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Review Article

Part II. high-dose methotrexate with leucovorin rescue for severe COVID-19: An immune stabilization strategy for SARS-CoV-2 induced 'PANIC'[1] attack

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ABSTRACT

Here, in Part II of a duology on the characterization and potential treatment for COVID-19, we characterize the application of an innovative treatment regimen for the prevention of the transition from mild to severe COVID-19, as well as detail an intensive immunotherapy intervention hypothesis.

We propose as a putative randomized controlled trial that high-dose methotrexate with leucovorin (HDMTX-LR) rescue can abolish 'PANIC', thereby 'left-shifting' severe COVID-19 patients to the group majority of those infected with SARS-CoV-2, who are designated as having mild, even asymptomatic, disease. HDMTX-LR is endowed with broadly pleiotropic properties and is a repurposed, generic, inexpensive, and widely available agent which can be administered early in the course of severe COVID-19 thus rescuing the critical and irreplaceable gas-exchange alveoli.

Further, we describe a preventative treatment intervention regimen for those designated as having mild to moderate COVID-19 disease, but who exhibit features which herald the transition to the severe variant of this disease. Both of our proposed hypothesis-driven questions should be urgently subjected to rigorous assessment in the context of randomized controlled trials, in order to confirm or refute the contention that the approaches characterized herein, are in fact capable of exerting mitigating, if not abolishing, effects upon SARS-CoV-2 triggered 'PANIC Attack'. Confirmation of our immunotherapy hypothesis would have far-reaching ramifications for the current pandemic, along with yielding invaluable lessons which could be leveraged to more effectively prepare for the next challenge to global health.

1. Hypothetical strategies for rescuing the severely affected COVID-19 patient

We submit that the best chance to rescue those afflicted with the severe variant of COVID-19 depends upon the decision to intervene with a corresponding assemblage of diverse immune modulatory agents, each directed toward a mechanistically distinctive component of the activated inflammatory network's machinery. Such an incredibly diverse combination of agents would require a synchrony of action in order to arrest the 'irrationally exuberant', poorly coordinated,

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[1] Prolific Activation of a Network Immune-Inflammatory Crisis [PANIC].

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and regulatory-deficient immune network in patients with severe COVID-19 symptoms.

This prolific activation, and expansive constellation of coincidentally activated inflammatory cascades, confers potentially destructive consequences upon host tissue compartments which harbor SARS-CoV-2 (i.e. bystander damage). In contrast to the application of a multi-component treatment strategy, the identification of a single agent endowed with pleiotropic mechanistic properties, and capable of reconstituting the normally coordinated immune-regulatory response characteristics to SARS-CoV-2, would represent a potential advance in our struggle to transition severely affected COVID-19 patients into the 'mild' phenotypic variant of this Coronavirus-mediated disorder.

2. Survival of the 'FOCUSED'

The categorical designation of 'mild COVID-19' signifies a considerably more 'focused' activation of the immune network compared to severely affected patients. The majority of mildly infected patients demonstrate a short-lived flu-like illness with minimal long-lasting effects or deficits, furthermore, others have such minimal symptoms as to be considered to be asymptomatic all together. Clinical manifestations, along with their magnitude and/or worsening, should give pause to consider the prospect of acute and directed interventions, which may potentially serve to mitigate symptoms and reduce the risk of advancing to a more severe clinical state with an associated worsening prognosis. Such issues can involve recalcitrant cough, impaired mucus clearance, perception of a diminished negative inspiratory force of breathing, and increased risk of nosocomial secondary upper respiratory tract infections, including, but not limited to *MRSA and enteric flora*.

Treating a *recalcitrant cough* may be as simple as offering dextromethorphan, but the addition of quinidine can reduce the former's metabolism and thereby prolong its action, A dose of 20 mg/10 mg respectively is the composition of Nuedexta which is FDA-approved for pseudobulbar affect, but can be of great utility in the circumstances of recalcitrant cough.

An expectorant, such as guaifenesin, may aid in *mucus clearance*, whereas a bronchodilator may improve *perception of a diminished negative inspiratory force of breathing*. Smooth muscle relaxation at the terminal bronchioles and activation of the beta-2 adrenergic receptor on lung mononuclear cells may skew the immune milieu from a TH1 to a TH2 anti-inflammatory phenotype. The intracellular biomarker, secondary to transmembrane signaling at the beta-2 adrenergic receptor, is an escalation of cAMP for this effect [1–4].

A bronchodilator in conjunction with inhalational steroids will likely reduce peripheral airway inflammation. Antibiotics, such as azithromycin, will mitigate *risk of secondary nosocomial upper respiratory tract infection and act as a protective agent against M. pneumoniae*, as well as other community acquired infection of the airway (but NOT all).

Interventions that help detect worsening, such as home finger pulse oximetry, may be useful. This multi-faceted interventional approach may prevent the transformation of the mild phenotype into the severe disease course and its correspondingly worse prognosis [Vignette 1].

The recovery phase for mildly to moderately affected COVID-19 patients appears to be temporally linked with the emergence of detectable SARS-CoV-2 antibodies; although their relevance to recovery (i.e. are they endowed with viral-neutralization effects or even protection in the absence of 'viral neutralization' in vitro) and the establishment of a durable remission is currently a matter of vigorous debate [5]. Notwithstanding the controversy surrounding the therapeutic relevance of SARS-CoV-2 associated antibodies, the donation of plasma derived from patients dichotomized to the mildly affected cohort, and administered to more severely affected patients, can exhibit impressive remission-exacting effects [5–7]. This salient observation, in conjunction with inadequate evidence to confirm the relevance of SARS-CoV-2 antibodies, makes it is entirely feasible to assume that one, or even a variety of plasma-derived soluble factors from recovered COVID-19 patients, may serve to promote recovery in severely affected patients.

Those dichotomized into the 'severe COVID-19' subgroup, because of their 'PANIC Attack' disease course, fail to organize the coordinated neutralization of SARS-CoV-2, in contradistinction to mildly to moderately affected patients who do in fact eventually orchestrate effective clearance of the COVID-19 pathoetiologic agent. Alternately, the severely affected cohort of patients will exhibit evidence of the coincident activation of both the innate and adaptive limbs of the inflammatory response network; which is in keeping with our definition of the 'prolific activation of a network-immune-inflammatory crisis' (i.e. PANIC), and provides an attractive explanation for why the most severely affected COVID-19 patients harbor stereotyped patterns of irreversible tissue destruction, including but not limited to, the obliteration of the life-sustaining process of gas exchange within the terminals of the bronchopulmonary tree.

