THE MOST PROMINENT ANTIVIRAL PHARMACEUTICALS USED AGAINST COVID-19 AND THEIR PERSPECTIVES

Nelson Durán[1,2*] (ORCID: 0000-0001-8372-5143)

Wagner J. Fávaro[1*] (ORCID: 0000-0001-5830-8938)

[1]Laboratory of Urogenital Carcinogenesis and Immunotherapy, Department of Structural and Functional Biology, University of Campinas, Campinas, SP, Brazil.
[2]Nanomedicine Research Unit (Nanomed), Federal University of ABC (UFABC), Santo André, SP, Brazil.

[*]**Correspondence**: Prof. Nelson Durán (E-mail: nelsonduran1942@gmail.com) and Prof. Wagner J. Fávaro (E-mail: favarowj@unicamp.br).

Conflict of Interests: The authors report the non-existence of any kind of conflict of interests.

Financial Support: São Paulo Research Council (FAPESP grant 2014/11154-1; 2018/10052-1) and the Brazilian National Council for Scientific and Technological Development (CNPq grant 552120/2011-1).

Individual Collaboration:
Nelson Durán: study design, bibliographic search, data analysis, interpretation of results, writing, review and approval of the final version of the manuscript.

Wagner J. Fávaro: study design, bibliographic search, data analysis, interpretation of results, writing, review and approval of the final version of the manuscript.

Abstract

Many well-known commercial pharmaceuticals have been used in the therapy against SARS-CoV-2, although in a random and experimental way, since this use is only based on the knowledge about culture cells *in vitro*. But, it is widely known that it is not correlated with assays *in vivo* conducted with in animals or with clinical trials in humans. Knowledge about treatments applied to previous pandemic infections caused by viruses such as MERS and SARS may be the most interesting strategy used to screen products with potential to act against SARS-CoV-2. Mechanistic aspects of virus infections and antiviral action mechanisms were herein analyzed based on the assumption that these commercial pharmaceuticals can eliminate the virus. Perspectives about these procedures, as well as about finished clinical trials and the ones yet in progress, were also addressed in the current study.

Keywords: COVID-19, Treatment, Mechanism of action, SARS-CoV-2

Introduction

The large number of clinical trials carried out to research likely therapies against COVID-19 emphasizes both the necessity and ability of the scientific community to generate high-quality proof, even during the Covid-19 pandemic. Unfortunately, treatments applied so far were not fully efficient (Sander et al., 2020).
It is well-known that inflammatory caspases represent a key function in innate immunity, since they reproduce cytosolic signals and induce dual responses (Jorgensen and Miao, 2015). Two important targets can be used to eliminate viruses, mainly SARS-CoV-2; the first one is pyroptosis, which is an important process triggered by caspase-1 - which activates and releases pro-inflammatory cytokines such as interleukin-18 (IL-18) and IL-1β. In addition, caspase-11 or caspase-1 are capable of activating some type of lytic, programmed cell death. Pyroptosis acts by removing the reproduction site of intracellular pathogens, like bacteria or viruses, and it makes them susceptible to phagocytosis and to be destroyed by subsidiary phagocytes. However, in several cases, abnormal systemic activation of pyroptosis *in vivo* can lead to sepsis. Emphasizing the ability of inflammasome recognition of viral infections, several pathogens have improved its abilities to prevent or disrupt pyroptosis (Durán and Fávaro, 2020a). These observations are also found in many other diseases (Ma et al., 2018; Liang et al., 2020) such as cancer (Durán and Fávaro, 2020b) and cardiovascular diseases (Zeng et al., 2019). The other target lies on RNA-dependent RNA polymerase (RdRp) because SARS-CoV-2 is a strand RNA virus, whose propagation is based on multiple subunit reproduction or/and transcription complex of viral proteins (Ziebuhr, 2005).

Although there are many other targets that can be used against SARS-CoV-2 (e.g., blocking the host target TMPRSS2 (ACE2 receptor), three of the most important ones (pyroptosis, RNA-dependent polymerase and nucleocytoplasmic trafficking of viral proteins), which are associated with pharmaceuticals used nowadays, will be addressed in the current study.

