

Doxycycline and Pentoxifylline for Mild and Mild-To-Moderate Covid-19

-Proposal for a clinical trial-

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ABSTRACT

We are proposing, for any interested investigator, a randomized open clinical trial for mild and mild-to-moderate Covid-19 comparing a treatment regimen with standard of care, and eventually with another investigational regimen, if ongoing or will be implemented. The patients could be hospitalized or ambulatory but not requiring admission to intensive care units and mechanical ventilation. The proposed therapeutic regimen consists of doxycycline (an antimicrobial having also anti-inflammatory properties, probable antioxidant and possible some antiviral effects), and pentoxifylline, a hemorheological compound used in occlusive arterial diseases but also having proven anti-inflammatory properties. Doxycycline is included in the WHO's list of essential medicine being an effective, safe, widely available, and inexpensive medication and widely accessible. It will be administered at a dosage of 100 mg orally twice daily for ten days. Pentoxifylline, in clinical use since 1972, and also widely accessible, will be given at a dosage of 400 mg orally, also twice daily, for ten days.

The primary outcome measures are: 1) Progression of disease to a severe form requiring intensive care admission and mechanical ventilation; 2) Fatality rates and 3) Time to clinical recovery.

This proposal was presented to the National Directors and Core Leads meeting of the Canadian Trials Network (CTN) on May 6, 2020. The interventional trial template suggested by CTN was used for designing the trial.

Key words: Covid-19, SARS-CoV-2, doxycycline, pentoxifylline, treatment, proposal

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1. INTRODUCTION AND BACKGROUND

The pandemic due to SARS CoV-2 infection is clearly recognized as an unprecedented global health crisis. Globally speaking, 188 countries and regions are affected. To date (June 21, 2020), it is estimated that 8 820 125 persons have been infected, and 464 952 deaths worldwide have been recorded. For US 2 255 801 individuals infected and 119 728 deaths and for Brazil 1 032 913 people infected and 49 976 deceased are listed on [Johns Hopkins CSSE dashboard](#) .

The clinical spectrum of the disease caused by SARS CoV-2 virus, Covid-19, varies from asymptomatic to mild, moderate clinical forms, ambulatory or those needing hospitalisation. Moreover, in about 15% of cases, severe forms of Covid-19 requiring admission to intensive care units due to the development of Acute Respiratory Distress Syndrome (ARDS) are reported. Approximately 5% of these patients require invasive mechanical ventilation or extracorporeal membrane oxygenation (ECMO). These percentages vary by age and comorbidities. Collectively, we are confronted with an infection that overwhelmed the health care resources in many countries.

As it was the case with Acquired Immuno-Deficiency Syndrome (AIDS) in late '80s, multiple treatments or approaches have been proposed to treat Covid-19. Up to this point, treatments have been employed without evidence of their efficacy and/or regard for patient safety and cost. In many cases, they are based on *in-vitro* or/and in non-human animals data or acquired from experience gained during previous epidemics with SARS-CoV or MERS-CoV.

Anecdotal cases and small-series reports prompted implementation of several small trials, often non-randomized. Some of the trials have already been completed with contradictory results. A total of 2816 trials have been recorded up to May 27, 2020 (doi:10.6084/m9.figshare.11961063.v1). Numerous trials are underway, including large accessible adaptive trials with a simple design and efficient data collection, such as SOLIDARITY launched by WHO and its counterpart, RECOVERY from United Kingdom, and DISCOVERY from Europe. Canada is part of the 10 country consortium for the SOLIDARITY trial and REMP-CAP (Canada, USA).

For the vast majority of these proposals the scientific basis is not entirely clear or is poorly justified, but some trials have shown encouraging results. However, given the urgency of the present situation, accelerated trial approvals and simplified designs and data collection have been proposed to accelerate progress. Protocols that provide data that is rapid and reliable now take on a greater urgency.

We are proposing a randomized open clinical trial to examine the therapeutic effects of doxycycline and pentoxifylline on patients with mild and mild-to- moderate clinical forms of COVID-19, requiring hospitalization or not, but not requiring admission to intensive care units. *The primary outcome measures are: 1) Progression of disease to a severe form requiring intensive care admission and mechanical ventilation; 2) Fatality rates and 3) Time to clinical recovery.*

This approach is based on the current knowledge of the pathogenesis of this disease, as well as on previous research and clinical and pharmacologic experience. There is also world-wide availability of both compounds.

This proposal aligns with World Health Organisation's priorities, as well as other's countries, to improve the speed for finding an efficacious treatment for COVID-19 that is safe, inexpensive and largely available and accessible.

1.1. Rationale and brief critical review

The Virus - Its Genome & Therapeutic Targets

SARS CoV-2 is an encapsulated ssRNA virus belonging to the beta-coronavirus family which also includes SARS CoV and MERS CoV [1]. Its genome was rapidly sequenced and made public, enabling collaborative studies, diagnosis testing, search for therapeutic targets, and vaccine development (<https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/>).

A recent phylogenetic network analysis of 160 complete SARS-CoV-2 genomes identified 3 central variants named A, B, C, distinguished by some amino acid changes. The A variant is the ancestral type [2].

The SARS CoV-2 genome of 30 Kpb contains 14 open reading frames (ORFs). At the 5' terminus, occupying two thirds of the entire genome, there is ORF 1a/ORF1ab which encodes for a poly-protein complex-replicase [3] containing polyprotein1a and polyprotein1ab. These proteins are autoproteolytic with resulting 16 nonstructural proteins (Nsp 1-16) forming the replicase/transcriptase complex. Each of them is or might be a potential therapeutic target as follows [3, 4, 5, 6]:

-Nsp-3 is a papain-like protease (PL-pro). Several products have been proposed, based especially on computer analysis and *in silico* docking. Among them, are chloroquine, formoterol, ribavirin and also doxycycline (included in our proposal) [4, 5].

-Nsp-5, the main SARS CoV-2 protease, is a 3 chemotripsin-like protease (3CL-pro). Its high resolution crystal structure has already been determined (protein Data Bank Identifier-6LU7). Therefore, many compounds are being presently studied as candidate inhibitors by computer virtual screening, molecular docking technology, conserved domain analyses, homology modeling, machine-learning algorithms, bioinformatics, etc. [7, 8]. This protease is different than HIV aspartyl protease, explaining the insignificant binding and only small clinical benefits with HIV protease inhibitors, such as lopinavir/ritonavir. The reported beneficial effects of lopinavir/ritonavir are due partly to other mechanisms than protease inhibition, such as heightening of endogenous retinoic acid [9, 10] as well as the possible proteasome/ubiquitin inhibition and consequent reduction of NFk- B activation.

For the SARS-Co-V, the 3CLpro, which is slightly different than that of SARS-CoV-2, cinanserin, a serotonin receptor antagonist, was shown to strongly reduce virus replication *in vitro* [11]. However, the development of this product was halted given its potential to induce malignant hepatoma in rats, although, no significant adverse events have been observed in humans [11].

Virtual screening of approved drugs for other indications and through a drug-target interaction deep learning, it was suggested that other existing approved drugs, including anti HCV drugs, velpatasvir, and ledipasvir are good candidates for COVID-19 [12,13,14].

-Nsp- 12 is a RNA-dependent RNA polymerase (Rd/Rp) and is the target of the nucleosid Remdesivir®, a promising drug in case reports and in an open compassionate clinical evaluation. However, a randomized, double- blind, placebo-controlled, multicentre trial showed no benefit [15]. In contrast, a preliminary report (just published) of the Adaptive Covid-19 treatment trial (ACTT-1) Remdesivir was found superior to placebo in shortening the time to recovery in hospitalized adults with Covid-19 [16]. Favipiravir, used with some clinical benefits in Ebola virus disease and approved for treating influenza was evaluated in China, and also in Italy to treat COVID-19 but until recently evidence was sparse. Preliminary results in an open trial on 80 patients (including both the experimental and the control groups) in China, suggested that it was more potent than lopinavir/ritonavir [17]. Some hepatitis C antiviral drugs.such as velpatasvir and ledipasvir seem to be potential inhibitors also of Rd/Rp [13].

