enzyme (ACE), angiotensin II (Ang II) and angiotensin receptor type 1 (AT1), activates several cell functions and

molecular signaling pathways related to tissue injury, inflammation and fibrosis. In sharp contrast, the RAS axis composed by angiotensin converting enzyme 2 (ACE2), angiotensin-(1-7) and Mas receptor exerts opposite effects in relation to inflammatory response and tissue fibrosis.[24]

In vitro, angiotensin II significantly increases the NADPH oxidase-4 (NOX4) level and reactive oxygen species (ROS) which is attenuated by NAC.[25,26] ACE2 deficiency has been shown in-vitro to increase ROS.[27,28] ACE2 overexpression has likewise been shown in-vitro to decrease ROS.[29] Thus, in conditions of chronic ROS it would be expected that both ACE and ACE2 would be more prevalent. This has been observed in patients with congestive heart failure where the level of soluble ACE2 was found to correlate with increased oxidative stress-mediated endothelial dysfunction.[30]

Tissue Fibrosis – Reduced by ACE2 Expression

Angiotensin II, the main effector peptide of the RAS (renin–angiotensin system), has been shown to be a key mediator of tissue fibrosis in a number of diseases, including chronic heart and kidney diseases and diabetes.[31] Recent studies indicate that angiotensin II may also play a central role in the pathogenesis of chronic liver disease and that the RAS is a promising potential target for anti-fibrotic therapies.[18] Even more recently, the RAS was demonstrated to mediate intestinal fibrosis in patients with inflammatory bowel disease and Angiotensin (1-7) was shown to reduce colonic myofibroblast proliferation in a dose-dependent manner.[32]

ACE2 Inhibits NOX4-Dervied ROS-Mediated Pathway to Reduce Fibrosis and Cell Death

The activation of the NOX-dependent generation of superoxide induced by Angiotensin II has emerged as a critical pathogenic factor in the development of pulmonary fibrosis.[33] NOX4-dependent generation of ROS (especially hydrogen peroxide [H2O2]) is required for TGF-β1-induced ECM generation and platelet-derived growth factor (PDGF)-induced migration of lung fibroblasts.[34,35] It was recently demonstrated that the ACE2/Ang(1–7)/Mas axis protects against lung fibroblast migration and lung fibrosis by inhibiting the NOX4-derived ROS-mediated RhoA/Rock pathway.[25]

In addition to reducing tissue fibrosis, activation of the ACE2/Ang-(1-7)/Mas pathway has been shown to reduce NOX4 expression and reduce oxygen glucose deprivation induced tissue swelling, ROS production, and cell death in the mouse brain with angiotensin II overproduction.[36]

Angiotensin (1-7) Prevents Pulmonary Fibrosis by Inhibiting TGF-β1-Smad Signaling Pathway

Accumulating evidence indicates that angiotensin (1-7) protects against pulmonary fibrosis in animal models but not only by the NOX4-derived ROS-mediated pathway. Angiotensin (1-7) also effectively inhibits epithelial-mesenchymal transition (EMT) induced by transforming growth factor- β1 *(*TGF-β1*)* in human alveolar epithelial cells by inhibiting TGF-β1 phosphorylation of Smad2 and Smad3 and suppressing the expression of downstream target genes of TGF- β1-Smad signaling (see figure 2).[37]

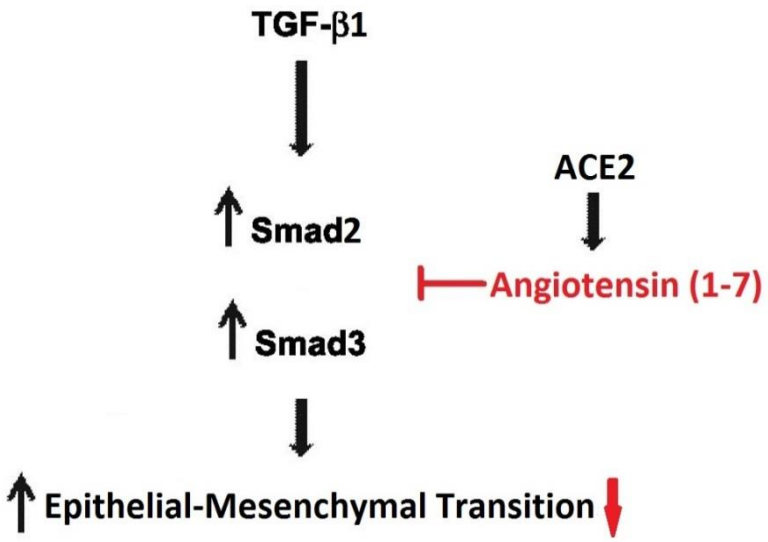

Figure 2. Schematic summary of the proposed mechanism of TGF-β1 induction of epithelial mesenchymal transition (EMT) in alveolar epithelial cells and inhibition by Angiotensin (1-7).[37]

ACE2 Protection against Thrombosis

Lung vascular injury is the most important initial cause of acute lung injury (ALI) and ARDS.[38] In the setting of both infectious and noninfectious lung injury, neutrophils accumulate in the lung microvasculature and become activated, leading to degranulation and the release of several toxic mediators, including proteases, reactive oxygen species, pro-inflammatory cytokines, and pro-coagulant molecules, which result in increased vascular permeability and a sustained loss of normal endothelial barrier function.[38]

There is growing evidence indicating that the counter-regulator axis of the renin-angiotensin system (RAS), composed of Angiotensin-Converting Enzyme 2 (ACE2), Angiotensin-(1–7) and the Mas receptor, has protective effects against thrombosis.[39]

The initial study demonstrating the direct antithrombotic effect of Ang-(1–7) was performed by Kucharewicz et al.[40] They observed that the intravenous infusion of Ang-(1–7) reduced the weight of the thrombus induced by the ligature of the abdominal vena cava in renovascular hypertensive rats.[40,41] However, Ang-(1–7) did not have any observed effect in normotensive rats.[41]

In addition to this activity on endothelial cells, evidence suggests that Ang-(1–7) may also directly modulate platelet activity. In 2008, the presence of the Mas receptor on platelets was detected and more importantly, it was demonstrated that the Mas receptor activation by Ang-(1–7) increased the production of NO in platelets.[42] Furthermore, it has been reported that incubation of platelet preparations with increasing concentrations of Ang-(1–7) reduced their adhesion to fibrillar collagen.[40]

ACE2 is also involved in the pathophysiological formation of a thrombus.[39] A decrease in ACE2 activity, but not in ACE2 protein expression, was associated with increased thrombus

formation in spontaneously hypertensive rats.[43] Supporting that finding, the pharmacological activation of ACE2 was associated with a marked reduction of thrombus formation in spontaneously hypertensive rats.[43] In addition, pre-treatment with,DX-600, an ACE2 inhibitor (see figure 3), increased the thrombus formation in both hypertensive and normotensive rats.[43] Moreover, ACE2 also modulates platelet activity, since its activation decreased platelet attachment to injured microvessels.[43]

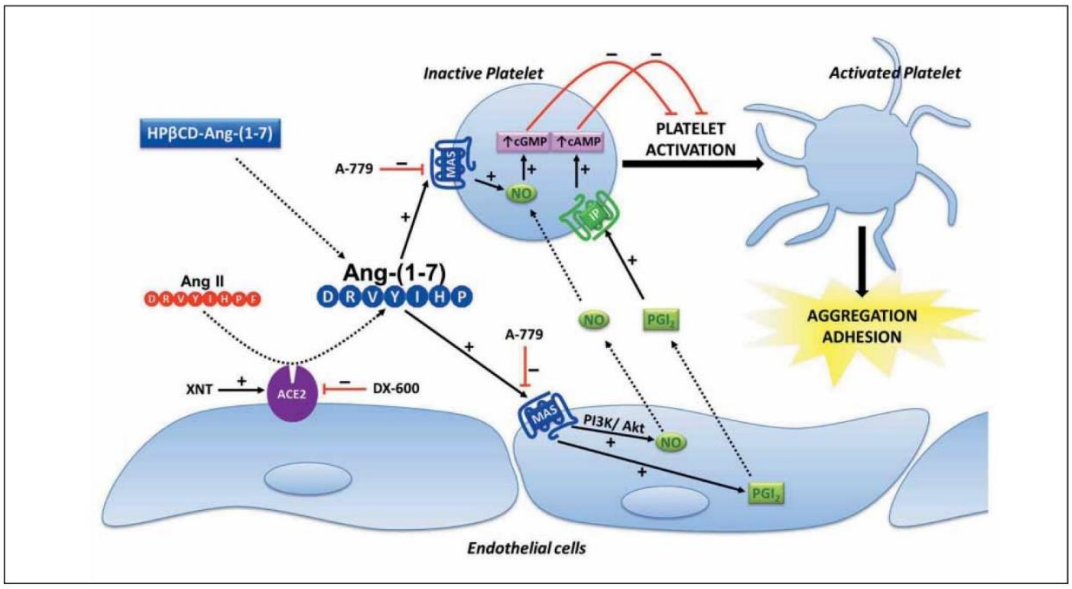

Figure 3. Schematic illustration showing the mechanism for the anti-thrombotic effect of ACE2/Ang(1-7)/Mas axis.[43]

7. ***NAC Reduces Oxidative Stress and Prevents Tissue Fibrosis and Thrombosis***

Mechanism of action of NAC – restoration of glutathione depletion from oxidative stress

N-acetyl-cysteine (NAC), is known to rapidly increase glutathione (GSH) synthesis in acetaminophen posioning[2]. GSH acts as a nucleophilic scavenger of electrophilic xenobiotic compounds and their metabolites, converting electrophilic centers via enzymatic conjugation into thio-ether bonds. In addition, GSH, as the only substrate of selenium-dependent GSH peroxidase[44,45] and also as a chemical reducing agent and antioxidant, has an extremely important role. By virtue of these features GSH is considered to be an outstanding protector of biological structures and function. GSH is synthesized intracellularly from the amino acids glycine, glutamate, and the thiol providing amino acid cysteine. Cell membranes in general are impermeable to the tripeptide glutathione. Hence, GSH is synthesized intracellularly only from the single amino acid constituents or their precursors.[44–46] Since glycine and glutamate are abundantly available in the intracellular space, an adequate supply and transmembrane transport of cysteine into the cell becomes the rate-limiting step in the biosynthesis of GSH. **The administration of exogenous N-acetyl cysteine (NAC) effectively meets intracellular needs of cysteine for**

the synthesis of GSH by supplementing precursor pools in a more physiological way.[44,47,48]

Any single GSH moiety, once it is conjugated to electrophils and transported out in this form, and also when oxidized, is not immediately available for subsequent needs. Oxidized glutathione is reduced once again to the functional form via the glutathione reductase pathway. Under these conditions the loss of glutathione remains reversible. Conjugated GSH, however, is exported from the cell and processed to mercapturic acids, which are excreted in the urine. In these reactions, GSH is lost irreversibly and can be replaced only via ex novo synthesis. Irreversible loss of GSH occurs preferentially following oxidative stress. Under conditions of intense oxidative stress, the rate of glutathione disulfide (GSSG) formation - through oxidation of GSH - is such as to surpass, and possibly exhaust, the intracellular glutathione reductase capacities; as a consequence, an accumulation of intracellular GSSG will result. GSSG in turn, being toxic to the intracellular milieu beyond a critical concentration, is exported from the cell and hence lost to the reductase pathway.[44,45,48]

NAC Inhibits NOX4-Dervied ROS-Mediated Pathway to reduce Fibrosis

In-vitro, Angiotensin II significantly increased the NOX4 level and ROS production in lung fibroblasts, which stimulated cell migration and a-collagen I synthesis through the RhoA/Rock pathway. These effects were attenuated by N-acetylcysteine (NAC). [25] In vivo, constant infusion with Ang(1–7) shifted the RAS balance toward the ACE2/Ang(1–7)/Mas axis, and alleviated bleomycin-induced lung fibrosis, and inhibited the RhoA/Rock pathway by reducing NOX4-derived ROS.[25] Thus, the NOX4-derived ROS pathway, which when inhibited appears to alleviate bleomycin-induced lung fibrosis in-vivo, has been shown to be inhibited in-vitro by NAC.

NAC Inhibits TGF-β Regulation of NOX4 to Reduce IL6 Release in Airway Smooth Muscle Cells

TGF-β induces the expression of NOX4 which is accompanied by an elevation of ROS levels and IL6 release from airway smooth muscles cells (ASMCs) which is reversed by NAC through inhibition of Smad3 phosphorylation (see figure 4).[49] **This suggests an important pathway by which NAC could reduce the severity of sudden acute respiratory syndrome in those infected with SARS-CoV-2 as it was recently observed in patients admitted to the hospital with symptomatic SARS-CoV-2 infection that IL-6 level was strongly associated with the need for mechanical ventilation, the maximal IL-6 level predicted for respiratory failure with high accuracy with the risk of respiratory failure 22 higher in patients with IL-6 levels greater the 80 pg/mL compared to patients with lower IL-6 levels.**[50]

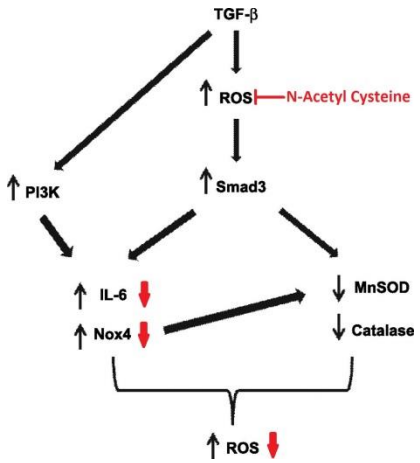

Figure 4. Schematic summary of the proposed mechanism of TGF-β mediated regulation of oxidant/antioxidant balance and IL-6 release in ASMCs and reversal by NAC.[49]

NAC Mitigates Toll-Like Receptor Activation to Reduce IL-1β and IL6

While the data in humans likely doesn't support NAC as effective in reversing progression of pre-existing idiopathic pulmonary fibrosis (IPF),[51,52] it is felt there may be specific clusters of IPF patients where NAC may have a role. In fact, NAC exposure was shown to be associated with improved transplant-free survival in ANA positive patients with usual interstitial pneumonia (UIP).[53] The mechanism in which this was felt possible was that NAC may have mitigated Toll-Like Receptor (TLR) activation by self-antigen/autoantibody complexes in ANA seropositive patients with UIP. The binding of SARS-CoV-2 to the TLR causes release of pro-IL-1β which is followed by production of active mature IL-1β, a mediator of lung inflammation and fibrosis.[54] In a guinea pig model, decreased expression of IL-1β was significant with NAC and was observed only in animals treated with NAC as compared to saline or ascorbic acid.[55] In a model of mouse ischemia-reperfusion, NAC prior to ischemia lead to a significant decrease in IL-1β levels after ischemia and significant decrease in IL-1β as well as IL-6 levels in bronchoalveolar lavage fluid after reperfusion.[56]

In a human placebo-controlled trial of oral NAC (600 mg twice daily) for 8 weeks in continuous ambulatory peritoneal dialysis patients, administration of NAC significantly diminished IL-6 ($p=0.002$) and IL-1 ($p=0.01$).[57]

Thrombosis – Reduced by NAC

Treatment with glutathione depletion agents has been shown in rabbits to significantly inhibit fibrinolytic activity in the arterial wall by induction of a significant reduction of the activity of tissue plasminogen activator and a significant increase in plasminogen activator inhibitor which suggests a modulatory role of glutathione in the release and/or clearance of components of the fibrinolytic system.[58] This suggests that NAC could have a role in preventing inhibition of fibrinolytic activity in disease states that could otherwise lead to a depletion of glutathione.