Pyroptosis

Recently, Zhao and Zhao (2020) have investigated PRRs (pattern recognition receptors) such as LRR, PYD and NACHT domains. These PRRs present protein-3 inflammasome, which is a complex consisted of the NOD-like receptor protein-3 - NLRP3 (NOD domain (nucleotide-binding oligomerization) NOD-like receptor NLRP3), which, in its turn, is the transcriber of apoptosis connected speck-like protein (ASC) and caspase-1. This kind of complex is crucial to hosts' defense against microbes since it stimulates IL-1β and IL-18 release and triggers pyroptosis, as previously mentioned. NLRP3 is capable of recognizing a whole diversity of PAMPs (pathogen-connected molecular patterns) and DAMPs (danger-connected molecular patterns) generated through viral replication; this process activates the NLRP3 inflammasome which is really of antiviral immunological responses and makes viral eradication possible. Unfortunately, many viruses use very sophisticated strategies to prevent the immune system from attacking the NLRP3 inflammasome (Sollberger et al., 2014). Few viruses have been reported to inhibit NLRP3 inflammasome stimulation to avoid innate immunity and enhance viral replication. The virus is capable of suppressing either the adjustment or the stimulation of NLRP3 inflammasome through a direct/indirect interaction.

Influenza virus NS1, paramyxovirus V and measles virus inhibit NLRP3 inflammasome induction and diminish IL-1β release. This interaction of viral protein and

NLRP3 avoid self-oligomerization and, at the same time, the enrolment of ASC, culminating in the blocks inflammasome activation (Zhao and Zhao, 2020).
Yang et al. (2020) conducted a study focused on investigating the history and epidemiology of SARS and emphasized several points, such as pathogenesis, epidemiology, clinical features, diagnosis and guidance of individuals infected with SARS-CoV-2.

At the first coronavirus contamination stage, epithelial and dendritic cells are activated and present a cluster of chemokines and pro-inflammatory cytokines, such as interleukins (IL-2, IL-6, IL-8, IL-1β), tumor necrosis factor (TNF), interferons (IFN-α/β), C-C motif chemokines and IP-10, among others. All these pro-inflammatory factors are modulated by the immune system. Thereby, excessive generation of all these chemokines ad cytokines accelerates disease progress. Interleukin IL-10 is generated by T-helper-2 (Th2) and has antiviral activity; however, SARS-CoV-2 infection is capable of significantly inhibiting it (Zhang et al., 2020a).

Thus, patients contaminated with SARS-CoV-2 present raised serum IL-1β levels (Huang et al., 2020), which is a strong symptom of cell pyroptosis. This factor may indicate that cell pyroptosis induction is implicated in the pathogenesis of SARS-CoV-2-infected patients.

It is well-known that both non-classical and classical pyroptosis indication can trigger IL-1β release, a fact that should be further investigated in SARS-CoV-2-associated pneumonia patients. Besides, both leucopenia and lymphopenia are observed in pneumonia patients, most SARS-CoV-2-contaminated patients show lymphopenia, that likely indicates that lymphocytes may be susceptible to cell pyroptosis throughout infection processes.

Chen et al. (2020a) have found the same conduct in many patients with SARS-CoV-2-associated pneumonia; nearly all patients who died presented lymphopenia (Durán and Favaro, 2020a; and references therein). Interestingly, Chang et al. (2020) reported that all assessed patients with confirmed SARS-CoV-2 diagnosis have recovered and presented scarcely elevated contents of inflammatory markers like the number of lymphocytes; all these facts were confirmed by Lipi and Plebani (2020) and Henry et al (2020). Besides, based on current data, SARS-CoV-2 presumably immunomodulatory effects leads to cell pyroptosis, mainly in lymphocytes, due to NLRP3 inflammasome induction. Steps involved in the induction of the signaling linking NLRP3, IL-18, IL-1β, and gasdermin D were suggested (Yang, 2020; Yang et al., 2020a).

A study review provided the pathophysiology of SARS-CoV-2 contamination and described the SARS-CoV-2/immune system interaction, as well as the sequential aid of dysfunctional immune responses to infection progression. Based on the current knowledge about coronavirus infections, the authors of the aforementioned study have pointed out the participation of these strategies in potential therapies focused on attacking viral infection and/or immunomodulation (Tay et al., 2020).

Many immunosuppressive therapies contributed at limiting immune-mediated damage in COVID-19 patients are at different evolution stages. Trials conducted with corticosteroids (e.g., prednisolone) to treat COVID-19 are currently in progress, although this class of therapy was not suggested during the 2003 SARS epidemic. An assay at clinical trial level, focused on testing the effectiveness of tocilizumab, a IL-6 antagonist is also in progress; sarilumab is also under investigation. Many others clinical trials are studying the effects of targeting granulocyte–macrophage colony-stimulating factor (GM-CSF), incorporating the application of gimsilumab, lenzilumab and namilumab. Cytosorb is another complement therapy that operates by absorbing a wide spectrum of cytokines, PAMPs and DAMPs with the view to reduce their circulating levels and