-Nsp-13 is a helicase nucleoside triphosphatase(NTPase) complex interacting with interferon signalling pathway, being an inductor of INF-regulatory factor (IRF) –dependant transcription. It seems to be also implicated in the modulation of the NF-kB inflammatory response. It might be one of the targets for 5-hydroxychloroquine [18, 19].

-Nsp-14 is an exoribonuclease interacting with purine biosynthesis pathway (affecting inosine 5' monophosphate dehydrogenase -IMPDH2- and might be inhibited by ribavirin [13]. Another drug with the same mechanism of action- Merimepodib (Vicromax™) developed as an HCV drug but failed in clinical trials- was shown to inhibit SARS-CoV-2. Clinical trials will start soon. The proof reading activity of Nsp-14 might be affected by a nucleoside analog-beta-S-D-N4-hydroxycytidine (NHC, EIDD-1931) through the induction of error catastrophe in the targeted virus [20].

-Nsp-15 is an endonuclease that may be a target of E3 ubiquitin ligase promoting/ regulating type 1 interferons. [21, 22].

The 3' terminus of the viral genome contains 13 ORF encoding for the 4 structural proteins:

- Spike glycoprotein (S) is the main protein necessary for the host receptor attachment and viral cell membrane fusion.
- Envelope (E) a small protein having a viral-human protein interaction [3,22].
- Membrane or matrix protein (M) responsible for transmembrane transport of nutrients and virion budding
- Nucleocapsid protein (N) interacts with host RNA.
- Accessory proteins and E interfere with host immune response [3, 21, 22].

-ORF 6 antagonizes INF signalling and ORF 10 interferes with the ubiquitination complex [21, 22].

Pathogenesis and other possible treatment targets

Rapid progress has been achieved in deciphering the SARS-coV-2 pathogenesis. At some stages of its life-cycle several putative effective drugs have been proposed or seem reasonable such as:

- a) *The attachment of the virus to its receptors through the spike protein*

The spike – S- protein of SARS CoV-2 has 2 subunits:

- S1 which interacts with the host protein angiotensin converting enzyme 2 (ACE2). This virus receptor binding might be affected by chloroquine, in addition to ACE 2 inhibitors, thus explaining in part some of chloroquine’s observed clinical effects [21].
- S2 is cleaved and activated by the host proteins transmembrane serine 2 (TRMSS2) and furin and serves for virus-host membrane fusion by its HR1 (heptade repeat) [23, 24].

This enables SARS CoV-2 to invade respiratory mucosa and other types of epithelial and endothelial cells. Two TRMSS2 inhibitors, Nafamostat® and Camostat® showed clinical effects in Mers-CoV infection and might be good candidates for treating SARS-CoV-2 [20, 23, 24].

- b) *Viral entry by endocytosis* mediated by the formation of endosome (when the virion surrounded by the cell membrane is internalized). Endosomal acidification is needed for the virion to become infectious. Chloroquine and hydroxy- chloroquine, which have a basic pH will prevent the acidification and consequently they might eventually prevent the endocytosis [25].
- c) *After uncoating, the RNA genome is released* and host machinery will translate the viral proteins including PLpro and 3Clpro, then transcribed by Rd/Rp. The transcription of the viral RNA negative strand will be followed by the translation of structural proteins.
- d) *Virion packaging and liberation by exocytosis.*

The compounds that might act at these stages have been described above (i.e. effects on PLpro, 3Clpro). The exit of the new virion might be inhibited by INF-beta and trials with it or in combination with other drugs are underway.

The new virion will be recognized by the antigen presenting cells (dendritic, NK, T-cells.) That will prompt the immune response. The activation of transcription factors NK-kB and IRF3 is followed by the release of type I interferons (alpha and beta) and the pro inflammatory cytokines such as TNF- α , IL-1, IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-17, macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemotactic protein 1 (MCP-1), MIP-1 [21]. Moreover, the neutrophil extracellular traps are also implicated in the immune response to SARS-CoV-2 [26].

The host immune response is vital for the control and resolution of infection. However, in some patients with advanced age or/with comorbidities, such as diabetes, hypertension, obesity, respiratory disorders, a dysregulated immune response might occur leading to a cytokines release syndrome also called “cytokine storm”, ARDS, multiple organ failures and eventually death[21,24].

Several older drugs might prevent or diminish this hyper-inflammatory response. Among them, both **doxycycline and pentoxifylline**, the combination of drugs we propose, might have a preventive effect (see their description below in the proposal; points 1.3 and 1.4).

Other drugs might diminish the levels of main proinflammatory cytokines such as IL-6 or its mRNA. Furthermore, in severe cases monoclonal antibodies to IL-6 receptors membrane bound

and soluble (tacilizumab, sirolizumab) or to Il-6 itself (siltuximab), proved, in a few cases, to be useful and therefore large clinical randomized controlled trails are underway [24]. However, their high cost and the potential for severe drug-associated events, especially autoimmune in nature, are huge barriers to their large utilisation.

1.2. Clinical presentation (briefly)

As shown above, the vast majority of infected persons are asymptomatic but the exact proportion of them is unknown. Among symptomatic patients the vast majority- approximately 80%- might have a mild and moderate clinical form. In mild cases an influenza-like illness manifested by various degree of fever ($< 38.6^{\circ}\text{C}$), fatigue, headache, sneezing, rhinorrhea, sore throat, cough, myalgias, gastro intestinal complaints, including diarrhea is usually encountered [27,28,29,30]. Hypo- or anosmia and loss of taste were reported rarely but a recent work showed that these symptoms might occur in 71% and 68% of patients, respectively [31]. However, these sensory impairments resolve after 2- 4 weeks, suggesting that they have been caused by the viral invasion of olfactory epithelium rather than of olfactory neurons [31]. The chest X-ray or the thorax CT-Scan or lung echography is usually within the normal limits.

In the moderate clinical forms, the fever could be higher than 39°C and the patient may have a more frequent cough, increased myalgias, fatigue, and dyspnea. The lung imagining could reveal, discrete alveolo-interstitial infiltrates, even as ground glass opacities, unilateral pneumonia affecting, in general, less than 50% of the lung fields [29, 30, 32].

Some patients need supplemental oxygen when the oxygen saturation is less than 96% but no less than 93% with 1-2 L O_2/min via nasal cannula with the FI O_2 of 22-30% [27, 28, 29, 30]. Most of these patients have underlying co-morbidities, as risk factors like obesity, diabetes, cardiovascular or pulmonary diseases, or immunosuppression due to a disease or its treatment [33, 34].

In severe cases, representing roughly 15% of symptomatic patients, the fever could reach 40°C , the oxygen saturation is less than 93% and the need for supplemental oxygen is constantly increased. Lung imagining show diffuse ground glass opacities, with or without bilateral or unilateral consolidation or pleural effusions and a microembolic process seems to be involved [33, 34, 35]. Clinical manifestations include respiratory distress, extreme fatigue, signs of myocarditis, elevation of liver enzymes, troponins and d-dimers. A critical illness may rapidly evolve in 5% of cases with significant respiratory distress, hemodynamic instability, development of an ARDS, altered mental status, necessitating admission to intensive care units, mechanical ventilation or even ECMO. Multiple organ failures may develop including acute kidney injury, CNS, cardiovascular and thrombo-embolic or haemorrhagic complications [36, 37, 38, 39, 40, 41, 42, 43]. All these severe complications will increase the fatality rate. ***In our proposed clinical trial, only patients with mild or mild-to-moderate clinical presentation will be included.***

1.3. Doxycycline

Doxycycline has a long history as a large spectrum useful antimicrobial agent being in clinical use since 1967. Its pharmacokinetics, pharmacodynamics, drug-drug-interactions and adverse effects are well-known. It is included in the WHO's list of essential medicine being an effective, safe, widely available, and inexpensive medication and, therefore, widely accessible.