In fact, in-vitro data exists that demonstrates that NAC reduces platelet activation induced by oxidized low-density lipoproteins.[59] The reduction in platelet activation by NAC appeared to be through its effect of increasing activity of the PI3K/AKT/mTOR pathway to reduce platelet autophagy thus inhibiting platelet aggregation (see figure 5).

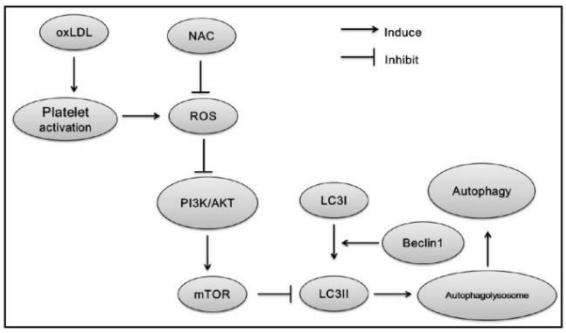

Figure 5. Schematic illustrating proposed mechanism by which ROS induce autophagy to promoted ox-LDL-induced platelet activating by inhibiting the PI3K/AKT/mTOR pathway is inhibited by NAC.[59]

In-vivo studies also demonstrate that NAC can reduce formation of thrombosis. In a mouse model of Type I Diabetes, in which the diabetic blood and brain became progressively more susceptible to platelet activation and thrombosis, the effects of diabetes were partly or completely reversed by NAC.[60]

In a large-vessel thromboembolic stroke model in mice, intravenous NAC administration was demonstrated to promote lysis of arterial thrombi resistant to conventional approaches such as recombinant tissue-type plasminogen activator, direct thrombin inhibitors, and anti-platelet treatments. It is important to note that NAC did not worsen hemorrhagic stroke outcome, suggesting that it exerts thrombolytic effects without significantly impairing normal hemostasis.[61] The results of this study demonstrate significant improvements in cerebral arterial occlusion events through a thrombolytic mechanism involving NAC. This effect appears to be mediated by the reduction of disulfide linkages that stabilize the high molecular weight forms of von Willebrand Factor (VWF).[62]

In well-established murine and baboon models for TTP, prophylactic administration of NAC was effective in preventing severe TTP signs in mice, but NAC was not effective in resolving preexistent acute TTP signs in mice and baboons.[63] Taken together with other studies, this suggests that NAC may be more effective in the acute setting or in preventing thrombosis rather than in the chronic setting.

In a human study of obese insulin-resistant subjects, NAC was found to reduce platelet aggregation ex-vivo; however, it required significantly higher concentrations of NAC with much longer exposer to NAC when compared to non-obese controls without insulin resistance.[64]

We therefore conclude that if NAC can reduce severity of SARS-CoV-2 infection in regards to thrombosis formation, it most likely would be effective if given early in the course of infection before significant thrombotic events have occurred and that higher doses and longer duration of dosing may be necessary in certain subjects such as the obese with insulin resistance.

8. ***NAC Reduces Viral Replication which is Increased by Oxidative Stress***

Oxidative Stress Increases Viral Replication

Inflammation has been shown to promote acute viral infection.[65] CMV virus enhancers were discovered, to an extent not previously recognized, to employ a path of least resistance by directly harnessing, within a short temporal window, the activation of anti-viral signaling in macrophages to drive viral gene expression and replication.[65]

Positive-sense single stranded RNA viruses have also been shown to utilize oxidative stress during infection to help control genome RNA capping and genome replication.[66]

NAC decreases Viral Replication through Reactive Oxidative Species Pathway

There is a plethora of in-vitro data demonstrating that NAC reduces viral replication, activation of latent virus or damage to infected cells in a multitude of viruses to include HBV,[67] CMV,[68] Coxsackievirsus B,[69] RSV,[70,71] H5N1,[72] EBV,[73] rotavirus,[74] HCV,[75] Rubella Virus,[76] rhinovirus,[77] KSHV,[78] porcine circovirus,[79] Enterovirus 71[80] and Adenovirus.[81] Additionally there is in-vitro data showing that reduction of oxidative stress by other anti-oxidants such as glutathione, which isn't easily absorbed orally unlike NAC, reduces viral replication in VZV[82] and HSV.[83,84]

The mechanism of action appears to be via the ROS/p53 signaling pathways.[85] Because this pathway is common to viral replication across a broad spectrum of viruses to specifically include positive-sense single stranded RNA viruses, we anticipate that NAC will likely also significantly reduce viral replication of SARS-CoV-2, a positive sense single stranded RNA virus.

b) Clinical rationale

1. ***Chronic Oxidative Stress is Associated with Conditions which Are Risk Factors for Severity of SARS-CoV-2 Infection and Death***

Patients with risk factors for increased oxidative stress or decreased cellular immunity, e.g., African ethnicity[86], male gender[87], elderly[88,89], obesity[90], hypertension[91], atherosclerosis[92], COPD[93], and DM[94] appear to have a much more virulent and potentially lethal SARS-CoV-2 infection.[95–97] In, fact recent evidence demonstrates that 94.3% of patients hospitalized in New York with SARS-CoV-2 infection had at least one high risk factor and 88% had more than one risk factors.[98]

Since ACE and ACE2 are both upregulated with oxidative stress and viral replication is increased with oxidative stress (see figure 6),[99] we hypothesis that severity of SARS-CoV-2 infection is likely far more virulent in patients with these conditions because the SARS-CoV-2 virus binding site, ACE2, serves as marker of those cells most hospitable for viral replication and the severity of tissue injury increases with ACE2 depletion (see figure 7).[100]

We further hypothesis that the earlier in the course of infection that attempts are made to reduce binding to ACE2 and to reduce oxidative stress the greater the potential exists to reduce the severity of the SARS-CoV-2 infection.

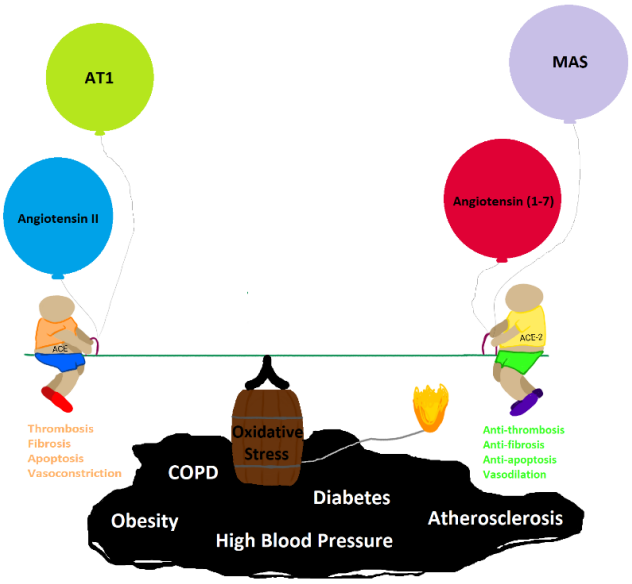

Figure 6: A cartoon drawing[99] of the increased but relatively balanced levels of ACE and ACE2 due to chronic oxidative stress.

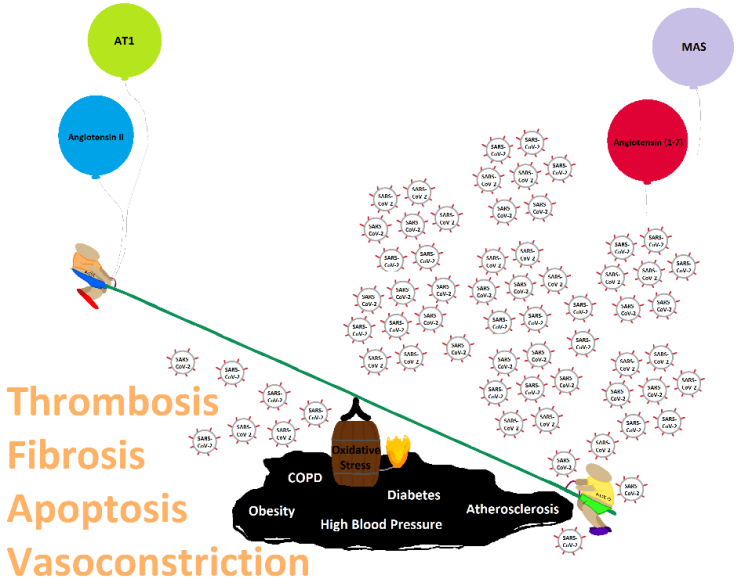

Figure 7: A cartoon drawing[100] of the increased severity of SARS-CoV-2 infection due to the sudden downregulation of otherwise increased levels of ACE2 due to conditions of chronic oxidative stress.

2. Clinical Pathology of SARS-CoV-2 Infection – Thrombosis and Fibrosis

Elevation of IL6 is Strongly Associated with Need for Ventilation and Respiratory Failure

Since the early descriptions, it appeared that the progressive worsening lung function of patients infected with SARS-CoV-2 is potentially driven by host immune response.[101] Recent analysis of 40 patients admitted to a single institution with PCR proven symptomatic SARS-CoV-2 infection were analyzed for baseline clinical and laboratory findings and elevated interleukin-6 (IL-6) was strongly associated with the need for mechanical ventilation (p=1.2·10-5).[50] In addition, the maximal IL-6 level (cutoff 80 pg/ml) for each patient during disease predicted respiratory failure with high accuracy (p=1.7·10-8, AUC=0.98). The risk of respiratory failure for patients with IL-6 levels of ≥ 80pg /ml was 22 times higher compared to patients with lower IL-6 levels.

Pathologic Findings - Massive Pulmonary Interstitial Fibrosis and Hemorrhagic Infarct

COVID-19 patients, and SARS patients, often develop a severe acute respiratory syndrome and appear to have typical ARDS pathology in the lung. [102] In a critical patient with COVID-19, histological examination demonstrated bilateral diffuse alveolar damage with cellular fibromyxoid exudates (figure 8A, B). The right lung showed evident desquamation of pneumocytes and hyaline membrane formation, indicating acute respiratory distress syndrome. The left lung tissue displayed pulmonary edema with hyaline membrane formation, suggestive of early-phase ARDS (figure 8B). Interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes, were seen in both lungs. Multinucleated syncytial cells with atypical enlarged pneumocytes characterized by large nuclei, amphophilic granular cytoplasm, and prominent nucleoli were identified in the intraalveolar spaces, showing viral cytopathic-like changes.[103] No obvious intranuclear or intracytoplasmic viral inclusions were identified. In fact, the pathological features of SARS-CoV-2 infection greatly resemble those seen in SARS and Middle Eastern respiratory syndrome (MERS) coronavirus infection.[102,104,105]

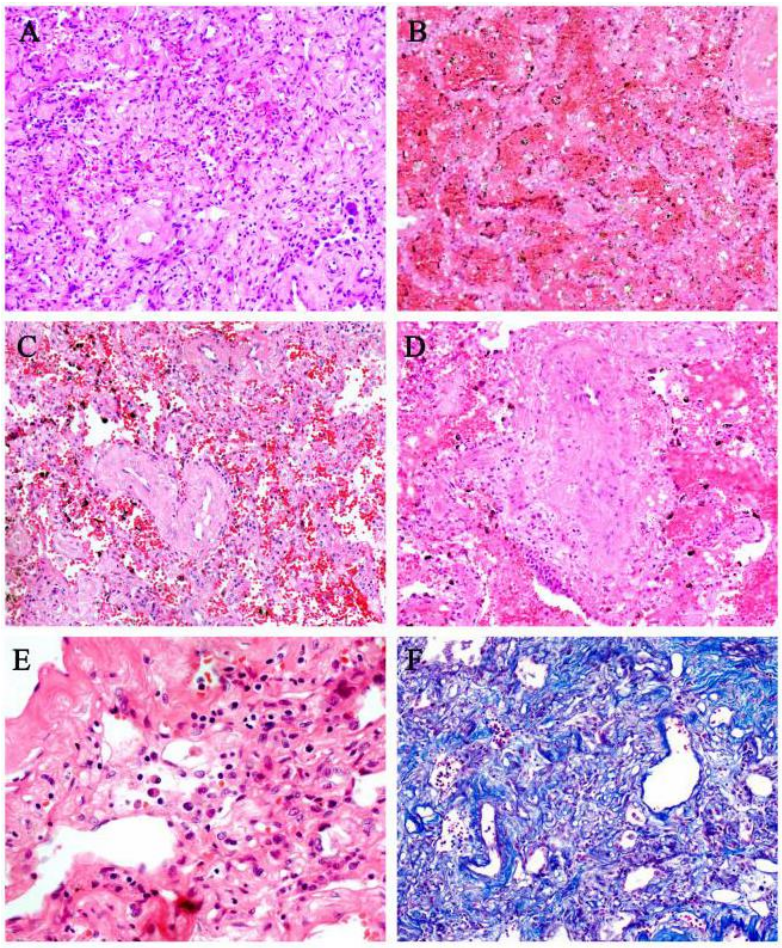

Figure 8. Pulmonary interstitial histopathology associated with critical patient

A: Massive pulmonary interstitial fibrosis. B: Pulmonary hemorrhagic infarct. C: Vascular wall thickening and lumen stenosis. D: Boangiitis obliterans are surrounded by inflammatory cells. E: Interstitial plasma cells infiltrating. F: Lung interstitial fibrosis by Masson stain.[102]

Microvascular COVID-19 Lung Vessel Obstructive Thrombo-Inflammation

In addition to the severe respiratory distress syndrome associated with SARS-CoV-2 infection, the infection appears associated with a transient increased risk of venous thromboembolic events. In the absence of major predisposing factors for venous thrombo-embolism, the severe COVID-19 pneumonia may be a precipitating factor for acute venous thrombo-embolism and acute pulmonary embolism.[106]

In a recent Dutch trial, a 31% incidence of thrombotic complications in 184 ICU patients with COVID-19 infections was reported.[107] It seems clear that more than standard anticoagulantion may be needed as a report of 388 patients from Milan in which 100% of ICU patients and 75% of general ward patients received thromboprophylaxis a cumulative rate of 21% of thromboembolic events were observed.[108]

In a study of patients with severe COVID-19 characterized by respiratory failure (n=5) and purpuric skin rash (n=3), examination of skin and lung tissue demonstrated there exists at least a subset of sustained, severe COVID-19 patients who experience a catastrophic microvascular injury syndrome mediated by activation of complement pathways and an associated pro-coagulant state.[109] Based on this study and observations of others, the use of MicroCLOTS (microvascular COVID-19 lung vessels obstructive thrombo-inflammatory syndrome) has been suggested as new name for severe pulmonary coronavirus disease 2019 (COVID-19). [101] It has been hypothesized that, in predisposed individuals, alveolar viral damage is followed by an inflammatory reaction and by microvascular pulmonary thrombosis. This progressive endothelial thrombo-inflammatory syndrome may also involve the microvascular bed of the brain and other vital organs, leading to multiple organ failure and death.[101]

Clinical Benefit from Anticoagulation

In a study of 449 patients with severe COVID-19, 99 of them received heparin (mainly with low molecular weight heparin, LMWH) for 7 days or longer. The D-dimer, prothrombin time and age were positively, and platelet count was negatively, correlated with 28-day mortality in multivariate analysis. No difference on 28-day mortality was found between heparin users and nonusers (30.3% vs 29.7%, P=0.910). But the 28-day mortality of heparin users were lower than nonusers in patients with SIC score ≥4 (40.0% vs 64.2%, P=0.029), or D-dimer > 6 fold of upper limit of normal (32.8% vs 52.4%, P=0.017).[110]

Not consistent with DIC rather Hypercoagulability with Severe Inflammatory State

In a separate study, whole blood from 24 patients admitted into the intensive care unit because of COVID-19 was collected and evaluated with thromboelastography by the TEG point-of-care device on a single occasion and six underwent repeated measurements on two consecutive days for a total of 30 observations. The results of this cohort of patients with COVID-19 were not consistent with acute DIC, rather they support hypercoagulability together with a severe inflammatory state.[111]

Recently, a group from Switzerland report a case of an ICU COVID-19 patient in which they observed a massive elevation of VWF, with VWF antigen at 555% (normal42- 136 %) and VWF activity of 520% (normal 42–168%), accompanied by an increase of Factor VIII clotting activity of 369% (normal 55–164%). They conclude, “We would like to emphasize the significance of anticoagulation in severe COVID-19 disease, by adding our observation of highly pathological data on anti-phospholipid-antibodies, von Willebrand Factor (VWF) and Factor VIII.”[112]

Taken together, given that NAC has demonstrated fibrinolytic properties, anti-platelet aggregation properties and the ability to reverse crosslinking of platelets through VWF, we propose that giving NAC early in the course of infection could go a long way to reduce the severity of a SARS-CoV-2 infection in regards to thromboembolic events.