improve immunopathology. Thalidomide is a pharmaceutics with immunomodulatory qualities; it has also been effectively administered to patient with COVID-19. Thus, two clinical trials have been recently implemented to test thalidomide potential to diminish lung injury. TNF (tumor necrosis factor) antagonism was indicated, although not tested, in SARS-CoV-contamination cases, but not in patients with COVID-19. An open-label, non-randomized study indicated that the combination of hydroxychloroquine (well-known antimalarial agent) and azithromycin (common antibiotic) might be useful to patients with acute COVID-19. While hydroxychloroquine's effect on direct virus suppression, as well as its anti-inflammatory and immunomodulatory activities are known, if these mechanisms play a role against COVID-19 remains unclear (Tay et al., 2020, and references therein). A scarcity of clinical information with ribavirin for SARSCoV-2 means its therapeutic function must be inferred from other nCoV information (Sander et al., 2020). Nitazoxanide was effective as antiviral activity *in vitro* against MERS and SARS-CoV-2 (Rossignol, 2016; Wang et al., 2020a). Awaiting further evidence, immunodulatory effect and the antiviral activity and safety profile of nitazoxanide requires further investigating it as alternative treatment for SARS-CoV-2. Another pharmaceutical called ivermectin has also appeared in some studies. A phase-III clinical trial focused on investigating ivermectin effectiveness against dengue virus infection was conducted in Thailand; results have shown that this pharmaceutical was safe and reduced the serum levels of viral NS1 protein, although it did not show significant clinical benefit to the investigated patients (Yamasmith et al., 2018).

It is known that extensive cytokine release due to immune system's reaction to viral contamination can lead to cytokine explosion and sepsis, which may lead to death in approximately 30% of severe COVID-19 cases. Uncontrolled inflammation condition lead to multiple organ injuries, which, in their turn, may lead to organ failure, mainly in hepatic, renal and cardiac functions. Patients contaminated with SARS-CoV who evolved to renal failure have died. Contrary, patients whose recruited cells healed the lung contamination, showed immunological response retreat and were healed. Meanwhile, dysfunctional immune reaction was observed in some cases due to cytokine explosion aimed at regulating extensive inflammation. Critical COVID-19 patients in need of rigorous hospital care presented exacerbated parameters such as significantly high G-CSF (granulocyte colony-stimulating factor) blood plasma levels, interleukins, such as IL-2, 7 and 10, IP-10, MCP1 and MP1α (inflammatory protein 1α from macrophage) and NTF (tumor necrosis factor). The IL-6 cytokine levels in these cases were also high, which led to higher death than survival rates. Importantly, there was a wide macrophage content of inflammatory monocyte from FCN1+ in the Broncho-alveolar material extracted from individuals with serous, although not mild, COVID-19. Likewise, individuals at advanced disorder stage have shown significant incidence of inflammatory monocytes (CD14+CD16+) in their peripheral blood in comparison to patients with mild COVID-19. These cellular systems release inflammatory cytokines and lead to cytokine explosion, incorporating MCP1, MIP1α, and IP-10 (Tay et al. 2020, and references therein).
Some of the main covid-19 trials were carried out with the following compounds: Chloroquine, hydroxychloroquine, nitazoxanide, thalidomide, ivermectin, remdesivir and favipiravir (pro-drugs) and prednisolone/dexamethasone (Figure 1).

Chloroquine and hydroxychloroquine

Chloroquine is a potent NLRP3 inflammasome-pathway inhibitor. Therefore, it can suppress mature IL-1β and IL-18 production and enhance the survival rates of patients with endotoxic shock by suppressing NLPR3 inflammasome activation, as well as IL-1β and IL-18 maturation. In addition, the inhibitory process of chloroquine on NLRP3 inflammasome activation comprises the inhibition of NLRP3, IL-1β, and IL-18 transcription by restricting in NF-κB and MAPK pathways, as well as the inhibition of NLRP3 complex assembly by restraining K+ efflux and ASC speck formation (see Figure 2).

These results indicate that chloroquine might be an auspicious therapy against LPS-induced endotoxin shock and other type of disease depending of NLRP3 inflammasome-related diseases (Chen et al., 2017)

Hydroxychloroquine has anti-inflammatory activity on Th17-related cytokines (IL-6, -17, and -22) in healthy subjects, as well as in rheumatoid arthritis (RA) systemic and lupus erythematosus (SLE) patients (da Silva et al., 2013). According to Borba et al. (2020), high (600 mg x 2 day) and low (450 mg x 2 day) chloroquine doses delivered for 10 days, in association with azithromycin and oseltamivir, were not safe enough to assure the continuation of the study group. Chloroquine did not show any apparent benefit to patients' lethality rate.