3. Anti-cytokine monoclonal antibody therapy

The observation of the cytokine release syndrome (CRS) appears to be, at least in part, secondary to the accelerated viral replication characteristics within the peripheral lung alveolar network, ultimately culminating in the massive release of newly assembled virions during cellular apoptosis, a process which also results in the release of pro-inflammatory mediators. Apoptosis resulting in the exaggerated and paroxysmal release of inflammatory effector elements is now referred to as *pyroptosis* (Preprint: Yang M. Cell pyroptosis, a potential pathogenic mechanism of 2019-nCoV infection. SSRN Electron J 2020). In fact, pyroptosis is the process most likely responsible for the pathobiologic underpinnings of the so-called 'cytokine storm' (representing an important mechanism among a constellation of pathobiological underpinnings, which collectively represent the new rubric, which we have defined as PANIC) which involves the rapid release of a broad repertoire of cytokine and chemokine effector elements including TNF-α, IFN-γ, IL-1β, IL-6, IL-12, IL-18, IL-33, TGFβ; CCL2, CCL3, CCL5, CXCL8, CXCL19, and CXCL10.

In conjunction with the CRS, the SARS-CoV-2 virus is further able to provoke what we believe to represent a redistribution of immune effector cells into the lungs, with the consequent intensification of tissue damage secondary to such cells releasing their corresponding effector elements, free radicals and reactive oxygen species, such as super oxide (O^-).

The viral spike glycoprotein which decorates the outside surface of SARS-CoV-2, and serves as the ligand for ACE-2-r, also happens to represent a strongly immunogenic portion of the viral architecture [8]. During the process of viral binding to the alveolar ACE-2-r, in addition to utlizing the endosomal pathway for cell entry, SARS-CoV-2 can enter cells via the fusion process. In vitro evidence suggests SARS-CoV-2 can also facilitate the generation of multinucleated cells [Fig. 3 in Part I], which are also known as syncytia [9].

Cells infected with SARS-CoV-2 express external spike protein, which can interact with anti-spike IgG (possibly also IgM) antibodies. The result is the establishment of antigen-antibody complexes capable of complement fixation, and ultimately complement-dependent cellular (alveoli epithelium representing the 'kill-target' in this case) cytotoxicity [see 'H' in Fig. 3 of Part I].

The anti-IL-6 monoclonal antibody, tocilizumab, has been shown to provide a mitigating effect upon the Cytokine Release Syndrome, in addition to reducing fevers, CRP levels, and improving the chest CT demonstrated lung abnormalities (e.g. the ground glass opacifications) in COVID-19 positive patients [10,11].

Tocilizumab is a humanized anti-interleukin-6-receptor (IL-6R) monoclonal antibody (mAb) that inhibits interleukin-6 (IL-6) signaling. However, the current understanding of the effects on mortality and morbidity of tocilizumab are still up for debate [12–20]. Similarly, the IL-1 receptor antagonist anakinra, may improve the clinical disposition of septic patients with COVID-19, especially those who harbor evidence

of the macrophage activation syndrome [21].

4. Biomarkers implicating SARS-CoV-2 triggered 'PANIC' attack

A primary aim of this 2-part series has been to advance and substantiate the hypothesis that the pathobiological underpinnings of severely affected COVID-19 patients are principally related to the 'PANIC' Attack. Specifically that such patients mount exaggerated and injurious inflammatory cascades spanning the broad dynamic range of the effector mechanisms which endow the human immune network. The unintended consequences of this process include bystander tissue injury, particularly destruction of the most distal and delicate microanatomy of the bronchopulmonary tree, given the proclivity of the SARS-CoV-2 spike glycoprotein binding to the robust expression of ACE2-r on the alveolar epithelium.

The pleiotropic mechanisms of tissue injury involved in severely affected COVID-19 patients mean that targeting a single regulatory cytokine (e.g. IL-1 or IL-6) is highly unlikely to adequately uncouple the confluence of convergent inflammatory cascades that appear to rapidly descend upon the bronchopulmonary tree, ultimately resulting in an irreversible and step-wise decrement of the alveolar gas-exchange apparatus.

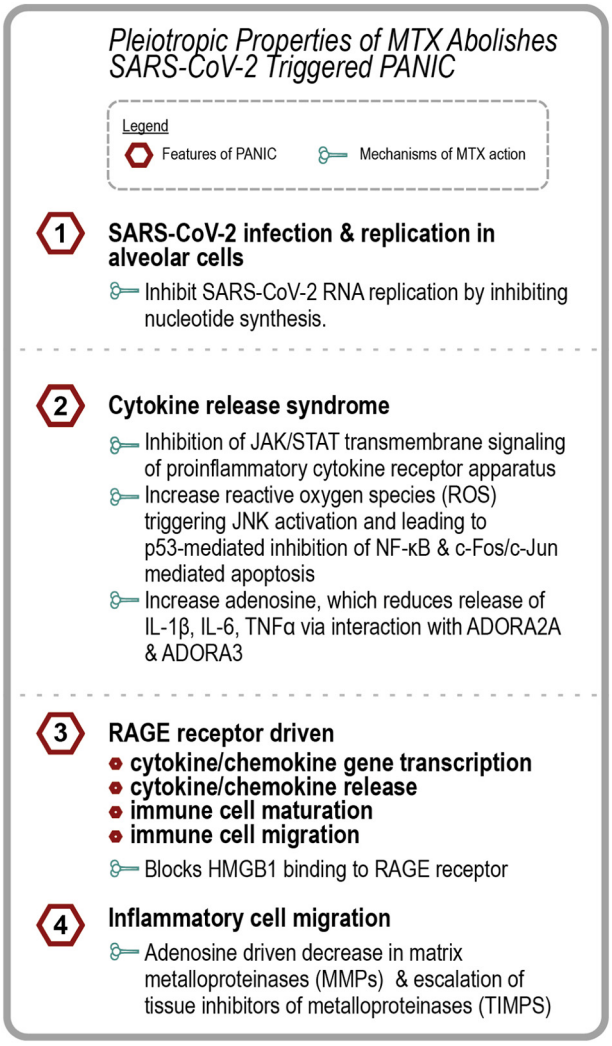
When a critical corpus of functional alveoli are lost, there is an accelerated failure to adequately load oxygen sufficient to deliver the substrate required for aerobic respiration. Virtually every system of the body requires aerobic respiration in order to maintain the constant bioenergetic balance between supply and demand of energy substrate for purposes of homeostasis. The respiration demands of a multiorgan system that operates across a dynamic range of metabolic circumstances with corresponding and rapidly fluctuating energy production and delivery characteristics must be met or catastrophe ensues.

Our hypothesis is that in the ~20% of SARS-CoV-2 infected patients with severe COVID-19, activation of innate and adaptive immune cascades working in synchrony inflict a 'PANIC Attack' upon the terminal lung fields. The outcome inevitably involves a hyperacute, and potentially diffuse, malfunction of the incredibly delicate functional interface between the alveolar epithelium and the single cell thick endothelial tubes, which comprise the capillaries derived from the pulmonary arteries and those which subsequently become the oxygen-rich blood vessels which enter into the pulmonary veins. We can ill afford to permit such a PANIC Attack to proceed without interruption, knowing that a catastrophic loss of alveoli will ultimately bring the severe COVID-19 patient to the pathophysiologic threshold beyond which the lungs will fail to both transfer and load oxygen onto red cell hemoglobin, and to expel carbon dioxide.