3. MERS-CoV infection Leads to Lung Fibrosis

Symptoms caused by MERS-CoV are similar to those of SARS-CoV infection but unlike SARS-CoV and SARS-Cov2, the MERS-CoV uses the species-specific receptor, human dipeptidyl peptidase 4 (hDPP4), for its entry into host cells.[113,114] In a stable murine model of hDPP4-transgenic mice infected with MERS-CoV, histopathological signs indicative of progressive pulmonary fibrosis, including thickened alveolar septa, infiltration of inflammatory monocytes, and macrophage polarization as well as elevated expression of profibrotic molecules and acute inflammatory response were observed in the lung.[115] In humans, DPP4 is continuously expressed in epithelial cells[116] and human tracheobronchial epithelium, bronchial epithelium and renal epithelium have undergone cytopathic changes when infected with MERS-CoV in culture.[117]

Patients with MERS often present to a hospital with systemic and lower respiratory tract (LRT) signs and symptoms of disease which usually include fever, chills or rigors, dry or productive cough, shortness of breath and one or more comorbidities including diabetes, chronic kidney disease, chronic heart disease, recent surgery, hypertension, chronic lung disease, asthma, obesity, smoking, malignant disease or steroid use.[117] These comorbidities are all associated with conditions of chronic oxidative stress and of interest DPP4 like ACE2 appears to be a marker for NOX4-derived ROS. Inhibiting DPP4 in high glucose-induce endothelial dysfunction,[118] cardiac hypertrophy ,[119] chronic kidney disease,[120] perivascular adipose tissue,[121] and oxidized-LDL- induced endothelial dysfunction,[122] has been demonstrated to reduce ROS and NOX-4 expression. In fact, in cardiac hypertrophy, it was demonstrated that the inhibition of DPP4 significantly suppresses angiotensin II induced increase in NOX4 mRNA.[119]

Therefore, due to high risk for severe acute respiratory distress, increased risk of severity due to very similar comorbidities, and receptor binding domains (albeit different for MERS-CoV) that serve as for markers of cells most likely to enhance viral replication via a NOX4-derived ROS pathway, MERS appears very similar clinically and pathologically to COVID-19.

4. SARS Leads to Lung Fibrosis and Heart Fibrosis with Downregulation of ACE2

SARS-CoV infection leads to Increased Pulmonary Fibrosis via the EGFR Signaling Pathway which is blocked by NAC

Many survivors of SARS-CoV infection develop pulmonary fibrosis (PF), with higher prevalence in older patients. Based on this observation a study using mouse models of SARS-CoV pathogenesis identified that the wound repair pathway, controlled by the epidermal growth factor receptor (EGFR), is critical to recovery from SARS-CoV-induced tissue damage. In mice with constitutively active EGFR [EGFR(DSK5) mice], it was found that SARS-CoV infection caused enhanced lung disease. Importantly, it was show that during infection, the EGFR ligands amphiregulin and heparin-binding EGF-like growth factor (HB-EGF) were upregulated, and exogenous addition of these ligands during infection lead to enhanced lung disease and altered wound healing dynamic.[123]

Of particular note, it has been demonstrated in non-small cell lung (NSCLC) cancer cells exposed to cigarette smoke extract (CSE) that CSE stimulated ROS generation leading to

EGFR-TKI resistance via promotion of EGFR signaling which was suppressed by NAC.[124] A similar effect of NAC has been demonstrated in human head and neck cancer cells and the induced cytotoxicity by a TKI was demonstrated via a NOX4-dervied ROS pathway.[125] In EGFR-sensitive NSCLC cell lines, exposure to oxidative stress has been demonstrated to result in tyrosine kinase inhibitor (TKI) resistance by abnormal activating EGFR phosphorylation and disrupting the classical dimer structure of EGFR.[126]

Thus, the signaling pathways discussed earlier, in which Angiotensin (1-7) inhibits pulmonary fibrosis and in which NAC inhibits IL-6 expression, appears to increase pulmonary fibrosis in mice infected with SARS-CoV and is inhibited by NAC in cancer cell lines.

SARS-CoV Infection Leads to Downregulation of ACE2 and Severe Acute Lung Injury

Additionally, in a study of ACE2 knockout (KO) mice and wild type mice treated with either SARS-CoV or recombinant SARS spike protein, wild-type mice exhibited significantly reduced ACE2 expression in the lungs with either treatment. These wild type mice showed increased severity of pathological conditions in acute lung injury. Treating ACE2 KO mice with SARS-CoV resulted in only a very low quantity of virus being recovered and a slight reduction in leukocyte infiltrate score relative to uninfected ACE2 KO mice. In contrast there was a significant increase in the leukocyte infiltrate score in ACE2 wild type mice infected with SARS-CoV compared to wild type mice not infected. Therefore, the downregulation of ACE2 expression in SARS-CoV infection may play a causal role in the pathogenesis of SARS, which provides a reasonable explanation for the progression of SARS patients into ARDS.[3,127]

SARS-CoV Infection Leads to Heart Fibrosis with Downregulation of ACE2

Many patients with SARS, in addition to suffering a severe acute respiratory syndrome, also suffered from cardiac disease including arrhythmias, sudden cardiac death, and systolic and diastolic dysfunction.[128,129] Autopsy heart samples from 20 patients who succumbed to the SARS crisis in Toronto were used to investigate the impact of SARS on myocardial structure, inflammation and ACE2 protein expression. Reverse transcriptase PCR analysis showed 35% had positive SARS-CoV genome detected in their heart. Importantly, the duration of the illness was significantly shortened in patients with detectable virus in their heart (3.9 versus 43.2 days; $p < 0.05$). Staining showed increased myocardial inflammation and interstitial fibrosis in patients who had SARS-CoV detected in their hearts. Additionally, the presence SARS-CoV in the heart was associated with marked down-regulation of ACE2 protein expression.[128]

Based on these autopsy findings, an in-vivo study was performed on mice which were infected with the human strain of the SARS-CoV and encephalomyocarditis virus and were examined for ACE2 mRNA and protein expression. Pulmonary infection with the human SARS-CoV in mice led to an ACE2-dependent myocardial infection with a marked decrease in ACE2 expression confirming a critical role of ACE2 in mediating SARS-CoV infection in the heart.[128]

5. ACE2 Protects from Severe Acute Lung Failure

Injection of recombinant human ACE2 (rhuACE2) protein into acid aspiration-treated ACE2 KO mice decreased degree of lung injury. Acid aspiration-treated wildtype mice were also rescued by rhuACE2. Catalytically inactive ACE2 did not rescue the severe lung phenotype in ACE2 KO mice and had no effect on the severity of acute lung injury in wild type animals. In contrast to ACE2 KO mice, ACE KO mice were partially protected against acute lung injury by acid-aspiration. In addition inactivation of ACE on ACE2 KO mice rescued the mice from severe lung failure. Thus it was shown that ACE promotes acute lung injury pathology and ACE2 alleviates it.[130]

6. *NAC Significantly Reduces Duration in ICU in ARDS and Ventilator Associated Pneumonia (VAP)*

It appears in chronic progressive conditions such as idiopathic pulmonary fibrosis, NAC may be unlikely to reverse lung fibrosis and its potential to prevent further progression of lung fibrosis is uncertain.[51,131] However, in conditions of acute pneumonitis and prevention of acute lung injury, NAC appears more promising.[132–135]

In a recent meta-analysis of five randomized trials in patients with ARDS demonstrated that time in the ICU was significantly shortened in patients randomized to receive NAC.[132] While this meta-analysis did not show a survival advantage to NAC, the numbers of patients in the trials was relatively small and the cause of respiratory failure was likely varied and perhaps not always due to viral pneumonia or other causes of oxidative stress. Additionally, the dose of IV NAC in these trials were significantly lower than the maximum tolerated dose of 450 mg/kg[136] and the number of days NAC was administered seems somewhat short in several of the trials. Despite these limitations it is quite notable that there was still significant benefit found in reducing the number of days in the ICU.

In a separate randomized double-blind placebo controlled trial for the prophylaxis of ventilator-associated pneumonia (VAP), in 60 mechanically ventilated patients at high risk of developing VAP, 30 were randomized to receive a dose of 600 mg twice daily via NG tube.[137] Patients treated with NAC were significantly less likely to develop clinically confirmed VAP compared with patients treated with placebo (26.6% vs. 46.6%; P = 0.032). Patients treated with NAC had significantly less ICU length of stay (14.36 ± 4.69 days vs. 17.81 ± 6.37 days, P = 0.028) and less hospital stay (19.23 ± 5.54 days vs. 24.61 ± 6.81 days; P= 0.03) than patients treated with placebo. Time to VAP was significantly longer in the NAC group (9.42 ± 1.9 days vs. 6.46 ± 2.53 days; P = 0.002). The incidence of complete recovery was significantly higher in the NAC group (56.6% vs. 30%; P = 0.006). No adverse events related to NAC were identified.

In our proposed trial of non-hospitalized patients, we propose oral dosing twice a day based on data demonstrating oral dosing results in increased levels of glutathione in bronchoalveolar lavage fluid one to three hours after administration of 600 mg of oral NAC which fell by 16 to 20 hours.[138]

7. NAC Reduces Tissue Damage from Viral Replication

Reduces TissueIn-Vivo Activity of NAC to Reduce Tissue Damage from Viruses

NAC has more recently been shown to reduce viral replication in animal models[139] and reduces apoptosis due to increased reactive oxidative species (ROS) in healthy cells of animals infected with virus (shown in the liver of mice infected with the dengue virus[139], lungs of mice infected with H9N2 swine flu[140], and in the intestines of piglets infected with a **lethal porcine coronavirus,** porcine epidemic diarrhea virus (PEDV)).[85,141,142]

Porcine Coronavirus, Porcine Epidemic Diarrhea Virus (PEDV), severity in 2013-2014

For the last four decades, PEDV infection has resulted in significant economic losses in the European and Asian pig industries, but in 2013–2014 the disease was also reported in the US, Canada and Mexico. As a result of the significant impact of PEDV, the US pig industry lost almost 10% of its domestic pig population after only a 1 year-epidemic period, amounting to approximately 7 million piglets.[143]

Porcine Coronaviruses Bind to the Aminopeptidase N Receptor on Small Intestine Paneth Cells Inducing Mitochondrial Damage and ROS Causing Paneth cell decrease and Accelerated Viral Replication

Transmissible gastroenteritis virus (TGEV) is an enteropathogenic coronavirus, that like PEDV, causes severe lethal watery diarrhea and dehydration in piglets. PEDV has been shown to recognize protein receptor aminopeptidase N from pig and human and sugar co-receptor N-acetylneuraminic acid.[144] TGEV shares aminopeptidase N as its receptor binding domain (RBD) but unlike, PEDV doesn't appear to use N-acetylneuraminic acid as a co-receptor.[145,146] Initial invasion by TEGV into Paneth cells through the aminopeptidase N (APN) receptor has been demonstrated to induce mitochondrial damage and ROS generation, ultimately causing Paneth cell decrease and loss of Notch factors (DII4 and Hes5), which are essential for Lgr5 ISCs self-renewal and differentiation. Interestingly, loss of Notch signaling induced goblet cells differentiation at the cost of absorptive enterocytes and promoted mucins secretion, which accelerated TGEV replication.[147]

Intestinal Damage from Porcine Coronaviruses Reduced by NAC

Studies have indicated that TGEV infection induces cell apoptosis in host cells. A more recent study, further investigated the roles and regulation of reactive oxygen species (ROS) in TGEV-activated apoptotic signaling. The results showed that TGEV infection induced ROS accumulation, whereas UV-irradiated TGEV did not promote ROS accumulation. In addition, TGEV infection lowered mitochondrial transmembrane potential, which was inhibited by ROS scavengers, pyrrolidinedithiocarbamic (PDTC) and N-acetyl-L-cysteine (NAC). Furthermore, the two scavengers significantly inhibited the activation of p38 MAPK and p53 and further blocked apoptosis occurrence through suppressing the TGEV-induced Bcl-2 reduction, Bax redistribution, cytochrome c release and caspase-3 activation. These results suggest that oxidative stress pathway might be a key element in TGEV-induced apoptosis and TGEV pathogenesis.[141]

NAC and PDTC have also been demonstrated to result in significant Inhibition of apoptosis induced by PEDV. Moreover, further inhibition tests were established to prove that p53 was regulated by ROS in PEDV-induced apoptosis.[85]

In addition to in-vitro data demonstrating inhibition of apoptosis by either porcine coronavirus, TGEV or PDEV, there is in-vivo data demonstrating significant effect of NAC. A trial of thirty-two 7-day-old piglets randomly allocated piglets to one of four treatments in a 2 × 2 factorial design consisting of a liquid diets with or without 50 mg/kg BW NAC supplementation and oral administration of 0 or $10^{4.5}$ TCID50 (50% tissue culture infectious dose) PEDV. On day 7 of the trial, half of the pigs (n = 8) in each dietary treatment received either sterile saline or PEDV (Yunnan province strain) solution at $10^{4.5}$ TCID50 per pig. On day 10 of the trial, d-xylose (0.1 g/kg BW) was orally administrated to all pigs. One hour later, jugular vein blood samples were collected, and then all pigs were killed to obtain the small intestine. PEDV infection increased diarrhea incidence, while reducing average daily weight gain. PEDV infection also decreased plasma d-xylose concentration, small intestinal villus height, mucosal I-FABP and villin mRNA levels but increased mucosal MX1 and GCNT3 mRNA levels ($P < 0.05$). Dietary NAC supplementation ameliorated the PEDV-induced abnormal changes in all the measured variables. Moreover, NAC reduced oxidative stress, as indicated by decreases in plasma and mucosal H2O2 levels. **Collectively, these novel results indicate that dietary supplementation with NAC alleviates intestinal mucosal damage and improves the absorptive function of the small intestine in PEDV-infected piglets.**[142]