COVID-19 patients hospitalized in metropolitan New York, who was treated with hydroxychloroquine, azithromycin, or both, did not shown significant differences in in-hospital mortality rate in comparison to patients who were not subjected to these treatments. But, the interpretation of these observations may have been restricted by the observational design (Rosenberg et al., 2020).

Based on another study conducted with hospitalized patients with Covid-19, hydroxychloroquine treatment was not related to either significantly lower or higher risk of patient intubation or death. The main analysis did not find important association between hydroxychloroquine administration and patient intubation or death. Results in multiple sensitivity analyses were similar and it was necessary conducting randomized regulated trials with hydroxychloroquine in individuals with Covid-19 infection (Geleris etal., 2020).

However, France, Belgium and Italy and recently USA have recently expressed their objection about administration of hydroxychloroquine to treat COVID-19 cases and banned its use. All of these countries were not the first to reject the use of this medicine. The World Health Organization (WHO) excluded the global trial with hydroxychloroquine a few days ago due to safety concerns. Similarly, Oxford University has paused a global trial a week after its implementation (https://www.wionews.com/world/covid-19-france-italy-and-belgium-ban-usage-of-hydroxychloroquine-301449) (accessed on May 28th, 2020). Nowadays, confusing data about hospital treatment were reported including a retracted paper posteriorly by Lancet (Mehra et al., 2020) and reopened the controversy about chloroquine use. However, health professionals are aware that this pharmaceutical is not safe to be used in an indiscriminate manner.

Nitazoxanide

Since nitazoxanide uncouples mitochondrial electron transport, the current study took into consideration the chance that mitochondrial effects, apart from DNA leakage, might intervene the antiviral responses. The defeat of pan-caspase inhibitor z-VAD (Selleckchem) in blocking nitazoxanide antiviral activities debates against pyroptosis or

apoptosis; both of them can be interceded by mitochondrial factors and are key elements of the nitazoxanide mechanism (Hickson et al., 2018).

Besides to its antiviral activity (Antony et al., 2020), nitazoxanide suppress the generation of pro-inflammatory cytokines TNF, IL-2, IL-4, I-5, IL-6, IL-8 and IL10 in peripheral blood mononuclear cells (PBMCs). Mice subjected to oral administration of nitazoxanide *in vivo* have reduced plasma IL-6 levels by 90% in comparison to vehicle-treated mice. The relevancy of all of the data to humans has not yet been investigated, however, these information indicate that nitazoxanide can enhance outcomes in patients infected with MERS-CoV by inhibiting the overproduction of pro-inflammatory cytokines such as IL-6 (Rossignol et al., 2016).

Clinical trials focused on investigating monotherapy with nitazoxanide are currently in progress (Clinical Trials. gov. Identifier NCT04348409-Aciduz-Brazil; NCT04343248-Romak Lab.-USA and NCT04359680-Romak Lab.-USA).

Thalidomide

Studies conducted *in vitro* or *in vivo* have shown that thalidomide damages the synthesis of TNF-α (tumor necrosis factor alpha). It raises peripheral blood CD8+ T cells, IL-12 levels, IFN-γ generation and cytotoxic activity. According to the study *in vitro* by Tabata et al. (2015), thalidomide was capable of decreasing IL-1β and IL-6 expression in human lung epithelial cells and helped preventing emphysema. A study conducted with animals has shown that thalidomide notably mitigated pulmonary fibrosis, oxidative stress and inflammation in mice's lungs. Similarly, thalidomide has decreased the production of TNF-α, IL-1β, IL-6, and modifying growth factor-β. Dogs with transplanted lung had better corticosteroids in early postoperative immunosuppression after the transplantation due to decreased incidence of pneumonia. Besides, it was published that thalidomide had anti-fibrotic effects against bleomycin-induced pulmonary fibrosis in rats. The anti-inflammatory activity of thalidomide on lung injury induced by H1N1 influenza virus in mice have shown that thalidomide significantly improved their survival rate, reduced the infiltration of inflammatory cells, as well as cytokine (e.g., IL-6, TNF-α), and chemokine (chemokine ligand 5, C-X-C motif chemokine 10) levels, and inhibited p-NFκB p6 activation. A combination of thalidomide and low-dose glucocorticoid therapy against Covid-19 has shown beneficial results (Dastan et al., 2020; and references therein).