We propose a potentially innovative hypothesis, whereby the application of an available, intensive, and highly pleiotropic immunotherapy, is characterized by commensurately synchronous and diametrically opposed actions serving to attenuate the specific sequence of SARS-CoV-2 triggered PANIC Attack mechanisms. We have employed this strategy for a number of fulminant and treatment recalcitrant inflammatory disorders of the CNS, that upon careful analaysis, suggests that the immune phenomenonlogy, whether triggered by a foreign microbe, an adjuvant-enriched vaccine, or the 'ignition' of a monumentally severe exacerbation of autoimmunity, all appear to exhibit common pathobiological mechanisms reminiscent of our definition for the SARS-CoV-2 triggered PANIC Attack.

Our proposed strategy may serve to abort the uncoordinated and poorly regulated host immune activities, which appear to be fundamentally germane to the development of severe COVID-19, and which determine its prognostic disposition if the PANIC Attack proceeds with impunity. Further, we present a vignette [Vignette 1] of a patient with initially mild COVID-19 disease, who began to exhibit what we suspected were transformational clinical features at high risk for advancing to severe COVID-19, and underscore our multi-modal intervention strategy for 'left-shifting' away from the transition to severe COVID-19.

Table 1
Coupling discrete mechanisms of PANIC with correspondingly matched mitigating therapeutic properties of high-dose Methotrexate.



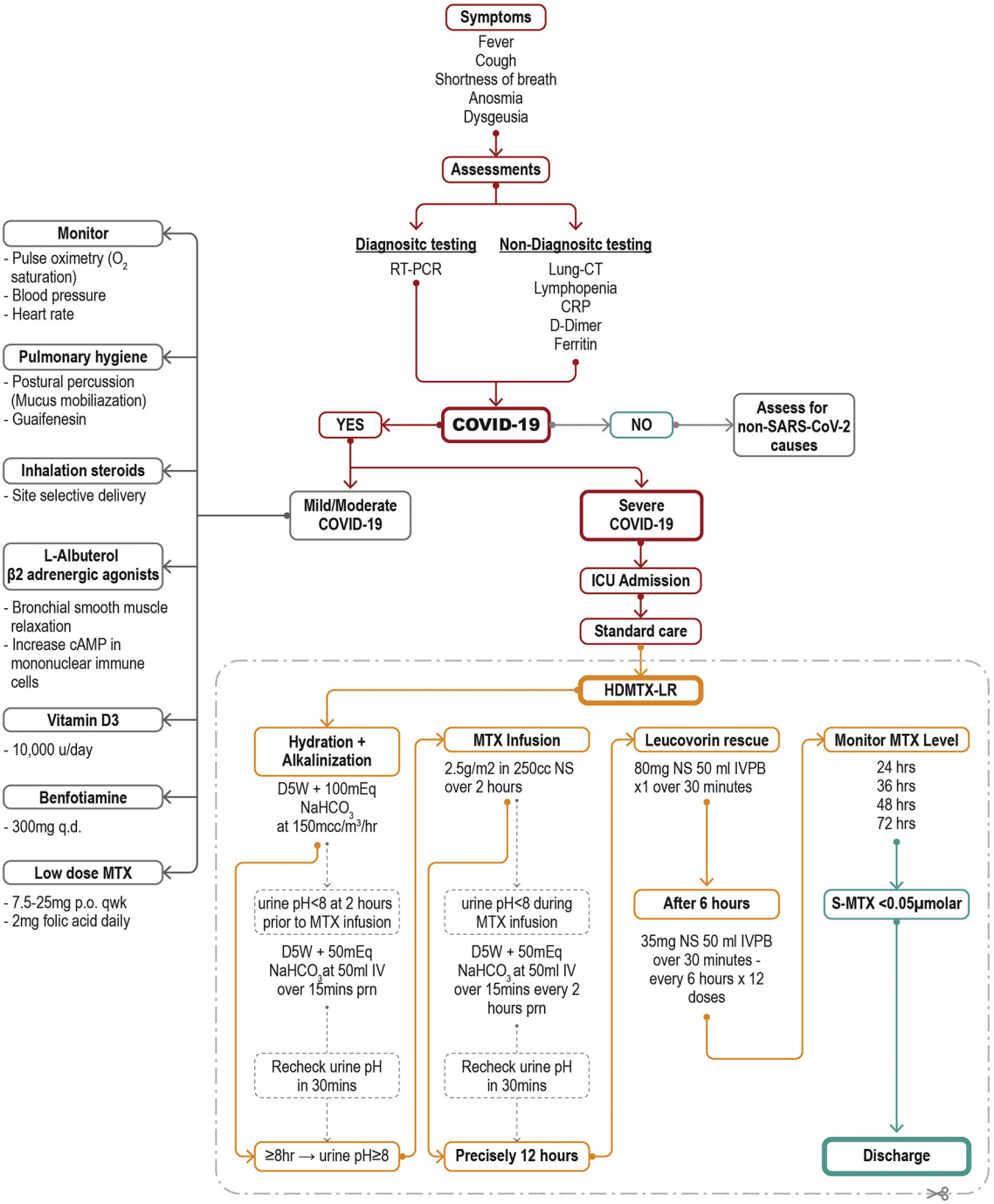
We follow with a second vignette which underscores the utility of the application of the HDMTX-LR protocol (the details of which are provided not only as a supplement at the end of the paper, but also are detailed in our flow diagram on dichotomizing mild/moderate COVID-19 from the severe variant, and their respective proposed treatment interventions). [Flow Diagram].

5. Hypothesis

5.1. Methotrexate & Leucovorin Rescue Will Abolish SARS-CoV-2-Induced PANIC Attack

5.1.1. A Mechanistically Pleiotropic Rescue Strategy For Severe COVID-19

We suggest that high-dose methotrexate with leucovorin rescue (HDMTX-LR), an anti-inflammatory and chemotherapeutic agent, is endowed with impressive mechanistically pleiotropic properties (Table 1), which are capable of mitigating, if not abolishing, each of the SARS-CoV-2 triggered mechanisms that compositionally make up the so-called 'PANIC Attack'. Further, this array of HDMTX-mediated coordinate actions may provide for the development of active immunity toward the SARS-CoV-2 infection, reflecting the very real prospect that such an intervention my serve to 'left-shift' the severe COVID-19 patient to the milder phenotype, along with a correspondingly improved prognosis. Our prior experience with the application of this intensive



Flow chart. Diagnostically Dichotomizing COVID-19 into mild/mod and severe variants along with corresponding treatment interventions, including the protocol for the application of intensive treatment intervention for the latter with high-dose MTX with Leucovorin rescue. The dotted line around this protocol allows the reader to 'cut-out' this section for easy access and lamination to be carried in the wallet, or posted for clinical staff to access when needed.

treatment strategy, and the nearly stereotyped remission in our patients, who, upon presentation, were codified as harboring treatment refractory syndromes of fulminant inflammatory disorders of CNS (e.g. multiple sclerosis (MS), neuromyelitis optica spectrum disorder (NMOSD), Sjogren's associated myelitis, post-vaccinal (dTap) encephalomyelitis, and a series of cases of monumentally severe post-viral encephalomyelitides). (See Flow chart.)