Lung Damage from H9N2 swine flu reduced by NAC

The effects of NAC on H9N2 swine influenza virus-induced acute lung injury (ALI)were investigated in mice inoculated intranasally with 10^7 50% tissue culture infective doses (TCID50) of H9N2 which demonstrated that pulmonary inflammation, pulmonary edema, MPO activity, total cells, neutrophils, macrophages, TNF-α, IL-6, IL-1β and CXCL-10 in BALF were attenuated by NAC. Moreover, NAC significantly inhibited the levels of TLR4 protein and TLR4 mRNA in the lungs. These results suggest that antioxidants like NAC represent a potential additional treatment option that could be considered in the case of an influenza A virus pandemic.[140]

8. NAC Dramatically Reduces Severity of H1N1 Influenza Viral Infection

In a study to evaluate the effect of long-term treatment with NAC on influenza and influenza-like episodes, 262 subjects (78% > or = 65 years old, and 62% suffering from non-respiratory chronic degenerative diseases) were enrolled in a randomized, double-blind trial involving 20 Italian Centers. Subjects were randomized to receive either placebo or NAC tablets (600 mg) twice daily for 6 months. NAC treatment was well tolerated and resulted in a significant decrease in the frequency of influenza-like episodes, severity, and length of time confined to bed. Both local and systemic symptoms were sharply and significantly reduced in the NAC group. Frequency of seroconversion towards A/H1N1 Singapore 6/86 influenza virus was similar in the two groups, but only 25% of virus-infected subjects under NAC treatment developed a symptomatic form, versus 79% in the placebo group. Evaluation of cell-mediated immunity showed a progressive, significant shift from anergy to normoergy following NAC treatment. [88]

9. Side effect profile of NAC

NAC has been used for over five decades and has an extremely favorable side effect profile. It has been studied extensively in innumerable Phase I, II and III trials. Phase I trials of oral n-acetyl cysteine have generally reported minor side effects to include intestinal gas, diarrhea, heartburn, bad taste, nausea, fatigue, flushing, stomach aches, constipation, burping, hiccups and bloating.[148,149] A recent meta-analysis of Phase III trials in patients with COPD receiving NAC revealed very common AEs of respiratory tract infection (10.85%); common AEs of GI disorders (4.16%) and pruritus (1.08%); and uncommon AEs of cerebrovascular disorders (0.9%), dizziness (0.72%) and musculoskeletal disorders (0.54%).[150]

A recent double-blinded placebo-controlled phase III randomized trial of 60 mechanically ventilated patients at high-risk of developing ventilator-associated pneumonia (VAP) were randomized between NAC (600 mg twice daily) and placebo via NG tube. Patients treated with NAC were significantly less likely to develop clinically confirmed VAP compared with patients treated with placebo (26.6% vs. 46.6%; P = 0.032). Patients treated with NAC had significantly less ICU length of stay (14.36 ± 4.69 days vs. 17.81 ± 6.37 days, P = 0.028) and less hospital stay (19.23 ± 5.54 days vs. 24.61 ± 6.81 days; P = 0.03) than patients treated with placebo. Time to VAP was significantly longer in the NAC group (9.42 ± 1.9 days vs. 6.46 ± 2.53 days; P = 0.002). The incidence of complete recovery was significantly higher in the NAC group (56.6% vs. 30%; P = 0.006). No adverse events related to NAC were identified in this trial.

A total of 262 subjects of both sexes (78% ≥65 yrs, and 62% suffering from non-respiratory chronic degenerative diseases) were enrolled in a multi-center, randomized, double-blinded trial. Subjects were randomized to receive either placebo or NAC tablets (600 mg) twice daily for 6 months. NAC treatment was well tolerated and resulted in a significant decrease in the frequency of influenza-like episodes, severity, and length of time confined to bed. Both local and systemic symptoms were sharply and significantly reduced in the NAC group. Frequency of seroconversion towards A/H1N1 influenza virus was similar in the two groups, but only 25% of virus-infected subjects under NAC treatment developed a symptomatic form, versus 79% in the placebo group.[88] The treatment with 600 mg NAC twice daily was well-tolerated in the large majority of subjects, with only 9% reporting adverse events, a figure which was not significantly different from that of the placebo group (5%).

A recent Phase I trial was conducted in which 30 subjects with retinitis pigmentosa received NAC in three cohorts of 10 subjects. Each cohort received 600, 1200 or 1800 mg of NAC bid for 12 weeks and then tid for 12 weeks. There were 11 drug related AEs found of which 9 were GI adverse events. 8 AEs resolved spontaneously without dose reduction and 3 AEs resolved with dose reduction from tid to bid dosing. The maximum tolerated dose in this trial was 1800 mg twice a day.[149]

10. Side effect profile of FAM

The main risks to Famotidine which have been occurred in greater than or equal to 1% of subjects in clinical trials are: headache; dizziness; and constipation.

Other adverse reactions that were reported in less than 1 % of subjects in clinical trials include: fever, abnormal weakness or lack of energy; fatigue; heart palpitations; elevated liver enzymes; vomiting; nausea; abdominal discomfort; decreased appetite; dry mouth; decreased platelet counts in your blood; swelling of the tissue around your eyes; rash; eye redness and irritation; difficulty breathing; muscle pain; joint pain; seizure; hallucinations; depression; anxiety; decreased interest in sexual intercourse; difficulty falling asleep; prolonged sleeping; dry skin; flushing; ringing in your ears; altered sense of taste; and difficulty in men with achieving erections capable of intercourse. The following have also been reported during post-approval use of famotidine: changes to your heart by effecting the rhythm in which it beats; jaundice; inflammation and possible damage to your liver; decreased levels of white blood cells, red blood cells or platelets which could be life threatening; allergic reaction which could be life-threatening; rapid swelling of soft tissues often involving tissue around the face, limbs or genitals; hives; muscle damage; muscle cramps; confusion; agitation; tingling, numbness, or burning sensation in the skin often involving hands, feet, arms, or legs; pneumonia; and rash and blistering which could be life threatening.

In a recent prospective trial of patients with Parkinson's disease, doses of famotidine were escalated from 80 mg/day to 120 mg/day to 160 mg/day. No significant adverse effects were observed.[151]

11. Possible Reduction in Absorption of NAC due to increased Gastric pH

It is conceivable that due to the reduction in gastric pH that oral NAC may not be absorbed as effectively when given in combination. In order to mitigate this theoretical possibility, we have proposed a phase I trial of the combination of the two investigational drugs with twelve possible combinations described in greater detail below. Three arms are with NAC alone. Additionally, while the trial design should allow us to evaluate the possibility of a decreased effectiveness of NAC in combination with FAM, we make the following observations to conclude that it is unlikely that the increased gastric pH will decrease the absorption of NAC.

It has been demonstrated that if NAC is absorbed in its protonated form, NACH2, this would have to occur in the stomach because very little NAC exists in this form at a pH greater than 4.0.[152] Thus, if NAC is only absorbed in the stomach then the combination with FAM could virtually negate any effect of NAC as very little might be absorbed. However, it was demonstrated in a study of radiolabeled NAC delivered orally to rats that NAC was incorporated into the intestinal and liver glutathione stores as early as 30 minutes after ingestion.[153] Therefore, since NAC exists predominately in its monoanionic form, NACH-, in a pH range from 4 to 9,[152] this study[153] would suggest that NAC can be absorbed effectively within the intestines and would likely be transported by some form of an anionic transporter, with the Organic Anion Transporting Polypeptide (OATP) B-21 a

potential candidate. If a significant amounts of NAC are transported in the intestines as it appears, then raising the gastric pH may have little effect since oral NAC is ingested in its functional form and doesn't depend on digestion by gastric enzymes that might be less efficient at a higher gastric pH.

12. Possible Synergistic Effect between FAM and NAC via enhancement of Lymphocytes and IL-2 Production

Famotidine use in a retrospective non-peer-reviewed report has observed to be to be associated with a significantly lower risk of death or intubation (10% versus 22%, $p < 0.01$) if it was given in the first 24 hours of a patient's admission to the hospital for a SARS-CoV-2 infection.[154] In this report the dose of famotidine was relatively low as famotidine users received a median of 5.8 days of drug for a total median dose of 136 mg (63 to 233 mg).

As was previously discussed IELs are a critical 1st line of defense in coronavirus infections, increased levels of IL-2 have been demonstrated to enhance the activity of IELs bovine coronavirus infection, and famotidine has been demonstrated to suppress histamine-inhibition of IL-2 production by IELs. NAC has the ability to be synergistic with FAM as it has also been demonstrated to increase significantly increase T-cell secretion of IL-2 and significantly stimulate growth of T-cells.[155] Therefore, the combination of NAC and FAM have the potential to synergistically create a positive feedback loop to enhance IELs defense against coronavirus infection with the potential to dramatically reduce the severity of the infection if given early enough in the course of infection.

13. Possible Synergistic Effect between FAM and NAC to counteract effect of IL-6 in Prolongation of the QT Interval

Increased pro-inflammatory interleukin-6 (IL-6) levels are associated with acquired long QT-syndrome (LQTS) in patients with systemic inflammation, leading to higher risks for life-threatening polymorphic ventricular tachycardia such as Torsades de Pointes. Inhibition of hERG by Interleukin-6 was demonstrated to underlie the risk for an acquired Long QT in Cardiac and Systemic Inflammation.[156]

In January of 2020, it was demonstrated that Thioridazine also induces cardiotoxicity vis ROS-mediated hERG channel deficiency which was felt might be the ionic mechanism for QT prolongation.[157] The authors further demonstrated that NAC significantly attenuated the hERG reduction induced by THIO and abolished the upregulation of ER stress marker proteins.

While there is some data that famotidine may prolong the QT interval,[158] the mechanism appears unrelated to hERG inhibition as there are several studies demonstrating that famotidine does not inhibit the hERG channel.[158–160] Not only may FAM have the ability to modulate the Mast Cell expression of IL-6 as a potential mechanism to reduce the risk of QT interval prolongation but the ability to modulate the release of histamine and block the H2 rector activation is likely to further reduce the risk of QT interval prolongation. It has been demonstrated that activation of the histamine H2 receptor exacerbates myocardial

ischemia/reperfusion injury by disturbing mitochondrial and endothelial function.[161] In fact famotidine was demonstrated to abolish the effect of H2R agonists on rat cardiomyocytes to induce apoptosis, increase mitochondrial permeability and increase caspase 3 expression.

A 2016 study demonstrates that macrolides induced ROS and increased the mitochondrial membrane permeability in rat cardiac myocytes.[162] The authors make the case based on these results and a body of literature about the relationship between MMP and QT interval prolongation that, this may be the mechanism in which macrolides can lead to QT interval prolongation. What is most fascinating about this study is that the authors demonstrated that NAC reversed the effect on the mitochondria from the macrolides.

In addition to this data in macrolides, there is also data in mice with a knockout NOS1Ap (neuronal nitric oxide synthase-1 adaptor protein), which is associated with QT interval prolongation and sudden death, in which oxidative stress was induced by an injection of doxorubicin. The knockout mice compared to WT mice not surprisingly had significant prolongation of QT interval, higher rates of ventricular arrhythmias, higher rates of death, and higher measured levels of H_2O_2. Administration of NAC to the knockout mice, significantly reduced mortality by doxorubicin injection, accompanied by prevention of QT interval prolongation.[160]

There is also data in rats which were irradiated while heart monitoring was performed. The rats were found to have cardiac injury as observed on the EKG along with significant increases in cytokine levels and oxidative stress. Pretreatment with NAC ameliorated the cardiac injury induced by irradiation.[163]

Thus, there are at least two potential mechanisms by which NAC and FAM may independently reduce a risk of QT interval prolongation that a SARS-CoV-2 infection may precipitate by dramatically increasing the level of IL-6.

In addition to the protection that NAC may provide from IL-6 and perhaps more importantly, NAC has been demonstrated to significantly reduce IL-6 levels in viral infections. NAC's ability to reduce IL-6 levels has been demonstrated in the H5N1 influenza A virus,[72] H92N2 swine flu virus,[140] RSV,[70] and HIV.[164]

14. Depletion of LC3-I is rate limiting in Coronavirus infection and NAC's demonstrated ability to dramatically increase LC3-II to LC3-I ratio

In addition to the mechanisms of actions previously described, the effect NAC exerts on autophagy could prove synergistic in the potential to reduce the severity of a SARS-CoV-2 infection. There is a body of literature since 2004 in coronaviruses to include mice (MHV),[165] pig (PEDV and TGEV)[166,167] and most notably the human coronavirus, SARS-CoV,[168] that demonstrates that coronaviruses create Double-Membrane Vesicles (DMV) from endoplasmic reticulum under oxidative stress. It seems that the coronavirus components are shuttled around in these DMVs. It has been demonstrated that depletion

of LC3-I can be rate limiting in viral replication.[169] It seems LC3-II normally decorates autophagosomes and LC3-I normally decorates lysosomes. It turns out that the coronaviruses DMVs are also decorated with LC3-I. In a 2018 in-vitro study of cardiomyocytes and in-vivo in rats, NAC increased the ratio of LC3II/I and reduced LC3-II autophagic flux.[59] These findings suggest that NAC triggers the accumulation of autophagosomes, which is the result either of increased synthesis or decreased degradation and that the availability of LC3-I for the formation of DMVs may be significantly decreased by NAC. **Therefore, by reducing ROS, inhibiting autophagy, inhibiting apoptosis, and potentially limiting LC3-I, these effects would potentially result in synergy between FAM and NAC to reduce the severity of a SARS-CoV-2 infection.**

Finally, because the dose limiting toxicity of NAC appears to be an anaphylactoid reaction, the combination has the potential to be synergistic if for no other reason than to allow higher doses of NAC to be tolerated orally through the action of FAM as an antagonist of the histamine-2 receptor.

ii) Clinical Significance

Rationale for a clinical trial

Given the existing biochemical data to suggest FAM inhibits suppression of intraepithelial lymphocytes by histamine and Mast Cell release of histamine; the correlation of severity of tissue fibrosis and thrombosis with ACE2 depletion; the ability of NAC to reduce oxidative stress, which appears related to severity of the SARS-CoV-2 infection; the ability of NAC to prevent tissue fibrosis, thrombosis and reduce viral replication; the favorable side effect profiles of FAM and NAC; the wide prevalence of SARS-CoV-2 infection and its substantial mortality rate; and, the lack of vaccine or proven therapies for non-hospitalized patients who have tested positive for a SARS-CoV-2 infection; a clinical trial of the safety and efficacy of the combination of NAC and FAM is justified. These medications are readily available and their combination does have the possibility of reducing the severity of infection and mortality for these patients (see figure 9).[170]