Thalidomide has inhibited pro-inflammatory cytokine IL-1 secretion by human primary keratinocytes and monocytes in a dose-dependent manner. Beside, this pharmaceutical also diminished the liberation of pro-angiogenic growth factor FGF2 and of several other proteins. The secretion of all these proteins demands caspase-1 activity. Proteolytic enzyme itself is activated by multimeric innate immune complexes called inflammasomes. Then, thalidomide has inhibitory effects on NLRP3 inflammasome, since it blocks caspase-1activity (Keller et al., 2009; Thi and Hong, 2017).

A positive-Covid-19 patient treated with lopinavir/ritonavir and, subsequently, with thalidomide, did not show any results. Laboratorial analyses showed a high increased C-reactive protein (CRP) level, and cytokine levels (interleukin IL-6, IL-10 and interferon (IFN)-γ), as well as significantly decreased T cell absolute value (including CD4 + T cells, CD8 + T cells, NK cells and B cells). Six days after thalidomide treatment implementation, IL-6, IL-10 and IFN-γ cytokine levels returned to the normal range.

Besides its ability to inhibit cytokine release and regulate immune functions, thalidomide can be used to relax COVID-19 patients for reducing their oxygen

consumption and alleviate digestive symptoms. Therefore, thalidomide may shed new light on an adjuvant treatment strategy focused on this potentially lethal viral disease. It is necessary conducting a randomized controlled trial focused on investigating the effectiveness of thalidomide use, in association with low-dose glucocorticoid therapy, to treat COVID-19 pneumonia (Chen et al., 2020). Actually, a clinical trial is already in course (Clinical trial.gov:NCT04273529-China).

Methylprednisolone and Dexamethasone

It is known that inhaled corticosteroids diminish the number of inflammatory cells (e.g., eosinophils, T-lymphocytes, mast and dendritic cells) at cellular level in any disease affecting individuals' airways. Corticosteroid activity lies on suppressing the recruitment of inflammatory cells in patients' airways by inhibiting the generation of chemotactic intermediates and adherence molecules and by suppressing the subsistence of inflammatory cells (e.g., T-lymphocytes, eosinophils, mast cells) in the airways. Epithelial cells are the major action site of inhaled corticosteroids (ICS), since they are capable of inhibiting several activated inflammatory genes in airway epithelial cells and the epithelial unity is recovered by normal ICS (Figure 3). Mucosal inflammation is overall rapidly inhibited due to significant reduction in the number of perceptible eosinophils within six hours; such an inhibition is linked to reduced airway hyper responsiveness. The reversal of this stage may take months to reach a threshold that may indicate improved structural changes in airways (Barnes, 2010).

Overall, corticosteroids dissipate through the cell membrane and link to glucocorticoid receptors (GRα) in the cytoplasm; next they translocate to the nucleus cell and trigger molecular effects. A GR homodimer is linked to a GRE (glucocorticoid response element) located in the promotor region of genes susceptible to steroids; this association is connected, or not, to gene transcription. Genes activated by corticosteroids comprise genes encoding anti-inflammatory proteins such as secretory leukoprotease inhibitor, β2-adrenergic receptors and the mitogen-activated protein kinase phosphatase-1 (MKP-1), which suppresses MAP kinase routes. These outcomes may enable the anti-inflammatory activity of corticosteroids. GR association with negative GREs may inhibit gene transcription; assumingly, this inhibition may play a key role in influencing many of the collateral effects caused by corticosteroids (Barnes, 2010).

Systemic treatments with glucocorticoids were largely applied to patients infected with coronavirus (SARS-COV) who developed severe respiratory complications during the SARS epidemic in 2003. The administration of pulsed high-dose methylprednisolone enabled clinical enhancement in SARS patients. A female patient subjected to bone marrow transplant due to acute myeloid leukemia was infected with SARS; she was subjected to treatment with oral prednisolone and ribavirin, which improved her lymphopenia, modified transaminases, chest X-ray and computed tomography results very fast (Lam et al., 2004). Interleukin-8, monocyte chemoattractant protein-1 and Th1 chemokine interferon-g-inducible protein-10 levels have decreased after 5-8 days of glucocorticoid therapy. Nevertheless, some data showed that pulsed doses of methylprednisolone presented high-risk factor associated with increased 30-day mortality. Besides, an observational study has shown that glucocorticoid treatment application in patients with MERS (Middle East respiratory syndrome coronavirus MERS-CoV) was associated to delay respiratory syndrome. However, the clinical therapeutics of systemic glucocorticoid therapy in patients with COVID-19 is not actually evident; thus, the systemic use of glucocorticoids, or not, in severe COVID-19 cases demands further investigation (Qin et al., 2020, and references therein).