In addition to its well-known antimicrobial effects (including bacteria, Chlamydia, Q fever, Rickettsia, Ehrlichia, Borrelia, Burkholderia, Mycoplasma, Anaplasma, malaria, filariasis, etc),

doxycycline, as well as other tetracyclines, has several proven immune modulating and anti-inflammatory activities [44,45]. Therefore, it is not surprising that the therapeutic potential of these compounds have been studied and even exploited in different clinical conditions, such as auto immune and chronic inflammatory illnesses, cancer or neurodegenerative conditions (e.g. relapsing-remitting multiple sclerosis) [45,46,47,48]. Antiviral and antioxidant properties have been also reported for doxycycline [49,50].

Some chemically-modified tetracyclines (lacking the antimicrobial activities) and doxycycline are already approved by Food and Drug Administration (FDA) for the treatment of rosacea (Aprilon™, Oracea™) [51], other chronic inflammatory skin diseases, periodontitis (Atridox™, Periostat™). Moreover, several compounds, including doxycycline and minocycline, are currently evaluated as adjuvant therapy for cancer and neurodegenerative disorders.

Several biochemical mechanisms are considered responsible for the above beneficial effects and are noted below:

a) *Inhibition of NF- κ B activation and its nuclear translocation.*

The NF- κ B resides in an inactive form in the cytoplasm, being attached to its inhibitor (I κ B). The activation of NF- κ B, induced by IL-6, is inhibited by tetracyclines (including doxycycline) by decreasing phosphorylation of its inhibitor, I κ B [47]. When the I κ B is phosphorylated, it is “detached” from NF- κ B and then degraded by the ubiquitin-proteasome system. Consequently, NF- κ B is no longer inhibited and it is translocated to the cell nucleus leading to increased transcription of genes responsible for TNF- α , IL-6 and other cytokines expression. Therefore, inhibition of its activation and nuclear translocation will decrease the levels of TNF- α , IL-6 and other cytokine implicated in the pathogenesis of multiple and diverse conditions including COVID-19 [75].

b) *Inhibition of matrix metalloproteinases*

The matrix metalloproteinases (MMP) are a group of 28 proteins with multiple and very different roles in the pathogenesis of chronic inflammatory conditions, cancers and infections [52, 53]. Their expression by macrophages is induced by IL-6 [52, 53]. On the other hand, disintegrin and metalloproteinase (ADAM) governs the shedding of endogenous interleukin-6-receptor, by a proteolytic release of the ectodomain, and consequently leads to increased IL-6 soluble receptors availability [54]. This will increase the IL-6 *trans* signalling via soluble receptors. The classical signalling, via membrane bound IL-6 receptors has anti-inflammatory effects involved in fever induction and acute phase response [53, 54].

The ability of tetracyclines to inhibit MMPs such as collagenases by multiple, direct and indirect, mechanisms has been documented for more than 25 years. [55].

Doxycycline and other tetracyclines are significant inhibitors, especially for MMP-2 and MMP-9 (whose expression is induced by IL-6 in macrophages). At subantimicrobial concentration doxycycline was shown to reduce MMP-9 and MMP-2 serum levels as well as of the high sensitivity C reactive protein (CRP) [56, 57].

In spite of earlier optimistic effects reported in the treatment of osteoarthritis [58, 59], a Cochrane review found that the benefits are minimal [60].

c) *Possible inhibition of SARS CoV-2 proteases.*

The recently solved crystal structure of the main SARS CoV-2 protease (3Cl-protease) and of papaine-like protease (PLpro) prompted computational screening of millions compounds from huge libraries for inhibitory effects [5, 11, 61]. Computer virtual screening analysis for therapeutic targets for SARS-CoV-2 suggested that doxycycline might have anti protease activity given its affinity binding to PL-pro (better than tigecycline, but less than ribavirin) and 3CL-pro [13]. In contrast, no significant binding to PLpro, 3CLpro and RdRp was noted for HIV protease inhibitors such as Lopinavir/ritonavir, whereas marginal binding was observed for amprenavir and nelfinavir [13]. Other virtual screening and molecular docking evaluations suggested that HIV protease inhibitors, especially saquinavir, amprenavir and lopinavir have good binding affinity for the SARSCoV and SARS CoV-2 main proteases [8].

Based on the current knowledge of the pathogenesis of SARS CoV-2 infection, development of COVID-19 and progression to severe forms, ARDS and eventual death, we would suggest that doxycycline is a reasonable adjuvant treatment alone or in combination for this condition.

Pharmacokinetics [62, 63].

Doxycycline is rapidly and almost completely absorbed (80-90%) following oral administration. Its absorption is not significantly affected by ingestion of food. The concomitant administration of doxycycline with dairy, antacids, calcium, magnesium supplements, iron products, and laxatives containing magnesium may decrease its oral absorption as a result of chelation with doxycycline, and consequently diminish its effectiveness. Peak plasma concentrations are attained in 1.5 to 4 hours. It is widely distributed into body tissues (including the lung) and fluids. Only small amounts diffuse into CSF, but it readily crosses the placenta and into breast milk. The average serum half-life is 18 hours after a single dose and 22–24 hours after multiple doses.

Serum concentrations and half-life may not be increased in patients with severe hepatic impairment or biliary obstruction. In patients with impaired renal function, neither increased serum half-life, nor accumulated serum levels have been observed. Hemodialysis does not affect serum half-life of doxycycline. It does not appear to be metabolized in the liver, but it is partially inactivated in the intestine by chelate formation. It is excreted into the gastro-intestinal tract via bile and 20%-40% of an oral or intravenous dose is excreted in feces and about 20–26% in urine.

Adverse effects

Doxycycline can cause gastrointestinal upset (nausea, loss of appetite, vomiting, diarrhoea) and might even cause “pill esophagitis”, particularly when it is swallowed without adequate fluid, or by persons with difficulty swallowing or impaired motility. Doxycycline is less likely than other antibiotics to cause *Clostridioides difficile* colitis.

An erythematous rash in sun-exposed parts of the body (photo sensitivity) has been reported to occur in 7.3–21.2% of persons taking doxycycline for malaria prophylaxis. This usually resolves upon discontinuation of the drug.

Despite crossing into breast milk, the actual risk of hypoplasia of dental enamel or dental staining of primary teeth is actually small. It is currently used in children for treatment of Q fever and also for tick-borne rickettsial diseases. A short course of treatment of up to 21 days could be given to children regardless of age. Other, much less frequent, adverse effects include pancreatitis,

increased intracranial pressure, DRESS (drug reaction with eosinophilia and systemic symptoms), Stevens-Johnson syndrome, erythrodermia, haemolytic anemia, thrombocytopenia.

Contraindications

It is contraindicated in patients allergic to doxycycline and other tetracyclines (minocycline, tygecycline) or tetracycline- chemically modified derivatives. It is also contraindicated for individuals taking *all tans* retinoic acid derivatives (may increase intracranial pressure).

1.4.Pentoxifylline

Is a methyl xanthine derivative in clinical use since 1972, as a hemorheological drug for the treatment of peripheral and cerebral vascular disorders, occlusive arterial diseases, vaso- occlusive crisis and hyperviscosity [64].It was approved by the FDA in 1984.

The hemo-rheological effect was attributed to the erythrocyte deformability, reduced plasma viscosity and decreased platelets reactivity aggregation [64]. Later on, it was documented that it is an effective inhibitor of circulating cachectin/tumor necrosis factor alpha (TNF- α), a key inflammatory mediator, known to modulate synthesis of other cytokines, and therefore an important pathogenic factor in a variety of illnesses.

Pentoxifylline for HIV infection

Since 1986, it has been suggested that TNF- α may play a pathogenic role in some clinical features characteristic of AIDS, such as cachexia, fever, premature ageing, HIV-associated encephalopathy, etc. In 1988, it was shown that a xanthate inhibited HIV replication *in vitro* [65]. Elevated levels of circulating of TNF- α have been reported in patients with AIDS [66] and the biological activity of TNF- α and its mRNA expression were shown to be suppressed by pentoxifylline [74].

It was not surprising, therefore, that some AIDS physicians, including myself, started to use pentoxifylline in addition to a reverse transcriptase inhibitor available at that time (AZT, ddI, ddC) in AIDS patients, early in 1989. Later on, it was reported that serum interleukin- 6 (IL-6) concentrations were elevated and associated with high TNF- α and immunoglobulin G and A concentrations in children with HIV infection [68].