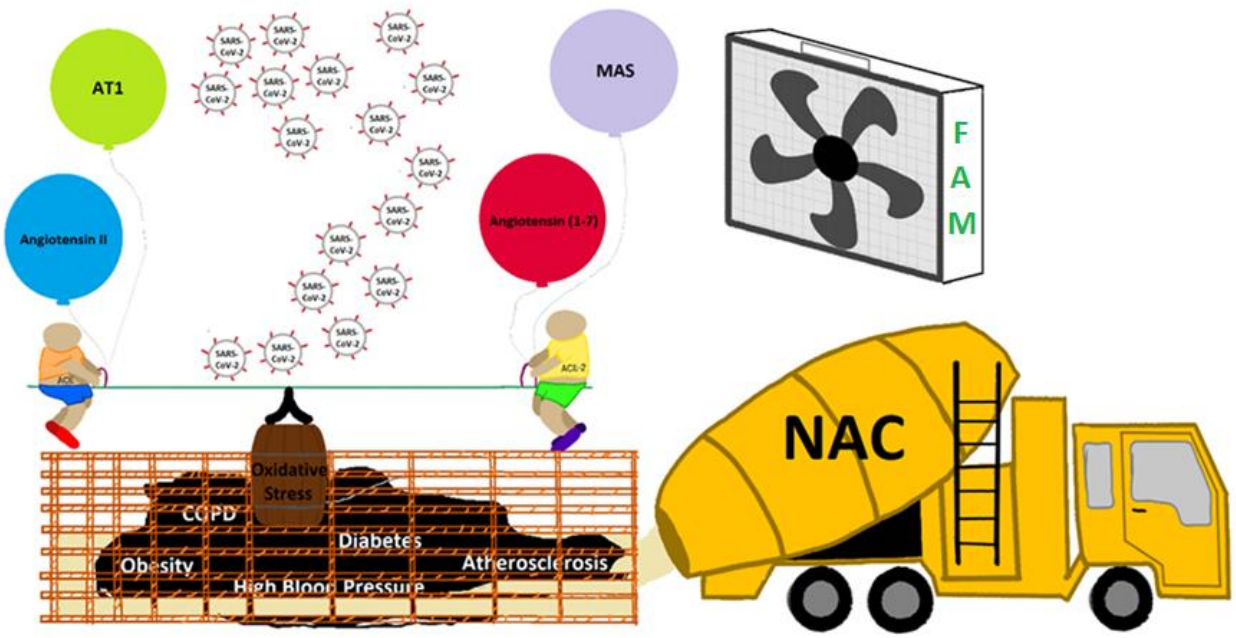

Figure 9: A cartoon drawing[170] of the putative effect of FAM to reduce inhibition of Intra-Epithelial Lymphocytes and of NAC to reduce severity of infection through reduction of oxidative stress and reduction of viral replication

III) RESEARCH DESIGN AND METHODS (including data analysis)

i) Study Design - Parallel Phase I/II Clinical Trial

The proposed parallel phase I/II design allows for simultaneously evaluating the safety and efficacy of combination dose levels and to select the optimal combination dose. The trial is started with an initial period of dose escalation, and then patients are randomly assigned to permissible dose levels. Bayesian posterior probabilities are used in the randomization to adaptively assign more patients to doses with higher efficacy levels. Combination doses with lower efficacy are temporarily closed and those with intolerable toxicity are eliminated. The trial is stopped if the posterior probability for safety, efficacy or futility crosses our pre-specified boundaries. This design has been shown in simulations to save sample size, to have better power and efficiently assigns more patients to doses with higher efficacy levels.[171]

Phase I Portion (Dose Escalation Phase)

The doses to be tested for famotidine are 20 mg thrice daily, 40 mg thrice daily, 80 mg thrice daily and no famotidine. The doses to be tested for n-acetyl cysteine are 600 mg thrice daily, 1200 mg thrice daily and 1800 mg thrice daily. The dose combinations are labelled 1 through 12 and are zoned as illustrated in Figure 10. The first zone has the combination of the lowest dose of each drug. The second zone has the combination of the lowest dose of each drug with the middle dose of each drug. The dose escalation starts with the first zone and follows with the second zone and then each combination of doses in the zone next closest to the coordinate

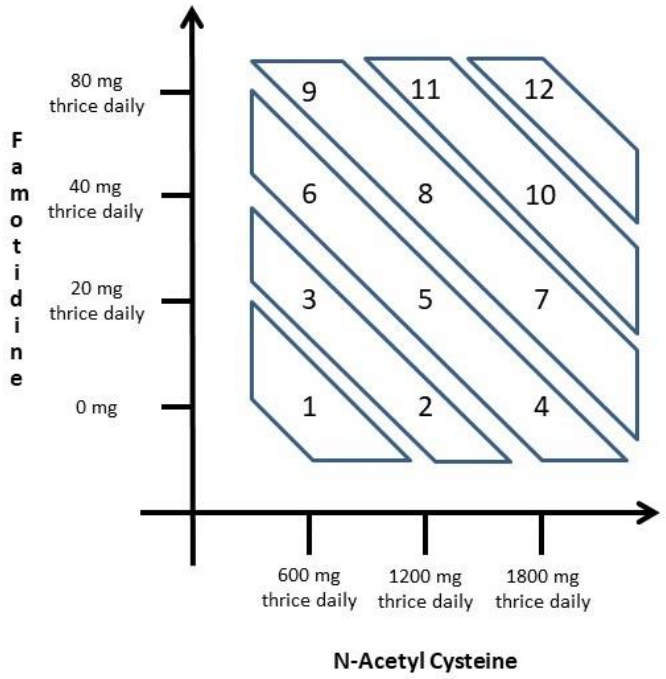

Figure 10. Dose Combinations of N-Acetyl Cysteine and Famotidine

origin shown in figure 10. Combination doses in the same zone are tested simultaneously using block randomization to control the number of patients for each open dose. Some combinations doses may close, in which case blocked randomization is applied to the remaining open combination doses in the same zone. A modified "3+3" design[172] will be used for the initial dose escalation.

To find acceptable dose levels any grade 3 or higher toxicity will be considered a dose-limiting toxicity (DLT). The modified "3+3" design proceeds as described below. Three patients receive dose level 1. Their responses determine the subsequent steps as described in the following decision rules.

(1) If two patients (2/3) experience DLT, then we close dose level 1 and all larger doses to testing.
(2) If no patient experiences DLT, then we designate dose level 1 as an admissible dose, and open doses in zone 2 for testing.
(3) If only one of the first three patients (1/3) experiences DLT, then we assign three more patients to dose level 1. In this case, we assign up to six patients to this dose, and the following decision rules apply:
    a. If only one of the six patients (1/6) experiences DLT, then we designate dose level 1 as an admissible dose, and open doses in zone 2 for testing.
    b. If two of the six patients (2/6) experience DLT, then we designate dose level 1 as an admissible dose, and doses in zone 2 and higher are closed to testing.
    c. If more than two of the six patients (>2/6) experience DLT, then we close dose level 1 to testing, and we also close doses in zone 2 and higher.

ii) Study Procedure

Subjects will be recruited from among patients at a Prisma Health-Upstate Emergency Department (ED) at either Greenville Memorial Hospital, Greer Memorial Hospital or Baptist Easley Hospital if testing for a SARS-CoV-2 infection is performed and the potential subject will be discharged to home from the ED. Potential subjects who express a desire to be evaluated for possible enrollment on trial will have their chart screened by one of the ED Investigators to ensure they meet all inclusion criteria without any exclusion criteria. If they have not had an eGFR measured within a year of enrollment, a blood draw will be performed to measure their eGFR. Female participants who are of childbearing potential, i.e., 15 to 55 years old and have not had their uterus surgically removed (i.e., hysterectomy) or both ovaries removed surgically, will have a urine pregnancy test performed. A pregnancy test will

not be performed if they would otherwise fit the category of childbearing potential described above but are of a confirmed postmenopausal state. After enrollment and while still in the ED subjects will have a blood draw for research labs that will be collected into two tubes. One tube will be sent to the main lab where it will be held. If the subject tests positive for a SARS-CoV-2 infection, they will be randomized within 24 hours of a positive test. A CURB-65 Assessment Form will be completed by the ED Investigator research staff member and a CAP-SYM 18 survey will be administered the ED Investigator. For male subjects an IPSS survey will also be completed by the ED Investigator. All subjects who have been enrolled will be provided with a signed copy of their Informed Consent Form, a copy of their signed Research HIPPA Authorization form, 30 copies of the daily diary CRF, 4 copies of the medication log CRF, an instruction sheet for the Roth Score and 40 Step Test and 4 sterile urine specimen containers.

The randomization process will be performed using the Randomization module within REDCAP®. This will be performed through use of the data entry form entitled, "COVID-19 Test Result and Drug Randomization Case Report Form." If the subject has a Rapid Test Performed in the ED and a positive result is obtained prior to discharge, the Co-Investigator who enrolled the subject will be able to access this form an perform the randomization. Otherwise, the randomization will be performed by the PI. Due to the trial design as a Phase I trial, the number of subjects on any arm can vary from zero to six depending on any dose limiting toxicities that may occur. Also, a new zone in the trial will not be able to open until all subjects in the previous zone have completed the trial. Therefore, the randomization process will be dynamic which REDCap® is incapable of handling directly but is capable of handling indirectly in a two-step process as described below. Prior to the start up the trial, a randomization lookup table will be uploaded into REDCap®, which will be nothing more than an ordered table from one to one hundred. While this may seem trivial, it is utterly essential in order to harness the power of REDCap® for a dynamic randomization process. After an investigator has performed the randomization task in REDCap®, the randomization number will be returned from this table. The investigator will then be required to lookup manually the arm to which the subject has been randomized in a file called "Dynamic Randomization Lookup Table" which they will only find within the "File Repository" in REDCap®. An example of this file can be found in the Appendix below. This file will be created prior to the trial initiation by the Lead Statistical Investigator initially assuming three subjects for each trial arm. Anytime, a change is needed to the "Dynamic Randomization Lookup Table" due to a DLT, the PI will suspend the ability for anyone to enroll or randomize a subject through the "User Rights" feature of REDCap® which can be used to restrict access by role to data entry forms. Once a decision has been made about either expanding or closing a trial arm due to a

DLT based on the trial rules and assuming the IRB and DSMB which will have also been notified of the DLT, have not imposed further restrictions, the PI will obtain from the Statistical Lead Investigator a new "Dynamic Randomization Lookup Table." The old "Dynamic Randomization Lookup Table" will be removed from the REDCap® "File Repository" but an electronic copy will otherwise be maintained by the Lead Statistical Investigator. The new "Dynamic Randomization Lookup Table" will be uploaded into the file repository and the User Rights to the enrollment and randomization data entry forms in REDCap® will be restored.

After the investigator has determined the trial arm to which the subject has been randomized through the two-step process as described above, the investigator will verify that they correctly accessed the "Dynamic Randomization Lookup Table" in the form entitled, "COVID-19 Test Result and Drug Randomization Case Report Form." The remainder of the form will be completed to include entry of the trial arm and verification that the correct prescription has been entered. The subject will be notified of the randomization and prescription entry. Whenever, a Randomization CRF is saved, the PI will receive an immediate electronic notification via REDCap® and the PI will then complete the Investigator Assignment Form in REDCap®, a copy of which can be found in the Appendix. It is through this form that the subject will be assigned to an individual Investigator and an individual Research Assistant for the remainder of their trial participation. The assigned Investigator and Research Assistant will be immediately notified electronically via REDCap®. The subject will be notified by the PI to which Investigator and Research Assistant they have been assigned. Unless the subject has declined a Vivify® electronic device, such device, the trial medication and paper copies of the medication log and daily diaries will be delivered to the subject's home within 24 hours of randomization by a courier who will have the appropriate training and PPE. If the subject has declined a Vivify® electronic device, they will be required to pick-up their study drugs and paper copies of their forms at an Upstate Pharmacy.

After a subject has been randomized to a combination of trial drugs the Prisma-Health lab will be notified to process the first tube of blood for a determination of the subject's blood type and Rh factor. The second tube will be sent to Kiyatec® to determine their Glutathione level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), and their tryptase level. The collected urine sample will also then be sent to Kiyatec® to measure the subject's F2-Isoprostane level, creatinine level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), n-methylhistamine level and TMPRSS2 levels. The research labs will be held, and the tests will only be performed if the subject is randomized to take an investigational

drug. Otherwise the specimens will be discarded. The lab evaluations to be performed by Kiyatec® will be kept frozen and typically run in batches of 40.

If the SARS-CoV-2 test results are negative after enrollment, the subject's participation in the trial will be completed and randomization to an investigational drug will not occur. An alert will be triggered within REDCap® with an immediate electronic notification to the PI both when a subject has been enrolled and when they have been randomized. When a zone has completed accrual, a text message will be sent by the PI to all investigators that the trial is temporarily closed to accrual, and the enrollment and randomization forms in REDCap® will be taken offline. When all subjects within the current zone have completed the trial, and the trial is not closed due to toxicity, the next zone will be opened. All Investigators will be notified by text message from the PI when a new zone is open and the enrollment and randomization forms in REDCap® will be placed back online.

Subjects will be expected to take the dose combination to which they are as assigned and as prescribed. Subjects will record dates and times each medication dose was taken on their log forms which will be collected weekly. Subjects will be contact at least weekly by a Research Assistant to monitor symptoms, verify compliance with study drugs and compliance with completing medication logs and daily diary. The Research Assistant will also administer a CAP-SYM 18 during this call. The Research Assistant will communicate any concerns and all findings to a study Investigator who will later call the subject to assess how the subject is doing, discuss any concerns and will monitor the subject in the performance of a 40 Step Test for those subjects provided with an electronic device capable of measuring an Oxygen Saturation level unless the Investigator is concerned about the safety of doing so. Subjects will collect a urine sample weekly. The urine samples and the daily diary and medication log CRFs completed by the subject will be delivered by the subject or a family member within 4 hours of collection by means of the COVID-19 testing car line at Greenville Memorial Hospital. The urine specimens will be sent to Kiyatec® to measure the subject's F2-Isoprostane level, creatinine level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), n-methylhistamine level and TMPRSS2 levels. Subjects will self-isolate per current CDC recommendations. Subjects will remain on medication for a minimum of 7 days and unless hospitalized will continue on the medications until it has been more than 10 days beyond the start of symptoms, symptoms have improved and the subject has been without a fever for at least 24 hours without the use of a fever reducing medication. If subjects are hospitalized, then the will instructed to discontinue taking the study medications. After discontinuing study drugs for any reason, the subject will have their blood will be drawn to measure Glutathione level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), and

their tryptase level; and a urine sample will be collected to measure the subject's F2-Isoprostane level, creatinine level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), n-methylhistamine level and TMPRSS2 levels.