5.1.2. A Novel, Host-Mediated Syndrome of Deleterious Immune Network Activation: PANIC

The observation of reminiscent 'PANIC Attack' mechanisms across our expanding set of fulminant inflammatory syndromes appears to be indistinguishable whether afflicting the CNS or the bronchopulmonary tree. These salient similarities have prompted us to extrapolate from our experience with treatment resistant, recalcitrant, and monumentally fulminant immune activation affecting our patients with MS, NMOSD, Sjogren's syndrome, and in a host of other circumstances where such catastrophic immune-mediated inflammation, can, if not identified and treated accordingly, obliterate the triggering agent's target tissue(s).

We have since utilized the same protocol in many patients, who we now believe in retrospect were suffering from a form of 'PANIC' Attack, except targeting the central nervous system. In Part I of this two-part publication, we reported a patient suffering from a severe adenovirus-

triggered PANIC Attack, characterized by cytokine storm, and an encephalomyelitis, with a PRESS-like pattern distribution of lesions [[44] in PART I]. His protracted and treatment-recalcitrant course had come to the point where a decision was made to consider cessation of care. However, it was finally agreed to try the HDMTX-LR regimen, which ultimately led to the complete recovery of the patient and resolution of all of the brain lesions which characterized his syndrome [Fig. 1 in Part I].

A more recent example considers the plight of a young accountant, who developed optic neuritis in August of 2017, approximately two weeks following a dTap vaccine and during the 3rd trimester of her first pregnancy. Declining steroids, for fear of early term delivery, she recovered in a self-limited fashion. However, by October she presented again, this time with optic neuritis in the fellow eye, in addition to some sensory and motor manifestations. Investigations revealed an elevated, blood-derived anti-myelin oligodendrocyte glycoprotein (anti-MOG) antibody at a titer of 1:1000, and consistent with a post-vaccinal, anti-MOG associated encephalomyelitis. Treated with corticosteroids, she failed to improve.

By December, our patient presented again with confusion, psychomotor slowing, and MRI demonstrating extensive and severe inflammatory lesions throughout much of the cerebral white matter. Coticosteroids once again were without effect, whereas the application of our HDMTX-LR protocol was tolerated without any adverse effects. This therapy rapidly attenuated the disease process, including normalization of her clinical manifestations, in conjunction with a remarkable disappearance of many of her white matter lesions [Fig. 2 of Part I]. A few months later, the appearance of new enhancing cerebral white matter leions prompted us to advance our regimen to a full course of plasma exchange (5-full volume exchanges; which took 8 days), followed by another course of HDMTX-LR. From the end of that course of therapy in early 2018 to the writing of this manuscript, there has been no recurrence, clinically or radiographically. In fact, most of the lesional burden has disappeared [Fig. 2 of Part I].

5.2. Calming 'PANIC' in Severely affected COVID-19 Patients

5.2.1. A Pleiotropic Immune Stabilization Strategy for SARS-CoV-2 Induced 'PANIC' Attack

The application of HDMTX for neoplastic and fulminant inflammatory syndromes of the CNS represents a critically important observation because, despite a steady-state blood to CSF ratio of MTX of about 30:1 [22], doses of 500 mg/m^2 or more are necessary to achieve CNS concentrations of MTX to be sufficiently efficacious against such disorders. The ability to escalate dosing of MTX while rescuing peripheral folate with leucovorin (folinic acid) represents a unique aspect of using this agent across a number of disease states.

5.2.2. Pleiotropic Mechanisms of Action for HDMTX

Methotrexate (4-amino-10-methyl folic acid) is both an analog as well as an antagonist of folic acid, but is capable of exerting its action via a wide spectrum of pleiotropic mechanisms, such that, it is considered by the WHO as an essential medicine [22,23] (Fig. 1; Table 1).

5.2.3. Cell Cycle S-Phase Inhibition via Folate-Dependent Enzyme Inhibition

Cells that are rapidly dividing, such as those associated with malignancy, autoimmunity, and during our proposed PANIC syndrome associated with SARS-CoV-2 infection, spend an increased time in the cell cycle synthesis (S) phase. However, MTX, in part, functions as an S phase inhibitor by depriving nucleoside precursors for both DNA and RNA synthesis and replication [24].

5.2.4. DNA and RNA Base Synthesis Inhibition

MTX accomplishes this via its potent inhibition of folate-dependent enzymes for both purine and pyrimidine base synthesis, with particular implications for the reproduction of Coronavirus virions [Fig. 1]. The vast majority of such rapidly dividing cells, and viral replication as with SARS-CoV-2, can manufacture base synthesis only by the de novo (i.e. from 'scratch') pathways involving inosine monophosphate dehydrogenase (IMDH) for purine, and dihydroorotate dehydrogenase (DHOD) for pyrimidine biosynthesis. Alternately, the vast majority of body cells can produce bases by either the de novo or via the salvage pathways, the latter characterized by the use of partially assembled or partially broken down bases [24].

Following its entry into the cellular cytoplasm, MTX is polyglutamated, which serves to both trap the drug within the intracellular compartment precluding its ability to efflux, and allow the drug to exert one of its principal actions: the inhibition of dihydrofolate reductase (DHFR), as well as other folate-dependent enzymes, such as thymidylate synthase, 5-amino-imidazole-4 carboxamide ribonucleotide (AICAR) transformylase (ATIC), and methylene tetrahydrofolate reductase (MTHFR) [[44] in PART I].

Interestingly, folate supplementation does not abolish either the anti-inflammatory, the chemotherapeutic actions, or the efficacy of MTX. This suggests that other mechanisms, independent of folate antagonism, must figure prominently in the potent actions of MTX. For instance, MTX administration provokes the release of adenosine, a potent anti-inflammatory agent that exerts regulatory effects upon neutrophils, macrophages, and T cells [24].