However, a recent study has shown that glucocorticoids used in combination with antibiotics have significantly diminished the viral infection. In this case, patients with severe COVID-19 pneumonia presented significantly increased number of inflammatory markers such as C-reactive protein (CRP), IL-6 and ferro protein (FER), which corresponded to the inflammatory reaction stage. Wang et al. (2020b) suggested that once the secondary infection happens in patients with severe COVID-19 pneumonia, besides methylprednisolone doses, full-dose antibacterial drugs (e.g. cephalosporin) should be immediately added to the treatment. Data have shown that this treatment was related to faster decrease in CRP and IL-6. Thus, early low-dose and short-term administration of corticosteroids was associated in patients with acute COVID-19 pneumonia with faster progress of clinical symptoms. In addition, methylprednisolone has been used in COVID-19 patients, in association with antibiotics, oseltamivir and oxygen therapy (Huang et al., 2020; Rosa and Santos, 2020). However, a recent study has shown that prednisolone was not capable of suppressing viral SARS-CoV-2 growth in infected VeroE6/TMPRSS2 cells. Moreover, another corticosteroid called ciclesonide, which is an inactive prodrug and activated by esterase in the lung to the metabolite des-ciclesonide, was highly effective in treating SARS-CoV-2 infection. These data strongly indicate that ciclesonide activity was specific to coronavirus; the authors of the aforementioned study have suggested that ciclesonide is a promising pharmaceutical to treat MERS or COVID-19 patients (Matsuyama et al., 2020).

Actually, clinical trials are already in course (Clinical trial.gov NCT04323592-Italy; NCT04343729-Brazil.

Recently, a clinical trial (randomized) has demonstrated that a low-price steroid named dexamethasone (low-dose) was able to prevent death in one over eight ventilated COVID-19 patients and one over of 25 patients receiving oxygen only. Over 11,500 patients have been enrolled from over 175 National Health Service (NHS) hospitals in the UK. (https://www.recoverytrial.net/files/recovery_dexamethasone_statement_160620_v2final.pdf) (Clinical trial gov.: NCT04381936). This study showed that dexamethasone diminished deaths by one-third in ventilated patients and by one fifth in patients receiving only oxygen. The medical staff implied that based on these outcomes, one death could be avoided by treatment of about eight ventilated patients or near 25 patients requiring oxygen alone.

Actually, there are several clinical trials in recruiting phase (NCT04325061-Spain; NCT04347980-France; NCT04395105-Argentina; NCT 04344730-Fance; NCT04327401-Brazil).

RNA-dependent RNA Polymerase

RNA-dependent RNA polymerase (RdRp) is another possible target against SARS-CoV-2. It is so, because SARS-CoV-2 is certainly a strand RNA virus, whose reproduction is based on a multiple subunit replication or/and transcription complex of viral unstructured proteins (nsp) (Ziebuhr, 2005). The main element of this structure is a subunit of catalytic activity (nsp12) of an RNA, which is RNA polymerase-dependent (RdRp) (Ahn et al., 2012; Te Velthus et al., 2010). It was observed that nsp12 has insignificant activity and that its functions need supplementary factors such as nsp7 and nsp8 (Subissi et al., 2014; Kirchdoerfer and Ward, 2019), which lead to increased RdRp scaffold binding and treatability. Remdesivir is one of the best antiviral targeting RdRps (Wang et al., 2020c; Holshue et al., 2020; Warren et al., 2016); this pro-drug is transformed into an active product derivative (triphosphate) form (RTP) in cells (Siegel et al., 2017). All structures related to RdRp were of rigorous structural biological

achievements (Kirchdoerfer and Ward, 2019; Zhai et al., 2005; Peti et al., 2005; Johnson et al., 2010; Gao et al., 2020), make available the complete design of the RdRp complex (Yin et al., 2020).

In order to understand the actual and in course antiviral activity against Covid-19 it will be an analysis of the actual pharmaceutics using on this disease.

Remdesivir

Remdesivir was effective against SARS and MERS in murine models (Holshue et al., 2020). It is an RNA polymerase-dependent RNA antagonist that leads to early viral RNA termination (Sheahan et al., 2017); this prodrug must undergo metabolization to enable its active form of GS-441524-triphospahate. Remdesivir presented excellent outcome during the West African Ebola virus epidemic (2013–2016) and the Kivu Ebola epidemic (2018) (Warren et al., 2015), a fact that also made this drug tested against COVID-19 (Wang et al., 2020b).