When Fazely reported that pentoxifylline exerted several beneficial effects in HIV infection [69], I began an open pilot trial in AIDS patients in conjunction with a reverse transcriptase inhibitor. Those patients have had taken the drug for 1 – 63 weeks. In general, at the dosage used (400 mg twice daily), pentoxifylline was well tolerated with minor or moderate gastrointestinal complaints. We noted in some patients a decrease in beta-2 microglobulin plasma levels and in a minority of them a transient increase in CD4+ T-cell count, but not enough to be considered for routine clinical use [70].

In 1994 Neuner showed that *in vivo* pentoxifylline down regulates IL-1, IL-6, IL-9, and TNF- α by human peripheral blood mononuclear cells (PBMC) [68]. Moreover, inhibition of TNF- α lead to a reduction of other inflammatory mediators such as IL-1 beta, IL-6, IL-8, GM-CSF [71].This effect occurred at the transcriptional level [71].

Mandell GL, in 1995, reported that pentoxifylline decreases the TNF- α production and inhibits its activity on neutrophils that otherwise could contribute to the pathogenesis of sepsis, adult acute respiratory distress syndrome (ARDS) and AIDS [72].

Dezube et al. reported that pentoxifylline decreases LPS-induced TNF- α production and its mRNA levels in PBMC in AIDS patients [73]. The common toxicity with pentoxifylline (at a high dosage of 800 mg thrice a day) was gastro intestinal. It was also shown that pentoxifylline is an effective inhibitor of TNF- α response in septic shock [74]. Inhibition of TNF- α production was correlated with intracellular levels of cAMP. It affected the production of other cytokines such as IL-1, IL-6, IL-10, IL-12, IFN-gamma.

An improvement of cell-mediated immunity along with a reduction in HIV viremia was shown to occur in asymptomatic HIV-infected persons receiving pentoxifylline. [75].

Fortunately, the introduction of combination antiretroviral therapies, starting with the HIV protease inhibitors, led to a control of this infection and almost no need for other adjunctive therapies.

Pentoxifylline as an immunomodulator in other clinical settings

In addition to HIV infection and AIDS, TNF- α is considered a key inflammatory mediator and it is known to modulate synthesis of other cytokines. Consequently, it is a recognized pathogenic factor in a variety of illnesses such as bronchial asthma, pulmonary fibrosis, and acute respiratory syndromes. It was shown that pentoxifylline was able to down regulate proinflammatory cytokines and proliferating cells in lung interstitium, and having also a bronchodilatory effect. So, it was logical to recommend it for respiratory syndromes expecting a clinical improvement. It should be used at an early stage when the inflammation is active and the diffuse alveolar damage is not yet installed.

Pentoxifylline was also evaluated in cerebral malaria, ischemic cardiomyopathy and cardiac insufficiency, septic shock, diabetic nephropathy, painful diabetic neuropathy, graft vs. host disease [76, 77]. In cerebral malaria it was used both in adults and children but with contradictory results [78].

In cardiac insufficiency, TNF- α plasma level is elevated and correlates with the functional class. In addition, TNF- α has negative inotropic properties [79]. In a randomized study in ischemic cardiomyopathy it was documented that pentoxifylline improved clinical status and radionuclide ejection fraction [79]. It competitively inhibited phosphodiesterase (PDE), resulting in increased intracellular cyclic AMP (cAMP), activation of protein kinase A (PKA), inhibition of interleukin-1 (IL-1) and TNF- α synthesis. The reduced inflammation was accompanied by decreased plasma markers of inflammation such as C-reactive protein (CRP) and of N-Terminal prohormone of Brain Natriuretic Peptide (NT-pro BNP), a marker for the severity of heart diseases including congestive heart failure [79]. Pentoxifylline's beneficial anti-inflammatory and antioxidant effects have been elegantly reviewed by McCarthy [80]. There are case reports on its adjuvant use for the treatment of osteoradionecrosis and osteomyelitis [81]. Moreover, the combination of pentoxifylline with doxycycline was reported useful in a few patients with either Behçet disease or generalized granuloma annulare [82, 83].

Pentoxifylline and antiviral effects

It was already reported that pentoxifylline might have an antiviral effect on other viruses than HIV, and for that reason it was used in HTLV-1-associated paraparesis with minor beneficial effects [84]. Furthermore, it was also reported that it might have an inhibitory effect on viruses such as HSV, hepatitis A and B, rotaviruses, West Nile virus, tick-borne encephalitis, vaccinia virus [84].

However, it was also reported that pentoxifylline promotes the replication of human cytomegalovirus both *in vitro* and *in vivo* [85].

Finally, it was also suggested that pentoxifylline might have a potential antiviral effect on SARS-CoV but afterwards it was documented that pentoxifylline did not inhibit SARS-CoV replication both *in vitro* and *in vivo*, in BALB/c mice, despite being useful in improving the viral-induced hyperinflammatory response [86].

Although, no antiviral effect of pentoxifylline was reported to date on SARS-CoV-2, collectively, the above data on its immunomodulatory effects and its long history as a hemorheological drug for the treatment of peripheral and cerebral vascular disorders, occlusive arterial diseases, vaso-occlusive crisis and hyperviscosity, justifies its evaluation in conjunction with doxycycline for the treatment of mild and mild-to-moderate clinical forms of COVID-19, aiming to prevent the clinical deterioration in these symptomatic patients.

Pharmacokinetic data [64, 87, 88]

In addition to huge clinical experience with pentoxifylline, its pharmacokinetics is well known. Following oral administration, it is rapidly absorbed and a mean absolute bioavailability of 33% was documented in healthy adults. The peak plasma level is attained within 1 hour. Both mean peak plasma concentrations and mean area under the curve concentration/time (AUC) are increased by food. They AUC is also increased in patients with hepatic cirrhosis as well as in persons older than 60 years.

It is extensively metabolized in the red blood cells and the liver and it is eliminated in urine (95%) and feces (4%). The half-life is about 0.4-0.8 hours. The elimination rate is prolonged in patients with cirrhosis and persons older than 60 years. Both pentoxifylline and its metabolites are distributed into breast milk but it is not known whether it crosses the placenta.

Adverse effects

The most frequent adverse reactions are nausea and vomiting, headache, dizziness. Rarely hypotension especially at higher doses (not used in this study), angioedema, arrhythmia, bleeding, elevation of liver function tests might occur.

Contraindications

Pentoxifylline is contraindicated in patients with intolerance to pentoxifylline or to other xanthine derivatives (theophylline, theobromine, caffeine), recent cerebral and/or retinal haemorrhage, active bleeding, peptic ulcer, pregnant or lactating women. Patients taking anticoagulants and platelet aggregation inhibitors should also avoid its use. It should be cautiously used in patients with hepatic insufficiency.

Potential risks and benefits to human participants

Both drugs are well known and safely used in the clinic for a long time: doxycycline since 1967 (being on the WHO's list of essential medicines) and pentoxifylline since 1972. The potential risks are the possible adverse events (see the above points 1.3 and 1.4) and no improvement of the clinical condition and outcome. No benefits could be guaranteed at this time for this combination treatment of COVID-19 and thus underlies this proposed trial.

2. STUDY OBJECTIVES AND DESIGN

2.1 Overall study design

This is a proposed randomized open-study comparing a treatment regimen with standard of care and eventually with another investigational regimen, if in place or will be implemented at the interested centres. The proposed therapeutic regimen consists of doxycycline (an antimicrobial having also anti-inflammatory properties and possible some hypothetical antiviral effects), and pentoxifylline a hemorheological compound used in occlusive arterial diseases but also having proven anti-inflammatory properties.