5.2.5. Adenosine Mediated Anti-Inflammatory Mechanisms

Adenosine promotes its action through interaction with its receptors ADORA2A and ADORA3, where transmembrane signaling activities result in a reduction of production and release of pro-inflammatory cytokines such as IL-1β, TNFα, IL-6, and a reduction in the production of matrix metalloproteinases (MMPs) (the transcription of each requires NF-κB), as well as an increase in production of tissue inhibitors of metalloproteinases (TIMPS). An additional action of MTX is the inhibition of NFκB. Methotrexate inhibits catabolism of adenosine and AMP, while increasing release of both ATP and ADP from inflammatory cells; an effect which modulates the bioenergetic activity, such that inflammatory cells are diminished in their ability to foment unchecked pro-inflammatory effector mechanisms [25,26].

5.2.6. JAK/STAT Signaling Platform Suppression

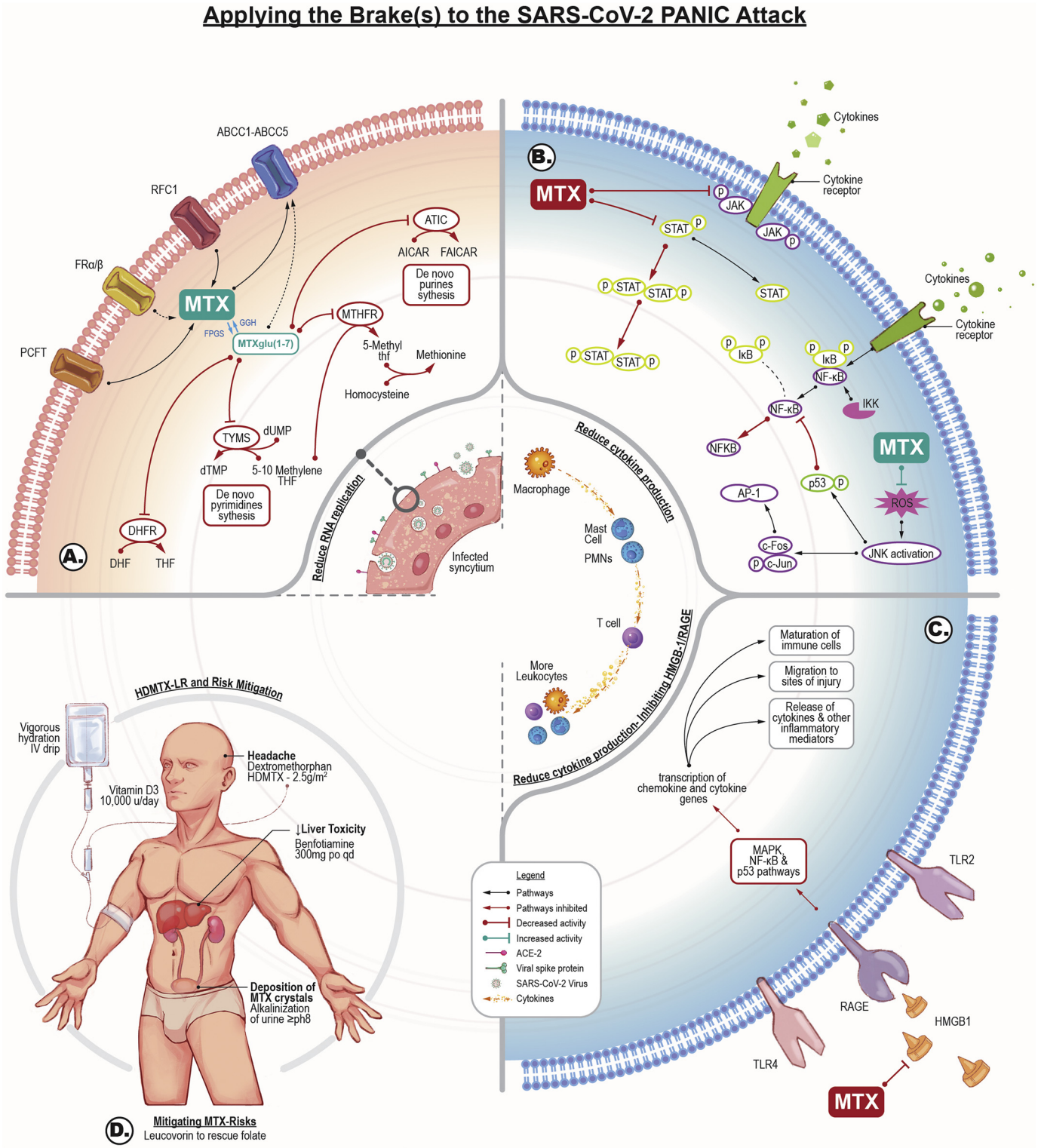
A significant pathway responsible for the transduction of multiple pro-inflammatory cytokines is the JAK/STAT signaling platform, which is potently suppressed by mechanisms independent of the folate inhibition effects of MTX, as such effects are not reversed by administration of folinic acid [Fig. 1B] [27].

5.2.7. Methotrexate Modulation of Reactive Oxygen Species

Methotrexate, through its modulation of reactive oxygen species (ROS), can induce T cell apoptosis, thereby reducing cytokine production and elaboration; with a prominent effect upon the reduction of IL-6 [28–31]. Further, MTX activates JNK, thereby activating downstream targets such as c-JUN and cFOS, constitutive elements of the AP-1 complex involved in the heightened expression of pro-apoptotic genes, which increase the propensity to apoptosis [Fig. 1].

5.2.8. Methotrexate Inhibits HMGB1 Binding to RAGE

The high-mobility group box chromosomal protein 1 (HMGB1) is elaborated by activation or injured immune cells, including monocytes, macrophages, and dendritic cells, and mediates inflammation [Fig. 1C]. One such example is called 'alarmin', which promotes acute inflammation followed by tissue repair [32–34]. The effects of HMGB1 is through its counterreceptor, a receptor for advanced glycan end products (RAGE), and is associated with cell maturation and migration to the site of injury, whereby chronic inflammation is perpetuated [35,36]. Methotrexate can inhibit HMGB1 by directly binding to RAGE or indirectly by inhibition of cytokine production of TNFα, IL-1, IL-6, IL-8.



(caption on next page)

5.2.9. Treatment Protocol for the Application of High-Dose MTX with Leucovorin Rescue

The proposed HDMTX-LR protocol has been the focus of prior investigation, the details of which have been published [Table and Fig. 1, Protocol, & Flow Diagram] [37]. Briefly, prior to therapy we ensure that the urine specific gravity is < 1.1010, urinalysis and urine culture are without evidence of infection, the serum creatinine (Scr) is below 1.4 mg/dl. Furthermore, complete blood count (CBC), and the comprehensive metabolic panel (CMP) to include liver functions and electrolytes, are assessed prior to administration of HDMTX-LR. For purposes of reducing the risk of MTX crystalline deposition in the kidneys, D5W and sodium bicarbonate 100 mEq are administered intravenously at a rate of 150 ml/m^2/h for at least 8 h prior to the administration of the HDMTX-LR protocol in order to achieve a urine pH of at least 8.0 before and during the entire HDMTX-LR infusion.