Remdesivir (RDV) and IFN-β have higher antiviral activity than lopinavir (LPV) and ritonavir (RTV) *in vitro*. Both prophylactic and therapeutic remdesivir applications have improved the pulmonary function, as well as reduced lung viral loads and severe lung pathology in mice. On the other hand, prophylactic LPV/RTV-IFN-β treatment has slightly reduced viral loads, although without affecting other disease parameters in the investigated model. Therapeutic LPV/RTV-IFN-β has enhanced the pulmonary function but it did not diminish virus replication or severe lung pathology in mice. This outcome provided evidence *in vivo* of remdesivir potential to treat MERS-CoV infections (Sheahan et a., 2020).

SARS-CoV-2 replication needs viral RNA-dependent RNA polymerase (RdRp), which is a target of the antiviral drug called remdesivir. The cryo-EM structure of the SARS-CoV-2 RdRp was reported either in its apo-form or in the complex with 50-base template-primer RNA and remdesivir can be a good solution under these conditions. This complex structure shows that the partial double-stranded RNA template is introduced in the central channel of the RdRp, where remdesivir is covalently added to the primer strand at the first replicated base pair and terminates the chain elongation. This structure provides critical insights about the viral RNA replication mechanism and a rational model for drug design to treat viral infections (Yin et al., 2020).

A clinical trial with double-blind, placebo-controlled and randomized of intravenous remdesivir was recently conducted with adult Covid-19 patients hospitalized with confirm of lower respiratory tract implication. All patients were randomly assigned to accept either remdesivir or placebo for up to 10 days. The total of 1,063 patients underwent randomization (ClinicalTrials.gov: NCT04280705-USA). Data and safety monitoring board advised early unblinding the results based on analysis findings that showed shortened recovery time in the remdesivir group. Initial results of 1,059 patients (538 treated with remdesivir and 521 with placebo) based on data available after randomization have indicated that individuals who received remdesivir recorded median recovery time of 11 days, whereas those who received placebo recorded median recovery time of 15 days. Based on Kaplan Meier's estimates for 14 days, remdesivir and placebo recorded 7.1% and 11.9% patient mortality, respectively. Severe adverse events were reported in the remdesivir (21.1%) and placebo (27.0%) groups subjected to randomization. Thus, remdesivir was more effective than placebo in shortening the recovery time in adult Covid-19 individuals hospitalized with evidence of lower respiratory tract involvement. However, given the high mortality rate observed despite remdesivir use, it is clear that treatments with antiviral drug alone are not likely to be

sufficient against Covid-19 (Wang et al., 2020; Beigel et al., 2020). A randomized, double-blind, placebo-controlled, multicenter trial conducted in China with 237 patients with laboratory-confirmed SARS-CoV-2 infection (ClinicalTrials.gov.: NCT04257656-China) has observed faster clinical improvement; however, adverse events were also reported in 102 (66%) of 155 remdesivir recipients and in 50 (64%) of 78 placebo recipients. Remdesivir treatment was interrupted early in 18 (12%) patients because of adverse events versus four (5%) patients who stopped placebo treatment early. The authors of the aforementioned study inferred that remdesivir was not related to statistically significant clinical benefits. However, further studies should be conducted in order to confirm the shorter time necessary for individuals subjected to early remdesivir treatment to present clinical improvement (Wang et al., 2020c).
Actually, clinical trials are already in course (ClinicalTrials.gov Identifier: NCT04365725-France; NCT04280705-USA; NCT04302766-USA).

Avifavir (Favipiravir)

Favipiravir (Fig.1) has been authorized as therapy to treat influenza in Japan. But, only suggested for new influenza (strains causing acute disease) instead than seasonal influenza (Shiraki and Daikoku, 2020).

The action´s mechanism is believe to be associated to the selective suppress of viral RNA-dependent RNA polymerase (Jin et al., 2013). Baranovivh et al. (2013) suggested that favipiravir caused lethal RNA transversion mutations, causing a no-viable viral phenotype (Baranovich et al., 2013). Favipiravir is a pro-drug which is metabolized to its reactive form, favipiravir-ribofuranosyl-5'-triphosphate (favipiravir-RTP), usable in both intravenous and oral formulations *(*Guedj et al., 2028). HGPRT (human hypoxanthine guanine phosphoribosyl-transferase) is presumed to play a crucial role in this activation activity. Interesting, that favipiravir acts as non-inhibitor of RNA or DNA synthesis in mammalian cells and is non-toxic to them. From 2014 is used in Japan, but, favipiravir has not been demonstrated to be efficient in primary human airway cells, causing doubt on its efficiency in influenza therapy (Yoon et al., 2018)
In 2020, favipiravir was used as treatment in China in emergent COVID-19 (Li and De Clercq, 2020) (https://www.fujifilmamericas.com.br/press/news/display_news?newsID=881796). A study on 80 patients comparing the use of lopinavir/ritonavir demonstrated that it diminished viral clearance period, and that 91% of patients had enhanced CT scans with almost no side effects. Unfortunately, this was not randomized double-blinded and placebo-controlled trial (Cai et al., 2020; Dong et al., 2020). Recently, the favipiravir has been approved for use in clinical trials of coronavirus disease in China (https://global.chinadaily.com.cn/a/202002/17/WS5e49efc2a310128217277fa3.html).