Responsibilities and those tasks and responsibilities delegated to co-investigators and all others participating in the conduct of this trial are specified in the Appendix in the Table with the heading, "Responsibilities and Delegated Tasks and Responsibilities"

iii) Inclusion / Exclusion Criteria

Inclusion criteria:

(1) Age > 18
(2) performance of a SARS-CoV-2 test within 1 day of enrollment

Exclusion criteria:

(1) All patients under 18
(2) Known allergy to N-Acetyl Cysteine
(3) Known allergy to famotidine or other H_2-receptor antagonists
(4) Pregnant or Nursing Mothers
(5) Laboratory Evidence or History of Renal Impairment (eGFR < 30 mL/min/1.73 m^2)
(6) Taking H_2-receptor antagonists, hydroxychloroquine or chloroquine.
(7) Patient has been admitted to the hospital prior to study enrollment

iv) Data Collection Methods

Please note that for ease of reference a figure is included in the Appendix of the mapping of the Data Collection Instruments to be used in REDCap® with the corresponding events as described in outline form below.

a. *Enrollment Data Collection Process*

Data will be collected in the ED by a study Investigator by taking a patient history, performing a physical exam and performing a chart review to ensure all inclusion criteria are meet and that no exclusion criteria exist. The Investigator will complete the Eligibility Assessment CRF, Demographics CRF, IPSS Questionnaire, CAP-SYM 18 CRF and the Post Enrollment Checklist directly into REDCap®. Adobe Acrobat pdf copies of these forms are included in the Appendix. It should be noted that these forms include branching logic that will indicate the subject is not eligible for enrollment if they do not meet the eligibility criteria, have any excluded conditions or have not had a pregnancy test if

required. Additionally, the Post Enrollment Checklist has branching logic to ensure that the subject has meet all eligibility criteria without any exclusion criteria being met and that all the forms mentioned above have been saved and marked with a status of "Complete." After the Post Enrollment Checklist has been marked complete, the PI will immediately be sent an email via a REDCap® alert which will indicate the Subject's study identification number and the name of the enrolling Investigator.

b. Post-Enrollment Data Collection Process

Within 3 days of a positive SARS-CoV-2 test for an enrolled subject, a research assistant will complete the Baseline Data form in REDCap®. The CBC, BMP, Coag, Crp, Liver Panel, Pregnancy Test, EKG, Blood Gas and PSA Forms will also be completed in REDCap® if any of the corresponding studies were completed within a year prior to enrollment and if so only the most recent value will be recorded. A Starting Medication Form will also be completed in REDCap® for every medication the subject is taking at the time of randomization. These Forms can all be found in the Appendix.

c. Randomization Data Collection Process

Within 1 day of a positive SARS-CoV-2 test, a subject previously enrolled will be randomized to combination of drugs by completing the COVID-19 Test Result and Drug Randomization Case Report Form in REDCap®. If the result is known before discharge from the ED this form will be completed by the Investigator in the ED and the PI will be immediately sent an email via a REDCap® alert which will indicate the Subject's study identification number and dose combination to which they were randomized. If the SARS-CoV-2 test result is returned after discharge home from the hospital, this form will be completed by the PI. This form also contains a checklist to document, the date of the positive result, that the prescription was sent to an Upstate Pharmacy, that the subject was contacted to pickup the prescription, that the subject was reminded to complete the medication log and daily diary forms. Within 24 hours of randomization, the PI will assign the subject to a non-ER study investigator and research assistant. The PI will notify the subject, investigator and research assistant of these assignments and document such in REDCap® using the Investigator Assignment Form.

d. Research Lab Data Collection Process

The subject's Blood Type and Rh factor will be entered by the assigned Research Assistant directly into REDCap® using the Blood Type Form. The Cytokine Panel results, tryptase level, glutathione level, TMPRSS2 level and n-methyl histamine level will all be provided by Kiyatec® in an Microsoft Excel file with fields to identify the Subject ID number, days since enrollment for which the specimen was collected and the specimen source (i.e., blood or urine). These files will be converted into a comma-separated values format and uploaded into REDCap® by the PI after verification that the field names correctly correspond to the field names in the form Cytokine Panel.

e. Outpatient Follow-up Data Collection Process

Subjects will be contacted weekly at minimum by the assigned Research Assistant and the assigned Investigator. It will be the responsibility of the assigned Investigator to ensure this happens correctly. Depending how a subject is doing clinically, an Investigator will have the discretion to perform more frequent contacts. These contacts will preferably be done with both a video and audio connection for which the Vivify® devices are capable. A telephonic contact will be discouraged but will be permissible for those who do not have a Vivify® device or for whom an acceptable audio-video connection cannot otherwise be established. Data will be collected and recorded for these contacts on the appropriate REDCap® form, i.e., Research Assistant Weekly Assessment, or Investigator Weekly Assessment. It will be the responsibility of the assigned Research Assistant to enter weekly data from the patient's medication log and daily diaries into the REDCap® forms, Daily Medication Log and Daily Diary. If any new medications are started or any non-trial medications are changed by either the Investigator or any other provider involved in the subject's care as documented in EPIC, the Research Investigator will document such changes using the REDCap® form, New Medications. At the end of every evaluation by the assigned Investigator, the Investigator will document in the REDCap® form their assessment which must be one of the following: 1, Continue on Study Without Change; 2, Have made arrangements for short interval follow-up with myself; 3, Discontinue Study; 4, Recommend Evaluation in ER; 5, Recommend Evaluation by Primary Provider; 6, Recommend Dose Reduction in one or both Trial Medications. If the Investigator Documents any assessment other then "Continue on Study Without Change", an electronic notification will immediately be sent to the PI notifying the PI of the Investigator's Assessment. If the Investigator's assessment is to recommend a dose reduction, they will be directed to document such on the REDCap® form, Trial Drug Adjustment. Within this form, the new dosages will be documented, to include the possibility of discontinuing either or both medications. Additionally, the Investigator will be required to identify whether the adjustment is being made due to a drug toxicity. If the Investigator documents that the adjustment is made due to a drug toxicity upon saving the form an immediate electronic notification will be made to the PI and Chair of the DSMB. The PI will then complete the REDCap form "NCI Adverse Event Serious Adverse Event CTCAE v5." This is an extensive form for which the PI will complete for any AE or SAE. It is comprehensive as it includes the entirety of the CTCAE v5 to include every possible grade of toxicity. Through branching logic, it minimizes the task of data entry only to the particular organ systems or laboratory values involved, an allows the PI to easily document all the data necessary for AE and SAE determination so that the IRB, DSMB and FDA can all be notified as necessary and within the required timelines as described more fully in the "Protection Against Risk" section below.

f. Hospital Follow-up Data Collection Process

If a subject becomes hospitalized, the subject's in-patient provider will be notified that the subject was on a clinical trial and of the trial medications the subject was on and any

other pertinent events that occurred while on trial. Also the in-patient provider will be notified of the trial requirement for a final blood draw to measure Glutathione level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), and their tryptase level; and a urine collection to measure the subject's F2-Isoprostane level, creatinine level, a cytokine panel (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), n-methylhistamine level and TMPRSS2 levels. These labs will be ordered in EPIC® by a study investigator. On a weekly basis the assigned Research Assistant will review the subject's chart in EPIC to review for any of the following: C Reactive Protein blood draw; EKG, Blood Gas; new medications; COVID19 NP swab; supplemental oxygen start or change; intubation; extubation; supplemental oxygen decreased or stopped; ICU admission; ICU discharge; SARS-CoV-2 serum antibody test; and, hospital discharge. A corresponding form for any such event will be completed in REDCap®.

g. Study Completion Data Collection Process

Upon completion of Study, the REDCap® form, Completion Data will be completed.

v) Data Analysis Methods

Data will be exported from REDCap® using the Data Export Tool into a format that can be analyzed using R, JMP and/or SAS (SAS, Cary, NC). The User Rights in REDCap® will restrict the use of the REDCap® "Data Export Tool" to only the PI, Epidemiologist and the Statistical Co-Investigators. The rights will be further restricted within REDCap® such that only De-Identified data can be exported from Data Export Tool. Therefore, because no date or time fields can be extracted from REDCap® as described above, any Data Entry Form within REDCap® that collects a date has been created to include a hidden calculation field to calculate and store the number of days between such a date and either the enrollment date or the medication start date. Some dates are calculated based on the medication start date to make data collection easier on certain forms; however, the number of days between enrollment and medication start is also calculated and stored so that any number of elapsed days can easily be determined from either. After the data has been exported from REDCap into an aforementioned statistical package and descriptive statistics have been generated and analyzed by the Statistical Investigators. These results will be shared with the Epidemiologist and all Investigators so that appropriate conclusions can be reached and ultimately disseminated.

vi) Limitations of the Proposed Study Design

This is a phase I trial and may be limited by unmeasured confounders such as clinical practice patterns of the providers ordering initial SARS-CoV-2 testing. It also may be limited as subjects are being recruited directly from the ED and thus may be more symptomatic or have other unmeasured cofounders as compared to the general population who may become infected with SARS-CoV-2.

IV) PROTECTION OF HUMAN SUBJECTS

i) Risks To The Subjects

The main risks to Famotidine which have been occurred in greater than or equal to 1% of subjects in clinical trials are headache; dizziness and constipation.

Other adverse reactions that were reported in less than 1 % of subjects in clinical trials include: fever, abnormal weakness or lack of energy; fatigue; heart palpitations; elevated liver enzymes; vomiting; nausea; abdominal discomfort; decreased appetite; dry mouth; decreased platelet counts in your blood; swelling of the tissue around your eyes; rash; eye redness and irritation; difficulty breathing; muscle pain; joint pain; seizure; hallucinations; depression; anxiety; decreased interest in sexual intercourse; difficulty falling asleep; prolonged sleeping; dry skin; flushing; ringing in your ears; altered sense of taste; and difficulty in men with achieving erections capable of intercourse. The following have also been reported during post-approval use of famotidine: changes to your heart by effecting the rhythm in which it beats; jaundice; inflammation and possible damage to your liver; decreased levels of white blood cells, red blood cells or platelets which could be life threatening; allergic reaction which could be life-threatening; rapid swelling of soft tissues often involving tissue around the face, limbs or genitals; hives; muscle damage; muscle cramps; confusion; agitation; tingling, numbness, or burning sensation in the skin often involving hands, feet, arms, or legs; pneumonia; and rash and blistering which could be life threatening.

The main risks to N-Acetyl Cysteine include but are not limited to the following: allergic reaction which could be life threatening; rash; itching; nausea or vomiting.

ii) Total Planned Enrollment

Estimated Number of Subjects: 42

We estimate a sample size of around 42 subjects for a 3+3 dose escalation period of our parallel phase I/II trial design. Based on our simulation of the phase I period of the trial and an assumption that a drug limiting toxicity ranges between 5% and 8%, we found an 83% chance that between 27 and 33 subjects would be required. During the adaptive phase of our trial with predicted rates of hospitalization ranging from 20 to 5% as shown in

iii) Does this study involve any of the following: prisoners, pregnant women, children, institutionalized individuals, or other populations that may be considered vulnerable?

No

iv) Does this study seek to exclude a specific racial, ethnic, or gender group? Include a compelling rationale if so.

No

v) Sources of Materials

Data will primarily be abstracted via chart review and patient's history and physical exam and by patient completed medication logs and daily diary of temperature and symptoms. Data of Oxygen saturation level and vital signs will also be obtained directly from the subjects Vivify® device.

V) ADEQUACY OF PROTECTION AGAINST RISKS

i) Recruitment and Informed Consent

See attached Informed Consent Form.

ii) Protection against Risk

Prerana Roth, M.D., will serve as the medical monitor and will chair an internal Data and Safety Monitoring Board to ensure the safety of all subjects and their data.

It is essential that all AEs, including serious adverse events (SAEs), which occur during the course of research subjects' involvement in a study are appropriately identified, documented, reported, and managed in order to ensure the subjects' continuing safety. This is the responsibility of the investigative site.

The PI will Complete in REDCap® an NCI Adverse Event Serious Adverse Evet CTCAE v5 form within 24 hours of notification of an adverse event by a subject, research assistant, co-investigator, outside provider or by any other means in which this information may be obtained. If the event is life-threatening or fatal, this form will be completed immediately, and the event will be reported immediately to Dr. Roth and the IRB. Whenever this form is completed by the PI, an email will be immediately sent Dr. Roth and any other individuals specified by Dr. Roth. Whenever this form is completed by the PI and a Serious Adverse Event has occurred, an email will be immediately sent via the REDCap® alert mechanism to the IRB Chair and any other individuals specified by the IRB. This copy of this form can be found in the Appendix and includes all the elements described below.

Adverse Event (AE)

An AE in this clinical trial is defined as any unfavorable change in a research subject that may occur during or after administration of the investigational drugs. There need be no causal relationship to the investigational drugs and may be due to the SARS-CoV-2 infection or for any other reason. This change does not have to be caused by the investigational product or study procedures to be described as an AE.

AEs may include:

- Physical signs or symptoms
- Abnormal laboratory values

- Changes in vital signs, physical examinations or an electrocardiogram (ECG)
- An increase in the frequency or intensity (worsening) of symptoms that were present before study enrolment
- Complications from procedures or a surgery
- Psychological, economic, or social harm

Causality Determination

All AEs will be assessed by either the sponsor-investigator and a determination of causality will be made as described below. Causality refers to the likelihood and extent that the investigational drugs being studied contributed to the development of an AE. Because the determination of causality involves medical decision making, only investigators as described above who have medical expertise will be making the causality or relationship determination. Any determination of causality will be reviewed by the Sponsor-Investigator and the Data and Safety Monitoring Board. If it is determined that an AE is related to the investigational drugs, required reporting to the IRB and FDA will be made in accordance with the required regulations.

When determining causality, the investigator will consider:

- **Elimination of other causes**
- **Effect of the drug or drug class**
- **Temporal sequence**
- **Chronic effects**
- **Cumulative effects**
- **Late effects**
- **Re-challenge**

Causality categories will include the following:

- ***Definitely related*: There is a certainty that the event is related to the investigational product.**
- ***Probably related*: There is high likelihood that the event is related to the investigational product.**
- ***Possibly related*: There is a likelihood that the investigational product is the cause of the event, but other causes cannot be ruled out.**
- ***Unlikely to be related*: It is not likely that the event is related to the investigational product, and other more likely causes are present.**
- ***Unrelated*: Evidence exists that the event is related to something other than the investigational product.**

Criteria for determining the causality category will be made as described in the below table.

Determination of Causality Category of AEs as Related to the Investigational Drugs	
Definite (must have all four [4])	1. Has a reasonable temporal relationship to the investigational drugs 2. Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions 3. Follows a known pattern of response to the investigational drugs 4. Disappears or decreases with reduction in dose or cessation of study drugs and recurs with re-exposure
Probable (must have three [3])	1. Has a reasonable temporal relationship to the investigational drugs 2. Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions 3. Follows a known pattern of response to the investigational drugs 4. Disappears or decreases with reduction in dose or cessation of study drugs and recurs with re-exposure
Possible (must have two [2])	1. Has a reasonable temporal relationship to the investigational drugs 2. Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions 3. Follows a known pattern of response to the investigational drugs 4. Disappears or decreases with reduction in dose or cessation of study drugs and recurs with re-exposure
Unlikely (must	1. Does not have a temporal relationship to the investigational drugs

have two [2])	2. Could readily have been produced by the subject's clinical state 3. Could have been due to environmental or other interventions 4. Does not follow a known pattern of response to the investigational drugs 5. Does not reappear or worsen with reintroduction of investigational drugs

Duration of AEs

Another important element for evaluation of an AE is the duration of the event. Onset of the event will be measured from the onset of signs and symptoms. Resolution of the event will be recorded as well, which we will define as the point when all signs and symptoms have subsided.
Some events continue or change in severity over time. Capturing this information is also important. Changes in severity such as when an event improves from severe to mild will be recorded as separate events, with separate intensities and durations collected. The ongoing nature of the event will be noted in the subject's research record.

Determining Severity

An important component in evaluating AEs is their severity. Severity refers to the intensity of the event and will be indicated as mild, moderate, or severe, as shown in the table below.