MTX at 2500 mg/m^2 in 250 ml of normal saline is administered

Fig. 1. Methotrexate has been recognized by the World Health Organization as an 'essential medication', principally due to its broad diversity of mechanisms of action. Methotrexate can be utilized at low doses to take advantage of its dynamic range of anti-inflammatory mechanisms (particularly beneficial in the management of rheumatoid arthritis), or at high doses to eradicate a range of malignancies, or in circumstances of fulminant inflammatory conditions where conventional approaches have been futile. In this figure we emphasize the pleiotropic and discretely targeted actions of high-dose methotrexate, as a hypothetical intervention for SARS-CoV-2 triggered PANIC Attack. The viral infection produces a severe variant of COVID-19 following the simultaneous activation of the diverse limbs of the human immune network (Fig. 1). As such, productive therapeutic strategies are likely combinations of different agents targetting distinctly different mechanisms of immune activation, or monotherapies endowed with a diversity of actions capable of counteracting the PANIC Attack triggered by SARS-CoV-2. In A) we showcase methotrexate as a potent inhibitor of the folate-dependent enzyme systems, especially those which are essential in rapidly dividing cells; such as those of the hyper-activated immune system, given that the cell cycle synthesis (S) phase requires nucleoside precursors for the synthesis of both DNA and RNA by the de novo pathways; and as such for RNA replication of the SARS-CoV-2 agent. Other body cells overcome this base synthesis blockade by utilizing the so-called salvage pathways, where partially assembled and partially broken down bases can be utilized to complete the synthesis; which are not available to rapidly dividing cells and microbes such as SARS-CoV-2. MTX is polyglutamated following entry into cells, a molecular modification which serves to prevent its efflux back out of the cell. Once inside the cell, this form of the molecule effectively inhibits dihydrofolate reductase among other folate dependent enzyme systems, which ultimately inhibits the de novo biosynthetic pathways for purines and pyrimidines (necessary for both DNA and RNA); and as such it reduces RNA replication of the virus. In B) we illustrate the inhibitory effects of MTX on multiple transmembrane signaling pathways involved in inflammation, including the JAK-STAT pathways that are activated by a host of inflammatory cytokines such as IL-2, IL-6, IL-12, IL-15, GM-CSF, and IFNγ. We also illustrate the role of the NFκB signaling pathway for a series of inflammatory mediators such as IL-1β, IL-6, TNFα, and for matrix matalloproteases. MTX inhibits the production of these inflammatory mediators, but also liberates reactive oxygen species (ROS) that then trigger JNK activation, which promotes p53 activity that inhibits NFκB (thereby blocking the effector functions of proinflammatory cytokines). Further JNK activation also activates the c-Fos and c-Fun pathways that culminate in pro-apoptotic gene expression and increased sensitivity to apoptosis. In C) we illustrate the ability of MTX to block HMGB1 binding to RAGE receptor, and thereby aborting the multiple downstream immune effector injury mechanisms that converge to destroy the lung alveoli in severe COVID-19. The attenuation of immune cell maturation, migration and transmigration across the alveoli epithelia (utilizing matrix metalloproteinases which digest fibronectin, collagen, and other matrix elements that constitute the integrity of tissue elements), and the release of cytokines and other inflammatory mediators by either active release mechanisms, or via their passive release secondary to the expulsion from damaged or dying cells are additional alveolar injury pathways that are blocked by MTX. In D) we emphasize a series of risk mitigation interventions that are crucial when using HDMTX-LR (all of which are further discussed in the text).

intravenously over 2 h. Precisely 12 h after the inception of the MTX infusion, we begin leucovorin (folinic acid) rescue with 80 mg given intravenously. Thereafter, leucovorin at 35 mg is administered intravenously precisely every 6 h for a total of 12 doses [Fig. 1 & Protocol]. Serum MTX concentration levels are assessed at 24 h, 36 h, 48 h, and 72 h after the administration of HDMTX-LR. Patient discharge cannot proceed until the MTX concentration falls below 0.05μmolar (typically requiring a period of 24–72 h).

Nausea and vomiting tend to occur during or shortly after the HDMTX-LR administration, hence anti-emetics (e.g. odansetron) are given 30 min before the infusion and as needed thereafter. Dextromethorphan (5-10 mg po prn every 2–4 h; or 20 mg with 10 mg of quinidine; Nuedexta daily) is utilized to treat infusion-related headaches. CBC, Scr, and CMP are assessed at baseline and then at weeks 1 and 4 following treatment with HDMTX-LR.

Penicillin derivatives, probenecid, fluoroquinolones, sulfonamides, aspirin, and non-steroidal anti-inflammatory drugs are known to decrease renal excretion of MTX and are therefore avoided during HDMTX-LR treatment. Vancomycin and trimethoprim/sulfamethoxazole are avoided immediately prior to, and during HDMTX-LR administration to avoid potential additive or synergistic nephrotoxicity, and possible myelosuppression [22].

A range of potential toxicities can be associated with the HDMTX-LR protocol, each of which, however, can be markedly mitigated, if not prevented or abolished, with careful attention to the precise proscriptions of our protocol, and the use of systematic and sequential stepwise transitions to each phase of the therapeutic plan [Protocol]. Most important is that the HDMTX-LR protocol can be safely employed, while reducing risk of toxicity by ensuring vigorous hydration (to avoid MTX crystalline deposition), urine alkalinization (crystal formation is more likely in acid pH of the genitourinary system), along with the precisely-timed administration of the leucovorin rescue (to prevent mucositis).

The addition of benfotiamine has been shown to reduce MTX mediated liver toxicity in those utilizing low dose weekly chronic dosing of the medication, and the addition of this agent to the high dose punctuated administration of MTX may also confer some tangible benefits, albeit a subject for further investigation. Further, adequate levels of vitamin D (to achieve 60-100 ng/ml) are known to exert immunomodulatory effects [38].

5.2.10. Management of Severe COVID-19:Practice Principles

The principal thrust of this paper is to emphasize that the SARS-CoV-2 induced severe variant of COVID-19 is not mediated by a single renegade cytokine or immunological phenomenon (e.g. the cytokine storm). Severe COVID-19 is principally recognized by the requirement for respiratory support, culminating in pressure ventilation, at which point the prognosis is poor, with most secumbing to the disease via the paroxysmal cessation of gas exchange, and the pathophysiologic signature of irreversible demise, treatment refractory hypoxic-ischemia. At this point, further interventions become an unnecessary and psychologically devastating exercise in futility for the patient, their family, and for the front-line care team.