The trial participants will be assigned on a 1:1 ratio by a computerized randomization. The primary outcome measures are the following:

- Number and % of patients progressing to a severe form necessitating intensive care admission and invasive mechanical ventilation;
- Case fatality rates;
- Time to clinical recovery

The study participants will be older than 18 years with symptomatic mild or mild-to-moderate clinical COVID-19 forms, needing or not (outpatients) hospitalization. A total of 150 participants per group should be enrolled to achieve a 95% power and a two-tailed type I alpha error rate of 0.05. Therefore, interested investigators would consider collaboration with other centres. The author of this proposal could be the facilitator. The study drugs are described at points 1.3 and 1.4, respectively. The study treatment will be administered for 10 days and the participants will be followed at 14, 21 and 28 days after the end treatment. The treatment could be interrupted in case of adverse reactions requiring discontinuation in the opinion of treating physician or progression of the disease necessitating admission to intensive care units. The study could be stopped at the recommendation of DSMC (to be nominated) for futility or no potential benefit.

2.2 primary objectives

- To evaluate the tolerability and safety of the proposed drug regimen
- To assess the efficacy in stopping or delaying disease progression, and eventual case fatality

Disease progression and need for intensive care admission are defined as follows:

- Increasing respiratory rate at ≥ 30 /min
- Decreasing resting-state oxygen saturation at $\leq 93\%$
- Persisting and increasing fever to ≥ 39.5 0C
- Increasing tachypnea
- Increased pulmonary infiltrates
- Increasing oxygen requirements of more than >5 L/min
- Needing ECG monitoring
- Onset of altered mental status
- Increase in abnormal lab tests including neutrophils, ALT, LD, troponins, d-dimers, lactate, NT proBNP
- Decrease of lymphocyte count to less than 0.3×10^9 /L (300/mL)

2.3 secondary objectives

- To assess the reduction of time to recovery
- To assess the reduction of in hospital-stay duration

The time to recovery will be calculated from the appearance of first symptoms to an asymptomatic state, no fever for 48 hours and a (or 2 at 48 h interval), negative PCR test for SARS-CoV-2.

2.4 exploratory objectives

- To evaluate a prognostic severity score
- To assess the effect on cytokines/chemokines serum levels
- To assess the changes in lymphocyte phenotyping

3. SELECTION AND ENROLLMENT OF PARTICIPANTS

3.1 Number of participants

We propose 150 participants per group: study treatment and standard of care.

If another study protocol will concurrently be conducted at the some centres, we propose to enroll also 150 participants in that group.

3.2 Inclusion criteria

- Persons older than 18 years of age
- Any sex/gender, race/ethnicity
- Covid-19 confirmed by RT-PCR for SARS-CoV-2
- Mild and mild-to-moderate clinical forms
- Needing or not to be hospitalized

3.3 Exclusion Criteria

- Participants with a known hypersensitivity/allergy to the doxycycline or other tetracycline derivatives (tygecycline, minocycline, etc.)
- Participants with known hypersensitivity/allergy to pentoxifylline or other xanthine derivatives (theophylline, theobromine, caffeine, etc.)
- Pregnant or breast-feeding women
- Individuals taking *all trans* retinoic acid derivatives (may increase intracranial pressure in conjunction with doxycycline).
- Participants who had a recent cerebral and /or retinal haemorrhage
- Active bleeding, peptic ulcer, recent surgery
- Patients with hepatic insufficiency
- Participants under anticoagulant therapy, platelets aggregation inhibitors or receiving other antibiotics (except Ceftriaxone)
- Participants who are actively participating in an experimental therapy study or who have received experimental therapy within the last month
- Participants who have an active uncontrolled infection in addition to COVID-19.
- Laboratory exclusion criteria:
 - Neutrophils lower than $10 \times 10^9/L$ (1000/mL)
 - Platelet count lower than $75 \times 10^9/L$ (75 000/mL)
 - Hemoglobin level lower than 90g/L

- Serum creatinine higher than 1.5 times the upper normal limit (UNL)
- Liver function tests (ALT,AST, Alkaline phosphatase, LD) values higher than 5 times the UNL

3.4 Strategies for recruitment

The trial protocol will be made available to all physicians, at each site, including those at the emergency rooms and those in the diagnostic clinics, who are implicated in the care of Covid-19 patients. They will be asked to inform the site research nurse or the site research assistant. The principal investigator should be available at any time if clarifications are needed.

4. WITHDRAWAL OF PARTICIPANTS

4.1. Withdrawal Criteria

A participant may be withdrawn from the study due to the reasons listed below including, but not limited to:

- A new health condition appears that is suspected to require care or medications prohibited by the protocol
- It is in the participant's best interest according to the Investigator's clinical judgment
- The participant decided to stop the medication and withdraw from the trial
- The DSMC's (to be nominated) recommendations to terminate the study for futility or no potential benefit. The withdrawn participants will not be replaced.

4.2. Procedures for discontinuation

A clinical, laboratory and radiologic evaluation is recommended, if possible, for all available participants who discontinued the trial at the time of discontinuation.

5. RANDOMIZATION AND BLINDING PROCEDURES

This is an open, randomized trial. No blinding is considered so far for this trial.

5.1. Randomization

The randomization will be performed as follows:

- 1:1 allocation by group of four: the proposed drug regimen vs. standard of care
- 1:1:1 allocation by group of 6: the proposed drug regimen vs. standard of care vs. another clinical protocol, if any, at a specific site

Permuted random blocks (of 4 or 6) will be generated by a computer-program and will be given to the pharmacist in charge. Otherwise, if possible, randomization will occur through *e-mail or (preferably) a web-site*. The site designated pharmacist will look for this information. For the outpatients, the research nurse will be in charge.

6. STUDY TREATMENTS

6.1. Investigational Product Description

Doxycycline tablets or oral solution: antimicrobial having also anti-inflammatory properties. This drug is in clinical use since 1972 and is contained on the WHO's list of essential medicines.

Pentoxifylline tablets: hemorheological drug with demonstrated anti-inflammatory effects. In clinical use since 1967

See the above points 1.3 (doxycycline) and 1.4 (pentoxifylline).

6.1.1. Expected Side Effects

- See the above points 1.3 (doxycycline) and 1.4 (pentoxifylline).
- Products monographs are listed in the References

6.1.2. Formulation, Storage and Handling

Both drugs to be used in this study are available as tablets. Doxycycline is also available as an oral solution. They should be kept at room temperature avoiding light exposure. The site pharmacy is in charge for the hospitalized patients. The outpatients will fill their prescription with their pharmacist who will provide to the patient the appropriate instructions and measures for storage.

6.2. Comparative Treatment

The comparator in this study is the standard of care including liquid repletion, supplemental oxygen, antipyretics or other symptomatic treatments as needed.

6.3. Study Product Supply and Accountability

For hospitalized patients the study medication should be supplied by the site pharmacy. The outpatients will receive a physician prescription to be filled at the participant's pharmacy.

6.4. Dosing and Administration

The trial medication will be administered twice daily orally:

- Doxycycline 100 mg twice a day for 10 days plus
- Pentoxifylline 400 mg twice a day for 10 days.

6.5. Concomitant Medications/Natural Remedies/Foods

Concomitant medications as standard of care is at the discretion of treating physician. However, it should not include natural remedies, corticosteroids, other antibiotics, except ceftriaxone, and amoxicillin/clavulanic acid if suspected community-acquired pneumonia. Concomitant medication will be recorded in the CRF.

6.6 Concomitant Alcohol, "Recreational drugs", and Vaping use

Alcohol, "recreational" drug use and vaping are prohibited during the study.

6.7 Prohibited Medications

Anticoagulant therapy, platelet aggregation inhibitors, *all trans* retinoic acid derivatives, any experimental drug are the exclusion criteria and are, therefore, prohibited during the study period.

6.8 Precautionary Medications and Procedures

Doxycycline should be taken with food and liquids before and after intake. Precautions are required if the participant has enteral or parenteral nutrition. Aluminum, bismuth, calcium and magnesium salts should not be given concomitantly (diminished effect of doxycycline due to complexes formation) but might be given, if necessary, 2 hours before or 2 hours following doxycycline administration.

6.9 Participant Access to Study Medication at Study Closure

There is no need to continue the study medication after the 10 days of therapy.

7. RISK MANAGEMENT

7.1. Acceptable Methods of Birth Control

For female participants of childbearing potential who are or who anticipate the possibility of becoming sexually active with a male partner during the study and for 2 weeks after study completion must practice a method of birth control considered acceptable by the trail physicians

Contraceptive measures should be reviewed with participants at all study visits over the course of the study.