Grading of Severity of AEs Based on Signs and Symptoms	
None	No signs/symptoms or within normal limits
Mild	Minor signs/symptoms; no specific medical intervention required; asymptomatic laboratory findings only, radiographic findings only; marginal clinical relevance

Moderate	Requiring minimal, local, or non-invasive intervention only
Severe	Significant symptoms requiring hospitalization or invasive intervention
Life-threatening or disabling	Complicated by acute, life-threatening metabolic, or cardiovascular complications (such as circulatory failure, hemorrhage, sepsis); life-threatening physiological consequences; or need for intensive care or emergent invasive procedure
Fatal	Causing death

Serious Adverse Event (SAE)

A grade of severe is not the same as serious. Serious is a specific term used in determining whether AEs must be reported to sponsors and regulatory agencies. SAEs, regardless of severity, demand immediate action and attention. A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:

- **Results in death;**
- **Is life-threatening (and puts subject at immediate risk of death);**

  N.B., The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

- **Requires inpatient hospitalization or prolongation of existing hospitalization.**
- **Results in persistent or significant disability/incapacity; or**
- **Is a congenital anomaly/birth defect.**

Medical and scientific judgement will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These will also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Reporting Requirements for AEs/SAEs

Clinical Co-Investigators and Sponsor-Investigator's Responsibilities:

All SAEs will be reported to the IRB within twenty-four (24) hours of their identification. If the event is life-threatening or fatal, the event will be reported immediately.

Expedited reporting to regulatory agencies is generally not required for events that are either:

- **Serious but expected**
- **Not reasonably related to the investigational drugs**

These latter events, if serious, will still be reported to the IRB. Because all SAEs are still adverse events, they will be recorded on a Form FDA 3500A, which is the MedWatch Mandatory Reporting form.

Sponsor-Investigator's Responsibilities:

The Sponsor-Investigator will report SAEs that are unexpected and associated with the use of the investigational drugs to the US FDA within specific time periods. This report is an Investigational New Drug (IND) Safety Report. The objective of rapid notification is to protect subjects participating in this trial of the investigational drugs.

Unexpected adverse event or suspected adverse reaction refers to an event or reaction that is not listed in the investigator's brochure or is not listed at the specificity or severity that has been observed; or, if an investigator's brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current IND application.

- ***Initial reporting*: The sponsor-investigator will report any suspected adverse reaction or adverse reaction to study treatment that is both serious and unexpected.**

  Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and will be reported to FDA as soon as possible but no later than 7 calendar days following the sponsor-investigator's initial receipt of the information.

- ***Follow-up reporting*: Any relevant additional information obtained by the sponsor-investigator that pertains to a previously submitted IND safety report will be submitted as a Follow-up IND Safety Report. Such report will be submitted without delay, as soon as the information is available but no later than 15 calendar days after the sponsor-investigator receives the information.**

All IND safety reports will be submitted on Form FDA 3500A and will be accompanied by Form FDA 1571. The type of report (initial or follow-up) will be checked in the respective boxes on Forms 3500A and 1571.

The submission will be identified as:

- **"IND safety report" for 15-day reports, or**
- **"7-day IND safety report" for unexpected fatal or life-threatening suspected adverse reaction reports, or**
- **"Follow-up IND safety report" for follow-up information.**

The report will be submitted to the appropriate Review division that has the responsibility to review the IND application under which the safety report is submitted. The sponsor-investigator will submit all safety reports electronically.

Once an IND Safety Report is filed with the FDA, the sponsor-investigator will notify the IRB, the Chair of the Data and Safety Monitoring Board and all the clinical co-investigators participating in this clinical trial.

Data and Safety Monitoring Board Responsibilities

The Data and Safety Monitoring Board in consultation with the IRB a will determine if the trial or trial arm needs to be temporarily or permanently closed or if any required modifications to the informed consent form and notification to research subjects are required.

iii) Potential benefits of this research to the subjects

The combination of famotidine and n-acetyl cysteine may be of potential benefit to the enrolled subjects as it may reduce the severity of their SARS-CoV-2 infection and potentially reduce the risk of hospitalization.

iv) Potential benefits of this research to society

Potential benefits to society include determination if the combination of famotidine and n-acetyl cysteine is safe and may improve clinical outcomes in patients suffering from SARS-CoV-2 infection.

VI) SUBJECT SAFETY AND MINIMIZING RISKS (Data and Safety Monitoring Plan)

i) Is this study a clinical trial?

Yes

ii) What is the Data Safety plan for this study? If this study is a clinical trial, please include a detailed description of the Data and Safety Monitoring Plan.

At the time of consent the Data Safety Plan will be discussed with the subject and the subject will be required to sign a CIRB Research HIPAA Authorization Form in addition the trial informed consent form. The trial consent form and the CIRB Research HIPPA Authorization Form will be kept together with all Paper CRFs in a locked room when not occupied by qualified research staff or in a locked cabinet. No PHI will be recorded on paper CRFs. All electronic data will be kept on the REDCap® server within the forms as described previously. A copy of the REDCap® Codebook can be found in the Appendix. Access to the REDCap® project for this trial will be restricted to study personnel. The ability to export data from REDCap® will be restricted to the Statistician Co-Investigators, the Epidemiologist and the PI. All patient identifier fields are identified in REDCap®, and data will only De-Identified Data can be exported. A figure depicting the assigned rights as organized by role with REDCap® can be found in the Appendix. No paper copies of an identification key will be created or maintained. It is anticipated that within 30 days of trial completion all database records containing any PHI will be de-identified.

VII) CONSULTANTS

i) Academic Partner Institutions

Clemson University, School of Mathematical and Statistical Sciences

Deborah Kunkel, PhD, (effort in-kind): Dr. Kunkel is a statistician with expertise in Bayesian statistical methodology. Dr. Kunkel will perform the mathematical derivations required for the design of the proposed parallel phase I/II trial. She and Dr. Rennert will oversee power calculations and all subsequent data analysis.

Clemson University, College of Behavioral, Social and Health Sciences

Lior Rennert, PhD, (effort in-kind): Dr. Rennert is an expert biostatistician with expertise in collaborative health research. Dr. Rennert has 10 years of experience with power calculations and application of statistical models for clinical trial data.

Corey Kalbaugh, PhD, (effort in-kind): Dr. Kalbaugh is an Assistant Professor at Clemson University and an expert epidemiologist. He will be instrumental in the writing, study design and execution of this research especially as it pertains to the phases following this trial and subsequent research trials as appropriate. Additionally, he will have a leading role in overseeing the analysis of the data.

University of South Carolina School of Medicine Greenville

David Pritchett: Mr. Pritchett is a second-year medical student at the University of South Carolina School of Medicine Greenville and has an undergraduate degree in Chemistry from Furman University. He will assist primarily with data collection.

Benjamin Hutto: Mr. Hutto is a second-year medical student at the University of South Carolina School of Medicine Greenville and has an undergraduate degree in from USC-Aiken. He will assist primarily with data collection.

Josiah McDonald: Mr. McDonald is a second-year medical student at the University of South Carolina School of Medicine Greenville. He will assist primarily with data collection.

Halee Bryant Vaughn: Ms. Vaughn is a fourth-year medical student at the University of South Carolina School of Medicine Greenville. She will assist primarily with data collection.

ii) Non-academic Partner Institutions

Timothy F. McTigue, M.D., (effort in-kind): Dr. McTigue is an ER Physician who is Board Certified in Internal Medicine and Pediatrics and is an independent contractor serving as a consultant in the intellectual development and writing of the trial. He also will assist in the data analysis.

VIII) FACILITIES AVAILABLE

Facilities include Greenville Memorial Hospital, Greer Memorial Hospital, Baptist Easley Hospital.

IX) APPENDICES

List of Separate Attachments

Schedule of Assessments

Medication Log Form (N.B., Provided to Subject)

Daily Diary (N.B., Provided to Subject)

Instruction Sheet for Roth Score and 40 Step Test (N.B., Provided to Subject)

Figure of REDCap® Data Entry Forms Mapped by Event

Table of Contents of REDCap® Data Entry Form Attachment

Responsibilities and Delegated Tasks and Responsibilities

Study Personnel and Roles

Figure of REDCap® User Rights

Prisma-Health Clinical Guidance

List of Separate Attachments

1. Prisma Health Upstate Informed Consent Form
2. CIRB Research HIPPA Authorization Form
3. REDCap® Data Entry Forms (See following Table of Contents)
4. Randomization Allocation Table Dev (Sample Used in Development Status)
5. Sample Dynamic Randomization Table (Sample Used in Development Status)
6. REDCap® Codebook
7. Sample NCI AE/SAE CTCAE v5 REDCap® form (with saved data)
8. NCI AE/SAE CTCAE v5 REDCAP® form (comprehensive with branching logic)

Schedule of Assessments

Schedule of Assessments: Trial of FAM and NAC for SARS-CoV-2				
Assessment	Enrollment (within 24 hours of a SARS CoV-2 Test)	Weekly after Randomization Until Hospital Admission or Study Completion	Upon Study Completion (If SARS CoV-2 Positive)	If Hospitalized and Until Discharge
Informed Consent	X			
Urine Pregnancy Test (If appropriate)	X			
Blood Type and Rh	X			
(Serum) Cytokines (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF), Tryptase and Glutathione Level	X		X	X (once upon hospitalization)
(Urine) F2-Isoprostane/Creatinine Ratio, TMPRSS2 Cytokines (IL-1β, IL-2, IL-6, IL-8, IL-10, IFNγ, TNFα and GM-CSF) and N-methylhistamine	X	X	X	X (once upon hospitalization)
CURB-65 Score	X			
CAP-Sym 18	X	X	X	
IPSS (men only)	X		X	
Weekly Review of Daily Medication Logs and Daily Diary		X		
Weekly Assessment CRFs		X		
Completion Data CRF			X	
Hospital Tracking CRFs				X

Medication Log Form

Medication Log Form: Trial of FAM and NAC for SARS-CoV-2

Subject Study Number: _____ Week #: _____

Day of Week Trial Started: _____(fill this in by hand under Day 1 below)

Dose of Famotidine 20 mg tablets Assigned to Taken Thrice Daily: 0 1 2 3
(Research Staff to Circle)

Number of N-Acetyl Cysteine Capsules Assigned to Take Thrice Daily: 1 2 3
(Research Staff to Circle)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Day of Week							
Date (MM/DD)							
Famotidine (20 mg tablet)							
1st Dose							
2nd Dose							
3rd Dose							
N-Acetyl Cysteine (600 mg capsule)							
1st Dose							
2nd Dose							
3rd Dose							

Instructions for Research Staff:

1. Print a minimum of 4 Sheets and enter Subject Study Number on each. Write in week numbers 1 to 4 on each sheet.
2. Enter Day of Week Subject is to start taking medicine. This should be the same for each sheet.
3. Under Day 1 enter the day of the week that subject is to start taking medicine. Under each subsequent Day enter the next day of the week.
4. Circle the assigned number of Famotidine tablets to take thrice daily.
5. Circle the assigned number of N-Acetyl Cysteine capsules to take thrice daily.

Instructions for Research Subject:

1. You will be assigned to take one, two or three Famotidine tablets three times a day. Please enter the time of day for the correct day for each dose you take.
2. You will take the N-Acetyl Cysteine capsules thrice a day. You will be assigned to take one, two or three capsules each time. Please enter the time of day for the correct day for each dose you take.
3. Please note that the days of each new week should correspond to the same day of the week you started on the trial which won't necessarily be a Monday or Sunday.
4. Please call the study coordinator if you have any questions. If you cannot get a hold of the study coordinator, then please call the Principal Investigator, John Joseph O'Connell, M.D., at any time day or night at (864)679-3900.

Daily Diary

Daily Diary: Trial of FAM and NAC for SARS-CoV-2

Subject Study Number : _____ Week #: _____

Day of Week Trial Started: _____ (fill this in by hand under Day 1 below)___

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Day of Week							
Date (MM/DD)	/	/	/	/	/	/	/

	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
Time	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__	__:__
Temperature	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F	___.__°F
O2 Sat														
Pulse														
Blood Pressure														
Roth Score														

Symptoms	0 indicates absence of symptom; 1 is mild; 3 is moderate; and 5 is severe													
Felt Feverish (0 to 5)														
Cough (0 to 5)														
Shortness of Breath/Difficulty Breathing (0 to 5)														
Chest Pain (0 to 5)														
Chills (0 to 5)														
Muscle Pain (0 to 5)														
Sore Throat (0 to 5)														
Other (Y/N) Specify Below														

Notes

Instructions for Research Subject:

1. Please take your temperature twice daily, once in the morning and once after noon. Please record your temperature in Fahrenheit to a single decimal point. If you take your temperature more than once during a time period, please record only the highest temperature during that time period. Please record the time you took your temperature.
2. Please record a value for every listed symptom twice a day on a scale of 0 to 5. 0 should indicate the absence of the symptom with 1 indicating mild, 3 moderate and 5 severe.
3. If you are experiencing any other symptoms, please indicate with a Y and describe below.
4. Please call the study coordinator if you have any questions. If you cannot get a hold of the study coordinator, then please call the Principal Investigator, John Joseph O'Connell, M.D., at any time day or night at (864)679-3900.

Instruction Sheet for Roth Score and 40 Step Test

Roth Score

The Roth Score has not been proven to be an effect measure of one's respiratory status and may lead to a false reassurance. It has not been previously studied in patients with a SARS-CoV-2 infection. If you feel your ability to breathe is worsening, you should seek medical attention immediately. We are asking you to perform this test daily for research purposes so that we can better understand the severity of a SARS-CoV-2 infection. It is possible that we may learn that changes in the Roth Score together with other objective measures may allow us to understand earlier predictors for severity of a SARS-CoV-2 infection.

To perform this test correctly you will need to take a deep breath followed by counting out loud from 1 to 30 in your native language, in a single breath, as rapidly as possible. You should stop your count when you reach 30 or take another breath whichever comes first. You will also need to use a physical or electronic stopwatch with a second hand and measure in seconds the time it takes between your count of 1 and the highest number you achieve. The result of the Roth score includes 2 measurements: (1) the duration of time elapsed between counting from 1 to 30 in 1 breath, or until the you take another breath; and (2) the highest number you reach in 1 breath. The first measurement is known as the "counting time" and this should be entered your Daily Diary in the row labelled "Roth Score" under the AM column. The second measurement is known as the "maximal count" and should be entered under the PM column. You only need perform this test once daily and ideally around the same time of day.

40 Step Test

The 40 Step Test should only be performed under the direct supervision of a health care provider and for the purposes of this trial will be done during your weekly virtual visit with one of the trial investigators. There is no evidence that this test is harmful; however, its safety has also not been conclusively proven. This test has not been studied previously in patients with a SARS-CoV-2 infection. This test is an exertional desaturation test and a determination will be made by the study investigator at the time of your weekly virtual visit. You will not have to perform this test if you are uncomfortable or unable to do so.

This test will only be administered if you were provided a pulse oximetry device. You will be asked to measure oxygen saturation prior to beginning this test. You will then be asked to walk 40 steps on a level surface, and you will then measure your oxygen saturation after completing your 40 step walk.