Herein we have underscored the serious nature of COVID-19, the manifestations and clinical and paraclinical factors which portend this ominous, and commonly irreversible, semiology. Further, we have dissected the manifold mechanisms, triggered by SARS-CoV-2. Mild to moderate COVID-19 is largely defined by the nature, magnitude, location, and whether such immune network responses are mechanistically coherent, and effective, while at the same time being sufficiently 'focalized'. Focalized responses signify a coordinately-regulated network immune response, one most capable of limiting the severity of end-organ tissue damage.

The primary goal of this communication is to help our front line workers recognize that despite the 'prolific activation of a network-mediated inflammatory crisis', or PANIC Attack, we do in fact have compositional immunotherapies, that when combined, may serve to abort or at least mitigate, suffering while promoting an improved prognosis. Alternately, we herein hypothesize that a single agent endowed with a plethora of diverse and site selective targeting capabilities, each of which is sufficiently robust so as to uncouple the SARS-Co-V-2 induced PANIC Attack, may be used to lead the counter-attack. It is precisely for this reason that the WHO has long considered MTX as a 'necessary' medicine.

5.2.11. Future Directions 'Available Now'

Remdesivir, a newly-approved antiviral therapy for COVID-19, is an IV formulation that could easily be given simultaneously with HDMTX-LR by the following rationale: Remdesivir is a nucleotide analog with broad-spectrum antiviral activity, formulated with a large amount (60:1 weight ratio excess) of the excipient sulfobutylether beta-cyclodextrin (SBECD, Captisol, Ligand Pharmaceuticals, Inc).

Captisol has been shown to prevent the nephrotoxicity of iodinated contrast [39]. Its nephroprotective effect includes the inhibition of apoptosis, in renal tubular cells, induced by numerous nephrotoxins. In addition, various cyclodextrins have been shown to reduce inflammation in other models [40–42]. It is conceivable that Captisol may act not only as a solubilizer for the antiviral agent, but may itself contribute to the efficacy of remdesivir in the treatment of COVID-19. Thus, remdesivir given with HDMTX-LR may provide synergism in the treatment of PANIC in COVID-19, while at the same time providing nephroprotection for methotrexate.

6. CONCLUSIONS: TIME IS TISSUE

About 20% of COVID-19 patients will ultimately advance to severe disease, with assimilation of the corresponding risk escalation of both morbidity and mortality. Most COVID-19 patients shall cycle through a number of clinical dispositions, each of which may be remediable, and potentially even mitigated with respect to 'left-shifting' the patient and their course, further away from those thresholds beyond which survival becomes increasingly more remote. To illustrate, we have prepared two scenarios as 'call out boxes' which contain an abbreviated vignette, followed by our specific plans of intervention [Box 1 and 2]. The goals for each scenario are to mitigate suffering, promote reconstitution of network immune coordinate regulation sufficient to prevent PANIC, but also to recognize and urgently treat PANIC when the features that define it are recognized by our front-line workers.

As a synopsis, the mildly affected COVID-19 patient is offered site-selected delivery of inhalational steroids followed by L-albuterol (R form of this agent, making it relatively lung selective). This promotes bronchial smooth muscle relaxation, as well as activates the beta-2 adrenergic receptor on immune cells, thereby rendering them diminished in their inflammatory activity via activation and escalation of intracellular cyclic AMP (essentially biasing immune cells from a TH1 to TH2 anti-inflammatory phenotype). Further, low-dose weekly MTX (7.5 to 25 mg taken po weekly along with 2 mg of folate daily) could be utilized in those patients with mild to moderate COVID-19, but who persist or exhibit what appears to represent a more protracted and slowly advancing clinical deterioration.

When compared to our HDMTX-LR intervention, low dose weekly oral MTX is not as prolific, nor as rapidly acting to exact remission. It

Box 1

Vignette Box 1	
Complaint	Intervention
Fever Infection	Azithromycin
Multiple Sclerosis Disease Modifying Therapy Change	1. The patient was lymphopenic on dimethyl fumarate (a recognized association) and with suspected COVID-19. The significant lymphopenia places this patient at risk for PML, although the lymphopenia can also be secondary to COVID-19. 2. A decision was made to discontinue the dimethyl fumarate, and to instead begin treatment with interferon beta 1a, taken SQ every two weeks (Plegridy). Further, given that the patient exhibited breakthrough on interferon in the past, it was also decided to treat concomitantly with teriflunomide; starting at 7 mg po daily for two weeks; then escalating to 14 mg daily thereafter. 3. Type I interferons exert anti-viral actions, and teriflunomide is an inhibitor of pyrimidine base synthesis, via the inhibition of the de novo biosynthetic enzyme, dihydroorotate dehydrogenase. Rapidly dividing cells and microbes such as the SARS-CoV-2 agent must utilize the de novo pathway for DNA and RNA base synthesis, whereas other body cells can use either de novo or salvage pathways (using partially assembled or partially broaken down bases for DNA and RNA synthesis).
Shortness of breath	1. Assess oxygen saturation with simple home finger pulse oximetry. Given parameters, patients can provide their managing physicians with crucial information as to whether the COVID-19 clinical course is deteriorating, and hence the need for hospitalization. 2. Supplemental Oxygen via nasal canula
Shortness of Breath (Chest Tightness)	1. Postural Percussion performed by husband to facilitate pulmonary hygiene (formerly pulmonary toilet to mobilize and promote mucus clearance) 2. Incentive Spirometry for deep breathing and exercise of respiratory muscles
Cough:Non-Productive	Dextromethorphan Dextromethorphan (DM) + Quinidine; Nuedexta; (the latter reduces the metabolism of DM). Quinidine is a derivative of quinine, from the Peruvian Cinchona tree, as are chloroquine and hydroxychloroquine; all of which have been observed to reduce viral replication and cytokine release in vitro.
Chest Tightness: A) Liquify mucus B) Mucus mobilization C) Bronchopulmonary inflammation D) Deep terminal airway inflammation	A) Guaifenesin bid for mucus liquification B) Consider Warm Steam Vaporizer C) Pulmicort Inhalational steroid D) Site-selective delivery of anti-inflammatory action into the terminal bronchopulmonary tree
Reactive Airway 1. Bronchial constriction 2. Beta-2 adrenergic receptor mediated immune skewing	1. Levalbuterol; also known as L-albuterol (Xopenex) and is the active form of the drug called R-albuterol. Fewer side effects than with racemic albuterol (which is a 50:50 mixture of R and S forms). Pharmacologically acts via the beta-2 adrenergic receptor on bronchial smooth muscle thereby relaxing the distal extent of the bronchopulmonary tree. Can reduce the 'tight lung' perception, and via bronchodilation can facilitate mobilization of mucus and avoid inspisated mucus organization. Two puffs TID. 2. Beta-2 adrenergic receptors are also expressed on circulating mononuclear cells. R albuterol binds to the beta-2 receptor and promotes conversion of cells of the TH1 proinflammatory phenotype to TH2 anti-inflammatory state [68–71]. Two puffs TID.
Elevated Blood Pressure	Blood pressure monitoring and correlate with headache.
1. Bioenergetics 2. Reduce MTX tissue injury	1. Benfotiamine 300 mg daily: Improved bioenergetics 2. reduced tissue damage from MTX and other inciting agents [94].
1. Vitamin D: 10, 000 units of D3 daily for promoting immune modulation/Tregs	Vitamin D may play an immunoregulatory role in COVID-19 patients [95].
Low Dose Weekly MTX (if needed) Daily Folic Acid	7.5-25 mg po q weekly 2 mg of Daily Folate