7.2. Risk Management

Risk minimization, management, and assessment procedures have been implemented in the study to minimize and assess potential risks to participants at this clinical study. This includes:

- (1) Specific study entry and exclusion criteria to ensure that participants who have underlying characteristics that potentially increase their risk for an adverse outcome are excluded;
- (2) Overview surveillance by an independent Data Safety Monitoring Committee;
- (3) On-going follow-up of one month for safety monitoring

8. CLINICAL AND LABORATORY EVALUATIONS

8.1. Clinical Evaluations

Medical history, medication history, physical examination, vital signs, ECG's, Chest X-Ray, or a thorax CT Scan, or lung echography will be performed at screening/ admission.

During the study: See *Section 9.1 Table 2: Schedule of Events*

8.2. Laboratory Evaluations and Specimen Collection

Table 1: Clinical Laboratory Tests

Hematology	Serum Chemistry	Urinalysis	Serology	Other
<ul style="list-style-type: none"> • Complete blood count • Lymphocyte phenotyping 	<ul style="list-style-type: none"> • Glucose • Creatinine • Sodium, • Potasium, 	<ul style="list-style-type: none"> • Urinalysis • Urine pregnancy test 	<ul style="list-style-type: none"> • Specimen for SARS-CoV-2 antibody testing <p>(to be conserved until test available)</p>	

- Plasma cytokines and chemokines (in interested centres)
- Magnesium,
- Calcium
- Lactate
- ALT,LD
- C reactive protein
- Procalcitonine
- Ferritin
- NT proBNP
- INR, PTT
- Fibrinogen
- Troponine
- D-dimer

The specimens for SARS-CoV-2 antibody testing will be collected as standard-of-care and processed and stored on-site through the local laboratory.

8.3. Prognostic severity score to evaluate the therapeutic response (optional)

See appendix 1: This could be completed by the research nurse. It is an exploratory evaluation of this score that was previously evaluated in HIV-infected patients with *Pneumocystis jirovecii* pneumonia (Vanhems P & Toma E. Chest, 1995; 107: 107-112).

8.4. Stored Research Specimens and Plans for Possible Future Testing

Two serum samples will be collected at the beginning and at the end of trial treatment. They will be stored at the site laboratory for future testing (when available) for antibodies to SARS-CoV-2.

9. STUDY PROCEDURES

9.1. Schedule of Events

Table 2: Schedule of Events

Visit Type	Screening	Study Visits					
		Baseline	Daily	Day3+ Day 7	Day 10: End of treatment	Safety, Efficacy follow up Day 14	Safety, Efficacy follow up Day 21 + 28
Visit Window	At admission or in less than 48 hours	At admission					
Procedures:							
Informed Consent	X						
Eligibility Assessment	X						
Medical History	X						
SARS-CoV-2 PCR	If not done				X	X	If previous positive
Vital signs		X	X	X	X	X	X
Laboratory		X		X	X	X	X
Stored serum specimen		X			X		
Lung Imaging	X	X		X*	X*	If need it	If need it
Adverse Events		X	X				X
Concomitant Medications		X	X		X	X	X

* If abnormal at baseline or persistent/increasing respiratory symptoms

9.2. Screening Visit: See Table 2

9.3. Baseline Visit as per screening visit. In most cases it will be done on the same day

9.4. Visit Day 10: this is the Final Treatment Visit –See table 2 (by telephone call if non-hospitalized or discharged)

9.5. Visits Day 14, 21 and 28 are follow-up visits and will be performed by telephone call if the patient was not hospitalized or was discharged

9.6. Visit Day 28 is the Final trial Visit.

The procedures to be conducted at all the visits are mentioned in the table 2.

9.7 Early Termination Visit

This visit will include all procedures to be conducted at the final study visit (see table 2). For participants who stopped the trial early, a 30-day post follow-up safety visit (phone call) will be done.

10. EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

10.1. Definitions

10.1.1. Adverse Event (AE)

During each follow-up visit with the participant, information on AEs will be gathered and documented accordingly. AEs will be graded as mild, moderate, severe or life-threatening and assessed by causality as probably related, possibly related, unlikely to be related or not related to the treatment regimen consisting of doxycycline and pentoxifylline.

Stable chronic conditions which are present prior to clinical trial entry and do not worsen are not considered AEs and will be accounted for in the participant's medical history.

10.1.2. Serious Adverse Events (SAEs)

An SAE is defined as an AE meeting one of the following criteria at any dose:

- Results in death during the period of protocol-defined surveillance
- Is a life-threatening event (defined as a participant at immediate risk of death at the time of the event)
- Results in in-patient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance.
- Results in persistent or significant disability or incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a congenital anomaly or birth defect.

Any other important medical event that may not result in one of the above outcomes, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in patient hospitalization.

Participants will be monitored during the 10 days study period for SAEs. If an SAE is ongoing at the time a participant discontinues/completes the study, the SAE will be followed until the Investigator agrees that the event is satisfactorily resolved, becomes chronic, or that no further follow-up is required.

10.2. AE Descriptions and Recording

10.2.1. Intensity

The AE will be graded according to:

- Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (http://rsc.tech-res.com/document/safetyandpharmacovigilance/table_for_grading_severity_of_adult_pediatric_adverse_events.pdf).
- Common Terminology Criteria for Adverse Events (CTCAE) (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm)]

10.2.2. Relationship to Study Treatment

For all collected AEs (including SAEs), the clinician who examines and evaluates the participant along with the site pharmacist will determine the AE's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded as: definitely related, probable related, possibly related, unlikely or not related. For the definition of the relationship see the Interventional trials template from Canadian HIV Trials Network

10.3. Reporting and Evaluation of SAEs and Other Clinically Significant AEs

10.3.1. SAEs

All SAEs which occur during the course of the study must be reported to site pharmacy within 24 hours of the site becoming aware of the event. Principal investigator will be responsible for reporting SAEs to FDA Med Watch www.fda.gov/medwatch/report.htm. and to their country Health Authority (e.g. Health Canada in Canada).

10.3.2. Other Clinically Significant AEs (if applicable)

Will be reported to the site pharmacy within 48 hours

10.4. Events Due to Disease Progression (if applicable)

Events that are judged to be unequivocally due to disease progression should be recorded in the source documents, but not reported as AEs. Accordingly, events that meet the criteria of SAEs but that are judged unequivocally due to disease progression should not be reported as SAEs. For example, if a participant is hospitalized or dies due to unequivocal disease progression, the events associated with the disease progression should not be reported as SAEs. See point 2.2 for the Criteria for disease progression

10.5. Follow-up for Adverse Events

Any AE that occurs between the time that a study participant is randomized and the time that s/he departs the study at the end of the final study visit (or at the time of early discontinuation of the participant from the study for any reason) will be captured and recorded. At each contact with the participant, the investigator (or designate) must seek information on AEs by specific questioning and, as appropriate, by examination.

AEs that had previously been reported by the study participant will also be reassessed for duration, intensity and possible reoccurrence. Assessment of safety will include clinical observation and appropriate laboratory, ECG or imaging monitoring.

All AEs (including SAEs) will be followed until resolution or until the investigator and the clinical/medical monitor are in agreement that the AE has resolved, stabilized or become chronic and no further follow-up is required.

10.6. Pregnancy Follow-up

If a participant becomes pregnant during the study, the Investigator must collect follow-up data regarding the pregnancy, birth, and status of the child and inform DSMC. Follow-up should be continued until study close-out at the study centre. After close-out, the investigator will continue to obtain follow-up information.

Pregnancy should be recorded as a protocol deviation since it is included in the exclusion criteria. Pregnancy is not an adverse event; however, any complication related to pregnancy would be considered an adverse event.

11. STATISTICAL CONSIDERATIONS

11.1. General Study Design

This is a randomized, open clinical trial comparing a treatment regimen consisting of doxycycline and pentoxifylline for 10 days with the standard of care. However, if at one study centre another therapeutic trial is already started or will be initiated in the same time, then the therapeutic regimen in this proposal will be also compared with the other investigational therapy if it is proposed to the same study population.