Figure of REDCap® Data Entry Forms Mapped by Event

Data Collection Instrument	Enrollment (1)	Post Enrollment (2)	COVID-19 Result - Randomize (3)	Research Labs (4)	Outpatient Follow-up (5)	Hospital Follow-up (6)	Study Completion (7)
Eligibility Assessment Case Report Form	✔						
Demographics	✔						
CURB-65 Case Report Form	✔						
International Prostate Symptoms Score Questionnaire Case Report Fom	✔						✔
CAP-SYM 18 Case Report Form	✔				✔		✔
Post Enrollment Checklist	✔						
Baseline Data		✔					
CBC		✔					
BMP		✔					
Coag		✔					
Liver Panel		✔					
C Reactive Protein		✔				✔	
Pregnancy Test		✔					
EKG		✔			✔	✔	
Blood Gas		✔			✔	✔	
PSA		✔					
Starting Medications		✔					
COVID-19 Test Result and Drug Randomization Case Report Form			✔				
Investigator Assignment			✔				
Medication Log							
Trial Drug Adjustment					✔		
Daily Medication Log					✔		
Daily Diary					✔		
New Medications					✔	✔	
Research Assistant Weekly Assesment					✔		
Investigator Weekly Assessment					✔		
Blood Type				✔			
Cytokine Panel				✔			
COVID-19 NP Swab Results						✔	
Hospital Admission						✔	
Supplemental Oxygen Started or Increased						✔	
Intubation						✔	
Extubation						✔	
Supplemental Oxygen Decreased or Stopped						✔	
ICU Admission						✔	
ICU Discharge						✔	
Hospital Discharge						✔	
Completion Data							✔
Antibody Results						✔	
NCI Adverse Event Serious Adverse Event CTCAE v5					✔		

N.B. Not all forms are required and most have very few data fields but allow for multiple instances and thus were created as separate data entry forms

Table of Contents of REDCap® Data Entry Form Attachment

Responsibilities and Delegated Tasks and Responsibilities

Responsibilities	Investigator-Sponsor	Clinical Co-Investigators	Epidemiologic and Statistician Co-Investigators	Pharmacy Co-Investigators	Consultants/ Senior Medical Mentor	Study Coordinator	Research Assistants	Data And Safety Monitoring Committee
Study design and general coordination of research	X							
Advise and Support in Study Design and Conduct of Research		X	X	X	X	X	X	X
Recruit eligible subjects	X	X			X			
Screen eligible subjects	X	X			X	X	X	
Obtain informed consent	X	X			X			
Conduct research procedures involving direct interaction with subjects	X	X			X			
Prescribe Study Drug	X	X			X			
Weekly Calls with Individual Subjects to Review Compliance with taking Assigned Study Drugs,	X	X			X	X	X	

Completing Medication Log and Daily Diary. Administer CAP SYM 18 Survey and Ensure Urine Specimen has been dropped off by Subject								
Weekly Review of Inpatients Records of Subjects who have been Hospitalized	X	X			X	X	X	
Coordinate clinical laboratory tests or support	X	X			X	X	X	
Coordinate research laboratory assays, tests, or support	X	X			X	X	X	
Coordinate Pharmacy support (i.e. storage, inventory, and dispensing of all investigational drugs used in the research)	X			X		X	X	
Data entry	X	X	X		X	X	X	
Data analysis	X	X	X	X	X	X	X	X
Storing/sharing research data	X	X	X		X	X	X	

Storing research protocol documents (including consent/information sheets)	X	X			X	X	X	
Storing/sharing specimens for current research	X	X			X	X	X	
Reporting to IRB, institutional officials, and /or sponsor - unanticipated problems involving risks to subjects or others, adverse events, and serious or continuing non-compliance	X	X			X	X		X
Establishing procedures to ensure subject privacy and confidentiality of research data	X					X		
Continuing Review by the IRB	X					X		
Post-approval monitoring of the conduct of the research	X	X			X	X		X

Data monitoring plan – coordination and communication	X	X			X	X		
Clearance of research-related publications or presentations	X							
Review of All Adverse Events	X	X			X			X
Authority to Temporarily Halt Conduct of Research	X	X	X	X	X			X
Weekly Calculations of Posterior Probabilities and Decision to Temporarily Close Treatment Arms due to Relative Ineffectiveness In Accordance with Trial Design and Pre-Determined Temporary Stopping Rules			X					
Permanent Closure of Treatment Arms or Trial Due to Futility or Dose Limiting Toxicities In Accordance with			X					

Trial Design and Pre-determined Stopping Rules								
Randomization to Assigned Treatment Arm at Time of Enrollment			X					
Sponsor Reporting Requirements to the U.S. FDA	X							

Study Personnel and Roles:

1. Sponsor-Investigator

John Joseph O'Connell, M.D.
Prisma Health-Upstate
Department of Medicine
Phone: (864) 679-3900 (office)
Email: John.O'Connell@PrismaHealth.org

2. Clinical Co-Investigators

Larisa D Coldebella Perino, M.D.
Prisma Health-Upstate
Department of Emergency Medicine
Phone: (864) 313-9576 (office)
Email: Larisa.Coldebella@PrismaHealth.org

Angelo Perino, M.D.
Prisma Health-Upstate
Department of Emergency Medicine
Phone: (864) 313-9493 (office)
Email: Angelo.Perino@PrismaHealth.org

Amanda Schnee, M.D.
Prisma Health-Upstate
Department of Medicine
Phone: (864) 455-9033(office)
Email: Amand.Schnee@PrismaHealth.org

W. Jeffery Edenfield, M.D.
Prisma Health-Upstate
Cancer Institute
Phone: (864) 679-3900 (office)
Email: Jeffery.Edenfield@PrismaHealth.org

Phillip Moschella, M.D., Ph.D.
Prisma Health-Upstate
Department of Emergency Medicine
Phone (864) 455-9033(office)
Email: Phillip.Moschella@prismahealth.org

Jennica Siddle, M.D.
Prisma Health-Upstate
Department of Emergency Medicine
Phone (864) 455-9033 (office)
Email: Jennica.Siddle@PrismaHealth.org

3. Epidemiologic Co-Investigator

Corey Andrew Kalbaugh, Ph.D.
Clemson University
College of Behavioral, Social and Health Sciences
Phone: (864) 656-6694 (office)
Email: coreyk@clemson.edu

4. Statistician Co-Investigators

Deborah Kunkel, Ph.D. (Lead)
Clemson University
School of Mathematical and Statistical Sciences
Phone: (864) 656-1656 (office)
Email: dekunke@clemson.edu

Lior Rennert, Ph.D.
Clemson University
College of Behavioral, Social and Health Sciences
Phone: (864) 656-7640 (office)
Email: liorr@clemson.edu

Alex Ewing, Ph.D.
Prisma Health-Upstate
Division of Research and Scholarship
Phone: (864) 455-1130 (office)
Email: Alex.Ewing@PrismaHealth.org

5. Pharmacy Co-Investigators

Michael Cohen, PharmD
Prisma Health-Upstate
Department of Pharmacy
Phone: (864) 455-7000 (office)
Email: Michael.Cohen@prismahealth.org

6. Senior Medical Mentor

W. Jeffery Edenfield, M.D.
Prisma Health-Upstate
Cancer Institute
Phone: (864) 679-3900 (office)
Email: Jeffery.Edenfield@PrismaHealth.org

7. Consultants

Arwen BL Declan, M.D., Ph.D.
Prisma Health-Upstate
Department of Emergency Medicine
Phone: (864) 455-7000 (office)
Email: Arwen.Declan@PrismaHealth.org

Timothy F McTigue, M.D.
Independent Contractor (in-kind effort)

Phone: (863) 221-8195 (office)
Email: tfmctigue@aol.com

Angela Orsky, DNP
Prisma Health-Upstate
Post-Acute Services
Phone: (864) 797-7784 (office)
Email: Angela.Orsky@PrismaHealth.org

8. Study Coordinator

TBD
Prisma Health-Upstate

Phone: (office)
Email

9. Research Assistants

Halee Bryant Vaughn
University of South Carolina
School of Medicine Greenville
Phone: (864) 455-7992 (office)
Email: habryant@email.sc.edu

Benjamin R. Hutto
University of South Carolina
School of Medicine Greenville
Phone: (864) 455-7992 (office)
Email: brhutto@email.sc.edu

David Y. Pritchett
University of South Carolina
School of Medicine Greenville
Phone: (864) 455-7992 (office)
Email: DavidYP@email.sc.edu

Josiah N. McDonald
University of South Carolina
School of Medicine Greenville
Phone: (864) 455-7992 (office)
Email: josiahm@email.sc.edu

10. Data and Safety Monitoring Committee

Prerana Roth, M.D. (Chair)
Prisma Health-Upstate
Department of Medicine
Phone: (864) 455-9033 (office)
Email: Prerana.Roth2@prismahealth.org

Figure of REDCap® User Rights by Role

Role name (click role name to edit role)	Username or users assigned to a role (click username to edit or assign to role)	Expiration (click expiration to edit)	Project Design and Setup	User Rights	Data Access Groups	Data Export Tool	Reports & Report Builder	Graphical Data View & Stats	Calendar & Scheduling	Data Import Tool	Data Comparison Tool	Logging	File Repository	Record Locking Customization	Lock/Unlock Records	Randomizatio	Data Quality (create/edit rules)	Data Quality (execute rules)	API	Create Records	Rename Records	Delete Records
—	**034509** (John O'Connell)	never	✔	✔	✔	De-Identified	✔	✔	✔	✔	✔	✔	✔	✔	🛡	Setup Dashboard Randomize	✔	✔	✖	✔	✖	✖
DSMB	[No users assigned]		✖	✖	✖	✖	✔	✔	✔	✖	✖	✖	✔	✔	🛡	✖	✖	✖	✖	✖	✖	✖
Epidamiologist	**ckalbaugh** (Corey Kalbaugh)	never	✖	✔	✖	De-Identified	✔	✔	✔	✖	✔	✖	✔	✖	✖	✖	✔	✔	✖	✖	✖	✖
Investigator	**035233** (Phillip Moschella)	never	✖	✔	✖	✖	✔	✔	✔	✖	✖	✖	✔	✖	✖	Dashboard Randomize	✖	✖	✖	✔	✖	✖
	040013 (Larisa Coldebella Perino)	never																				
	041219 (Arwen Declan)	never																				
	043733 (Jennica Siddle)	never																				
	043900 (Amanda Schnee)	never																				
	aao17a (Angela Orsky)	never																				
Medical Student	**040483** (Halee Vaughn)	never	✖	✔	✖	✖	✔	✔	✔	✖	✖	✖	✔	✖	✖	✖	✖	✖	✖	✔	✖	✖
	044435 (Josiah Mcdonald)	never																				
Research Coordinator	[No users assigned]		✔	✖	✖	✖	✔	✔	✔	✔	✔	✔	✔	✖	✖	✖	✔	✔	Export Import	✔	✖	✖
Statistician	**dkunkel** (Deborah Kunkel)	never	✖	✔	✖	De-Identified	✔	✔	✔	✖	✔	✖	✔	✖	✖	Setup Dashboard	✔	✔	✖	✖	✖	✖
	jae13b (Joseph Ewing)	never																				
	lrennert (Lior Rennert)	never																				

PRISMA HEALTH

Viral Symptom Management Telehealth Workflow

Justification:

- A large portion of clinical decompensations from COVID-19 occur later in disease process, often day 7-9.
- In order to monitor these patients more closely in the ambulatory setting and to best meet their needs before they show up in an ED in florid respiratory failure, the following workflow has been outlined.
- Patients without a PCP/medical home may also feel lost about who to turn to with questions while quarantined at home.
- Additionally, some patients with positive results are not aware of the importance of self-quarantine, and for that reason, there is a need to reinforce guidelines for when patients can resume activity and social interaction.

Existing Process:

- All COVID-19 outside of the ED or inpatient setting is done at either Greenville Memorial Hospital's drive-through tent or Patewood's Team Member Care Center in the Prisma Health Upstate Market.
- Both positive and negative test results are received by the Prisma Health NP pool who review the results. Nurses and NPs communicate results to the patients via phone call. In that phone call, patients are instructed as to next steps and self-care.

Proposed New Additional Process:

- On a daily basis, the WQ for outstanding results is updated with tests collected the prior day.
- This WQ will be reviewed by the Nurse pool, under the leadership of the Business Health NPs. They will review the listing to determine if the patients have a PCP yet or not.
- For patients with a PCP, an InBasket message will be sent to the PCP's Clinical Pool with the following message:
    - "Your patient has been tested for COVID-19 as he/she is currently experiencing the signs and symptoms of the illness. Please schedule a video visit with this patient for Day 2 (tomorrow) as well as day 5 and 8. At each of these visits, please monitor the patient for worsening symptomatology, answer his/her questions, and ensure that he or she is maintaining quarantine. If at any time the patient experiences worsening of symptoms and further evaluation face-to-face is indicated, the patient may either be seen at the practice with appropriate PPE or may be directed to an MD360 for urgent care, unless his/her symptoms are severe enough to merit attention in an ED."
    - If a COVID-19 Positive patient or PUI will be sent to MD360 following any of these virtual visits, please refer to the workflow below.

PRISMA HEALTH

- For patients without a PCP, the Business Health NP or nurse pool will direct the patient to the Physician Finder "New Patient Specialist team" in addition to submitting a Referral to Primary Care.
    - The New Patient Specialist will schedule the patient with a PCP and send the following message:
    - "This patient is new to you. He/she has been tested for COVID-19 as he/she is currently experiencing the signs and symptoms of the illness. Please schedule a video visit with this patient for Day 2 (tomorrow) as well as day 5 and 8. At each of these visits, please monitor the patient for worsening symptomatology, answer his/her questions, and ensure that he or she is maintaining quarantine. If at any time the patient experiences worsening of symptoms and further evaluation face-to-face is indicated, the patient may either be seen at the practice with appropriate PPE or may be directed to an MD360 for urgent care, unless his/her symptoms are severe enough to merit attention in an ED."
    - If a COVID-19 Positive patient or PUI will be sent to MD360 following any of these virtual visits, please refer to the workflow below.

Patients in need of in-person care will either be seen at a practice site or be referred to an MD360 location:

- There is onsite x-ray and testing available.
- If the PCP is referring a patient to MD360 for further evaluation, the PCP must notify MD360 by phone prior to sending the patient.
- PCP will inform the MD360 provider of the patient's current status and assessment needs.
- Patient will be advised to call the MD360 front desk prior to entering the facility.
- MD360 team member will wear appropriate PPE and meet the patient at the door. Will escort the patient directly to an exam room for further care.

PRISMA HEALTH

Tip Sheet for Telehealth Encounter with COVID-19 Positive Patients and PUIs

The following questions are a guideline for the ambulatory provider to discuss in a telehealth encounter with a COVID-19 positive patient or a PUI. These visits are to be conducted on Day 2, 5, and 8 after a positive test. The goal is to help guide whether a patient needs a higher level of care.

1. What was your temperature today and yesterday?
    - *If still febrile on day 8, do another follow-up on day 12*
2. Are you able to get to the bathroom without getting SOB?
    - *If no and different from patient baseline → send to ED*
3. Do you feel light headed or dizzy?
4. Have you been able to eat and drink some?
    - *If not able to eat and also dizzy, check CMP for AKI/transaminitis vs consider ED*
5. Are you having any kind of chest pain?
    - *If yes – take more detailed history to sort out if it's musculoskeletal with coughing.*
    - *If patient has cardiac history and chest pain, consider ED*
6. Are you having nausea, vomiting or diarrhea?
7. Do you feel like you are getting worse, better, or about the same? If feeling worse, do another telehealth visit the next day
    - *How is your cough? (If initially present)*
    - *How is your sore throat (If initially present)*
8. Any other symptoms?
9. Remind patient to stay quarantined

X) REFERENCES/LITERATURE CITATIONS

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