Box 2

Vignette Box 2

Prevention, Mitigation, or Abolishment of the SARS-CoV-2 Triggered PANIC

A 55-year-old University School of Medicine hospital physician intensivist, and unit Director, has been carefully and systematically managing mild, moderate, and severely affected COVID-19 patients since March 15, 2020. During this time he has consistently utilized PPE consisting of gloves, disposable gown, N95 masks with facial shields, and head and shoe covers. On April 22, his unit is visited by a senior member of the COVID-19 Federal Safety Task Force, refuses to wear a mask of any type, so that he is certain to 'express his abundant respect' by not covering his face while he offers his congratulations to the unit Director, as the visit was orchestrated to recognize the heroic efforts of this senior faculty member, in the management of the most severe cases of COVID-19 in the region. The conversation between the two men is at a distance of about 3–4 ft from each other. In a lapse in judgment the unit Director removes his mask in order to exhibit his respect for the senior task force representative.

Within 24 h, the unit Director has developed a fever of 101.4 F, a dry, non-productive cough, chest discomfort with inspiration, profound fatigue and malaise, increased work of breathing, myalgias and exercise intolerance. Laboratory assessment reveals elevated D-Dimer, a CRP of 3.75, CK of 1500, lymphopenia (absolute number = 400), ferritin of 700, and an O_2 saturation of 88%. Chest CT reveals multilobar ground glass lesions consistent with COVID-19; while the RT-PCR for SARS-CoV-2 is negative. There is a history of hypertension, currently being treated with Losartin.

Complaint	Intervention
Fever Infection	Azithromycin
Shortness of breath	1. Assess oxygen saturation (80%) 2. Supplemental Oxygen 4 L via nasal canula
Shortness of Breath (Chest Tightness)	3. Postural Percussion to facilitate pulmonary hygiene (formerly pulmonary toilet to mobilize and promote mucus clearance) 4. Incentive Spirometry for deep breathing and exercise of respiratory muscles
Cough:Non-Productive	Dextromethorphan Dextromethorphan (DM) + Quinidine; Nuedexta; (the latter reduces the metabolism of DM). Quinidine is a derivative of quinine, from the Peruvian Cinchona tree, as are chloroquine and hydroxychloroquine; all of which have been observed to reduce viral replication and cytokine release in vitro.
Chest Tightness: E) Liquify mucus F) Mucus mobilization G) Bronchopulmonary inflammation H) Deep terminal airway inflammation	E) Guaifenesin bid for mucus liquification F) Consider Warm Steam Vaporizer G) Pulmicort Inhalational steroid H) Site-selective delivery of anti-inflammatory action into the terminal bronchopulmonary tree
Reactive Airway 3. Bronchial constriction 4. Beta-2 adrenergic receptor mediated immune skewing	3. Levalbuterol; also known as L-albuterol (Xopenex) and is the active form of the drug called R-albuterol. Fewer side effects than with racemic albuterol (which is a 50:50 mixture of R and S form). Pharmacologically acts via the beta-2 adrenergic receptor on bronchial smooth muscle thereby relaxing the distal extent of the bronchopulmonary tree. Can reduce the 'tight lung' perception, and via bronchodilation can facilitate mobilization of mucus and avoid inspisated mucus organization. Two puffs TID. 4. Beta-2 adrenergic receptors are also expressed on circulating mononuclear cells. R albuterol binds to the beta-2 receptor and promotes conversion of cells of the TH1 proinflammatory phenotype to TH2 anti-inflammatory state [Ref; Weiner Harvard]. Two puffs TID.
Elevated Blood Pressure	Blood pressure monitoring and correlate with headache.
3. Bioenergetics 4. Reduce MTX tissue injury 2. Vitamin D: 10, 000 units of D3 daily for promoting immune modulation/Tregs	3. Benfotiamine 300 mg daily: Improved bioenergetics 4. reduced tissue damage from MTX and other inciting agents
Prepare for HDMTX-LR Protocol (Consult the Flow Diagram for detailed instructions)	

can, nevertheless, exert a constellation of attenuating influences in response to the SARS-CoV-2 triggering of the broad expanse of the host immune networks.

Pulmonary toilet (now referred to as pulmonary hygiene) can loosen and expectorate mucus in the distal bronchopulmonary tree, which is facilitated by the performance of postural percussion of the back and sides of the thoracic cage. For chronic cough, dextromethorphan + quinidine may be prescribed, the latter reducing the metabolism of dextromethorphan while also providing a derivative of quinine from the cinchona tree. Quinine, like chloroquine and hydroxychloroquine, has demonstrated interrupted viral synthesis in vitro, as well as the release of cytokines. Vitamin D3 taken daily, and at a dose commensurate to achiving a blood level of 60-100 ng/ml, can promote immune regulatory networks, and improve energy (reduce fatigue) [43].

Ultimately, rather than targeting individual cytokines and their receptors, the HDMTX-LR protocol provides a broader spectrum of protection against the cytokine storm described herein, as well as the other mechanisms that collectively we now define as a PANIC Attack. Whether triggered by immune network dysregulation in autoimmune disorders, or secondary to a foreign microbe capable of triggering PANIC, the resultant process occurs in a significant proportion of infected individuals. The pathobiological underpinnings which dichotomize those destined to experience mild vs severe COVID-19, shall, and already is, the subject of vigorous debate, and extensive investigations by the most gifted and talented physicians, scientists, and physician-scientistis from around the globe. We truly are in this together, and by working together, we have our best prospects to conquer this pandemic, while also preparing for the next 'PANIC' Attack(s).

Author contributions

Elliot Frohman: conception, critical revision of manuscript for intellectual content.

Nicole Villemarette-Pittman: critical revision of the manuscript for intellectual content.

Roberto Alejandro Cruz: critical revision of manuscript for intellectual content.

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Elizabeth S. Rowe: Conception critical revision for intellectual content.

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Elizabeth Rowe: Nothing to disclose.

Vernon Rowe: has a financial interest in Captisol-Enabled Iohexol being developed by Ligand Pharmaceuticals, Inc.

Thomas Varkey: Nothing to disclose.

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Appendix A. Supplementary data

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