11.2. Sample Size Considerations/Justification

The sample size calculation could not be realistically estimated at this point in time. Depending on standards of care or/and different investigational treatments, the reported rates of progression from mild and mild-to-moderate clinical forms to severe status, admission to intensive care (for which there is no standard criteria) and intensive mechanical ventilation are variable from one study to another, and from one location to another. It could be between 15 to more than 30%, according to my estimation.

Considering that 20% of hospitalized patients could progress to severe forms with standard of care, but only 5% in those receiving Doxycycline and Pentoxifylline in addition to standard of care, 123 patients per group, for a total of 246 should be enrolled to have a 95% power and a two-tailed Type I error rate of 0.05. Considering the drop outs for intolerance or other reasons of 27 patients per group a total of 300 patients should be enrolled: 150 per group
In case of 3 arms: 450 patients will be considered.

The time required to complete the enrollment of patients would probably be around 100 days, but could be prolonged if an important decrease in the incidence of new cases will occur. Also, the implementation of other concurrent trials must be considered.

11.3. Outcome Measures

Primary:

1. Number and % of patients progressing to a severe form necessitating intensive care admission and invasive mechanical ventilation
2. The effect on case fatality rates
3. The time to clinical recovery.

Secondary:

1. Time to improvement of radiologic findings
2. Effects on inflammatory markers (CRP, Ferritin), lymphocytes and ALT levels

Exploratory:

1. Changes in a prognostic severity score
2. The effects on cytokines/chemokines serum levels
3. The changes in lymphocyte phenotyping.

11.3.1. Analysis of Primary Outcome Measures

Consultation with a biostatistician is recommended before implementing the trial

For the primary outcomes and the first secondary outcome the differences between the study group and the standard of care group will be assessed by using Mann Whitney non parametric test. The Kruskal-Wallis test will be used in case of 2 study regimens and a standard of care group followed (if P values were <0.05) by Dunnett's multiple comparison test. Changes over time for repeated measures will be assessed by analysis of variance using Friedman's test followed by Dunnett's posttest for multiple comparisons when p values were < 0.05 . Correlation between different parameters will be analyzed with Spearman's rank correlation test.

The time to clinical recovery and time to improvement of radiologic findings will be assessed by using the log-rank test. The Kaplan-Meier estimates and the log-rank p-values will be plotted. All p values will be two-sided.

11.3.2. Analysis of Secondary and Exploratory Outcome Measures

Changes over time for repeated measures will be assessed by analysis of variance using Friedman's test followed by Dunnett's posttest for multiple comparisons when p values were < 0.05 .

11.3.3. Analysis of Exploratory Outcome Measures

Mann Whitney non-parametric test will be used

11.4. Summary of Demographic and Baseline Data

The demographic and baseline data will be summarized as medians and interquartile ranges (25-75).

11.5. Interim Analyses

An interim analysis could be performed once 100 participants (50 in each group) will finish the 10 day treatment.

The primary outcome measures only will be examined.

At the recommendation of DSMC the trial could be terminated for efficacy, for safety concerns and eventually for futility.

12. STUDY ETHICAL CONSIDERATIONS

12.1 Ethical Conduct of the Study

This study should be conducted in accordance with the ICH-GCP Guidelines, applicable regulations and the principles in the Declaration of Helsinki. The Investigator will be thoroughly familiar with the appropriate use of the study treatment as described in the protocol and *product* monograph.

12.2 Informed Consent

All participants will be given detailed oral and written information about the study. Consent forms describing in detail the study medications, study procedures and risks will be given to each participant and written documentation of informed consent is required prior to starting study medication/intervention. Participants must sign an informed consent document that has been approved by a REB/IRB prior to any procedures being done specifically for the trial. Each participant should have sufficient opportunity to discuss the study, have all of their questions addressed and consider the information in the consent process prior to agreeing to participate. Participants may withdraw consent at any time during the course of the study without prejudice. The informed consent form will be signed and dated by the participant and the investigator or delegate. The original signed informed consent form will be retained in the participant's study files and a copy will be provided to the participant.

The informed consent process must be conducted, and form signed before the participant undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study.

12.3 Confidentiality

All participant-related information including Case Report Forms, laboratory specimens, evaluation forms, reports, etc. will be kept strictly confidential. All records will be kept in a secure, locked location and only accessible to research staff. Participants will be identified only by means of a coded number specific to each participant. All computerized databases will identify participants by numeric codes only, and will be password protected.

Upon request, and in the presence of the investigator or his/her representative, participant records will be made available to the study sponsor, monitoring groups representative of the study sponsor, representatives of funding groups, and applicable regulatory agencies for the purpose of verification of clinical trial procedures and/or data, as is permissible by local regulations.

12.4 Institutional Review Board, Ethics Committee, or Research Ethics Board

The IRB, Ethical Committee or REB will review all appropriate study documentation to safeguard the rights, safety, and well-being of the participants. The study will be conducted only at sites where ethics approval has been obtained. A copy of the protocol (including protocol amendments), all versions of informed consent forms, other information to be completed by participants such as survey instruments or questionnaires, and any proposed advertising/recruitment materials must be reviewed and approved by the REB/IRB of each participating centre prior to implementation of the trial. The investigator will notify the REB/IRB of serious adverse events as applicable. The

investigator will seek prior ethics approval for any protocol deviations except when the change is intended to eliminate an immediate hazard to participants. In this case, the protocol deviation will be promptly reported.

12. General Trial Conduct Considerations

12.1. Adherence to Protocol

12.1.1. Protocol Amendments

All protocol amendments will be reviewed and approved and if applicable submitted to the applicable regulatory agencies for prior approval or notification. The Investigator must sign and date the amendment prior to implementation. All protocol amendments must also be submitted to the ethics committee.

12.1.2. Protocol Deviations

No deviations from this protocol will be permitted without the prior written approval of the Investigator, except when the modification is needed to eliminate an immediate hazard or hazards to participants. Any deviations that may affect a participant's treatment or informed consent, especially those increasing potential risks, must receive prior approval from the REB unless performed to remove an immediate safety risk to the participants. In this case it will be reported to the REB and to the investigator immediately thereafter. Any departures from the protocol must be documented.

12.2. Monitoring & Auditing

12.2.1. Data Safety Monitoring Committee (DSMC) should be nominated

DSMC will review the enrollment rate

DSMC will review the data at an interim analysis when the first 50 patients in each group finished their follow-up (1 month after the 10 day treatment is over)

The DSMC could recommend that trial should be stopped earlier:

- if it is concluded that the proposed therapeutic regimen is safe and efficacious as supported by the accumulated data and the pre-defined statistical analyses;
- for futility if no signals of efficacy or inefficacy after 100 patients in each group finished their follow-up;
- for safety concerns;
- for a slow rate of enrollment.

12.2.2. Study Monitoring

The Investigator will perform ongoing study site monitoring at 1- to 2-week intervals during enrollment to ensure quality assurance. Once enrollment is complete, the study site monitoring will be performed at 2- to 4-week intervals.

Protocol deviations will be monitored and recorded by the Investigator. Details regarding patient accrual and ineligibility are specified in a separate form than the CRF.

Early Termination of the Trial

As shown above the following conditions will lead to an early termination of the trial:

- if it is concluded that the proposed therapeutic regimen is safe and efficacious as supported by the accumulated data and the pre-defined statistical analyses.
- for futility if no signals of efficacy or inefficacy after 100 patients finished their follow-up
- for safety concerns
- for a slow rate of enrollment (less than 3 patients /week)

12.3. Record Keeping

12.3.1. Data Collection

The Investigator must maintain detailed records on all study participants. Data for the study will be recorded in the participant's chart and entered into CRFs. Applicable data from the participant's chart should be recorded in the CRFs completely and promptly, taking time to correct any mistakes. Copies of CRFs will remain at the clinical site at the conclusion of the study.

12.3.2. Source Documents

The Investigator must maintain adequate and accurate source documents upon which CRFs for each participant are based. They are to be separate and distinct from CRFs except for cases in which the Investigator has pre-determined that direct data entry into specified pages of the participant's CRF is appropriate. These records should include detailed notes on:

- Oral and written communication with participant regarding the study treatment (risks/benefits)
- Participation in trial and signed and dated informed consent forms
- Inclusion and exclusion criteria details
- Visit dates
- Adverse events and concomitant medication
- Results of relevant examinations
- Laboratory printouts
- Participant's exposure to any concomitant therapy (start/stop dates, dosing details)
- Reason for premature discontinuation (if applicable)
- Enrollment number
- Methods of contraception and fertility status (if applicable)
- Compliance/non-compliance protocol deviation information.

12.3.3. Data Management

Instructions concerning the recording of study data on CRFs will be provided by the site principal investigator. Each study site is responsible for submitting the data in a timely fashion.

Detailed aspects of data handling will be described in the Data Management Plan.

12.3.4. Record Retention

The local REB/IRB and Institutional requirements for record retention timelines should be mentioned once known.

INVESTIGATOR AGREEMENT

Protocol Title: Doxycycline and Pentoxifylline for mild and mild-to moderate COVID19

Protocol No.:

Version No.: 1.3

Date: July- 2020

This clinical study will be conducted in accordance with applicable Health Canada regulations, ICH guidelines on current GCP, and the Declaration of Helsinki.

I confirm that I have read and understand this protocol and I agree to conduct this clinical study in accordance with the design and specific provisions of the protocol, with the exception of a change intended to eliminate an immediate hazard to participants. Any deviation from the study protocol will be documented in the case report form.

I agree to promptly report to the applicable ethics boards any changes in the research activity and all unanticipated problems involving risks to human participants or others. Additionally, I will not make any changes in the research without prior ethics and sponsor approval, except where necessary to ensure the safety of study participants.

Name

Signature

Date (dd-mmm-yyyy)

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*Appendix 1.***Prognostic severity score to evaluate the therapeutic response (adapted from Vanhems P & Toma E: *Chest*,1995;107:107-12)****1. Fever:**

- 0= Less than 37.6⁰C
- 2= Greater than 37.6⁰C but less than 38.5⁰ C
- 4= Greater than 38.5⁰ C but less than 39.5⁰ C
- 6= Greater than 39.5⁰ C

2. Cough:

- 0 = None
- 1= Mild; coughing less than 1/15 min
- 2= Moderate: between 1/15 and 1/5 min and cough interfering with sleep
- 3= severe: 1 or more/5 min and cough interfering with both conversation and sleep

3. Fatigue :

- 0= None
- 1= Diminished daily activities
- 2= Have to sit down after 5-10 min
- 4= Have to stay in bed

4. Respiratory rate:

- 0= Less than 16/min
- 2= 17-22/min
- 4 = 23-28/mi
- 6= 29-39/min
- 8 = More than 40/min

5. Dyspnea:

- 0 = None
- 2= Forced to slow walking pace
- 4= Forced to interrupt walking
- 6= Forced to stop every few minutes or after about 9 m
- 8= Breathlessness with dressing or undressing
- 10=Breathlessness at rest

6. Oxygen saturation at resting-state

- 0 = More than 96%
- 2= 96%-94%
- 8= 93% or less

7. Lung imaging (Chest X-Ray, CT-SCAN, Lung echography)

- 0 = Normal
- 2= Discrete pulmonary infiltrate; ground glass appearance or not
- 4= Pulmonary infiltrates; ground-glass appearance; unilateral consolidation; covering less than 50% of lung fields
- 8= Bilateral pneumonia and pulmonary infiltrates in more than 50% of lung fields

8. Laboratory

0 = No abnormal results

2= For each abnormality: leukopenia (< 3000), lymphopenia (<1000), thrombocytopenia (< 100 000), elevated LD, CRP, ferritin, NT-proBNP, D-dimers, troponins

9. Comorbidities:

0= None

2= for each condition: diabetes, obesity, cardiovascular disease, hypertension, chronic lung disease, hemato -oncology, HIV infection, immunosuppression, solid organ transplant

Protocol synopsis

Full Title	Doxycycline and Pentoxifylline for mild and mild-to-moderate COVID19
Short Title	DoxyPenCovid-19
Protocol and Version No.	1.2
Clinical Phase	3
Study Duration	Enrollment period: July -31 September 2020 Study period: July- 31 October 2020
Sponsor-Investigator	TBD
Number of Centres	At least 3-4 in each country if possible
Study Design	Randomized, open label
Primary Objective	<ol style="list-style-type: none"> 1) Evaluate tolerability & safety of proposed drug regimen 2) Assess efficacy in stopping or delaying disease progression, and reduction of case fatality
Secondary Objectives	<ol style="list-style-type: none"> 1) To assess the reduction of time to recovery 2) To assess the reduction of in hospital-stay duration
Exploratory Objectives	<ol style="list-style-type: none"> 1) To evaluate a prognostic severity score 2) To assess the effect on cytokines/chemokines serum levels 3) To assess the changes in lymphocyte phenotyping
Sample Size	<p>N = sample size calculation could not be realistically estimated at this point in time.</p> <p>We hypothesize that 20% of hospitalized patients will progress to severe forms (with standard of care), but only 5% in those receiving DoxyPen in addition to standard of care. Thus, 123 patients per group, for a total of 246 will be enrolled to have a 95% power and a two-tailed Type I error rate of 0.05 alpha error. Considering the drop-outs for intolerance or other reasons of 27 patients per group, a total of 300 patients will be enrolled: 150 per group. In case of 3 arms: 450 participants will be considered.</p>
Randomization	<p>1:1 allocation by blocks of four: the proposed drug regimen vs. standard of care</p> <p>1:1:1 allocation by blocks of 6: the proposed drug regimen vs. standard of care vs. another clinical protocol, if any, at a specific site</p> <p>A computer randomization by block of four was prepared (by E.T.) and will be given to the pharmacist in charge.</p> <p>A randomization by blocks of 6 will be prepared if another clinical trial is or will be implemented.</p>
Study Population	<p>Symptomatic patients infected with SARS-CoV-2 (positive PCR test) with mild or mild-to-moderate clinical forms</p> <p>Older than 18 years</p> <p>Needing or not hospitalization</p>

	<p>Any sex/gender</p> <p>Any race/ethnicity</p> <p>Able to give an informed written consent</p> <p>Have no other acute active medical condition</p>
Investigational Product Description	<p>Doxycycline capsules plus</p> <p>Pentoxifylline tablets/capsules</p>
Control	<p>1.Standard of care</p> <p>2. Another investigational regimen-if any</p>
Administration and Dosing	<p>Doxycycline-100 mg orally twice daily</p> <p>plus</p> <p>Pentoxifylline-400 mg orally twice daily</p>
Duration of Treatment	<p>10 days</p>
Outcome Measures	<p>Primary:</p> <p>1.Number and % of patients progressing to a severe form necessitating intensive care admission and invasive mechanical ventilation</p> <p>2.The effect on case fatality rates</p> <p>3.The time to clinical recovery</p>
	<p>Secondary:</p> <p>1.Time to improvement of radiologic findings</p> <p>2. Effects on inflammatory markers (CRP, Ferritin), lymphocytes and LD levels</p>
	<p>Exploratory:</p> <p>1. Changes in a prognostic severity score</p> <p>2.The effects on cytokines/chemokines serum levels</p> <p>3.Changes in lymphocyte phenotyping</p>
Statistical Analysis	<p>For the primary outcomes and the first secondary outcome the differences between the study group and the standard of care group will be assessed by using Mann Whitney test. The Kruskal-Wallis test will be used in case of 2 study regimens and a standard of care group followed (if P values were <0.05) by Dunnett's multiple comparison test.Changes over time for repeated measures will be assessed by analysis of variance using Friedman's test followed by Dunnett's posttest for multiple comparisons when p values were < 0.05. Correlation between different parameters will be analyzed with Spearman's rank correlation test.The time to clinical recovery and time to improvement of radiologic findings will be assessed by using the log-rank test. The Kaplan-Meier estimates and the log-rank p-values will be plotted. All p values will be two-sided.</p>