Italy, in March 2020, Italy authorized favipiravir for research use against COVID-19 and has initiated trials in three locals most involved in the contamination (https://www.ilfattoquotidiano.it/2020/03/22/coronavirus-il-veneto-sperimental-antivirale-giapponese-favipiravir-ma-laifa-ci-sono-scarse-evidenze-scientifiche-su-efficacia/5745426/).

There are in progress to initiate in three hospitals in Massachusetts, USA , trials with favipiravir (https://advances.massgeneral.org/research-and-innovation/article.aspx?id= 1171). In a first week of May 2020, a trial is initiating in London, UK. (https://www.standard.co.uk/news/uk/uk-coronavirus-patients-trial-japanese-covid19-drug-a4429731.html)

The favipiravir was also approved for the therapy of COVID-19 in the hospital installations in Russia on May 29, 2020, after taking place open-label randomized clinical trial had recruited 60 patients on. As claimed by the government clinical trial registry as COVID-FPR-01 is predicted to enroll 390 patients overall and end by December 31, 2020. On May 30, 2020, the Russian Health Ministry authorized a generic form of favipiravir called Avifavir. Russian Direct Investment Fund (RDIF) supported the exploration of Avifavir and established it high efficiency in the first stage of clinical trials (https://www.bnnbloomberg.ca/russian-health-ministry-approves-anti-coronavirus-drug-avifavir-1.1443601); https://www.reuters.com/article/ us-health-coronavirus-russia-cases/russia-plans-coronavirus-vaccine-clinical-trials-in-two-weeks-report-idUSKBN2360BJ).

Then, avifavir or favipiravir appears as a potential to treat Covid-19 in Russia and probably soon in England, Italy and USA.

Nucleocytoplasmic trafficking of viral proteins

Ivermectin

Protein signal-dependent targeting inside and outside the nucleus is regulated by components belonging to the importin (IMP) family of transport receptors, which are capable of identifying targeting signals in a load protein and of mediating transit over the complexes of nuclear pores in nuclear wrapping. This process is essential to activate cell division and differentiation; however, it is also crucial for viral reproduction and disease development. The phosphorylation process plays a key role in regulating viral protein nucleocytoplasmic trafficking and other post-translational transformations. Nucleocytoplasmic trafficking mechanisms are modulated by IMPs/EXPs (export members of the IMPβ superfamily) levels and distribution, as well as by the number and/or components of nuclear pore complexes (NPCs). One of the most investigated nuclear transport modulation mechanisms refers to the phosphorylation process near the NLS/NES (nuclear localization signal/nuclear export signal) and to change identification through IMP/EXP; however, transformations such as esterification (e.g., acetylation, ubiquitinoylation, among others) have also been reported to modulate the nucleocytoplasmic trafficking of cellular proteins (e.g., p53) and tumor suppressors ($p110^{Rb}$), survivin, PTEN (phosphatase and tensin homolog on chromosome 10), nuclear factor NF-κB and MEMO (NF-κB essential modulator). It is clear that viral proteins are often post-translationally transformed, and fully incorporate the action of cyclin-dependent kinases(CDKs), which account for the cell cycle-dependent regulation of nucleocytoplasmic trafficking (Fulcher and Jans, 2011; and references therein).

Ivermectin was the central point of a phase-III clinical trial conducted to investigate dengue virus (DENV) infection in Thailand in 2014–2017; results have shown that a single oral dose a day was safe and significantly reduced serum viral NS1 protein levels, although it did not lead to changes in viremia or presented clinical benefits (Yamasmith et al., 2018).

A study has demonstrated that ivermectin was capable of dissociating the pre-formed IMPα/β1 heterodimer, beside avoiding its generation by linking to the IMPα armadillo (ARM) repeat domain to enable IMPα thermal stability and α-helicity. Ivermectin has also inhibited a non-structured protein 5 deriving from dengue virus (NS5)-IMPα association in cell context (Yang et al., 2020).

Caly et al. (2020) have shown that ivermectin had antiviral action against SARS-CoV-2 clinical isolate *in vitro*; a single dose was capable of controlling viral replication within

24–48 h under that condition. It was hypothesized that it may have happened due to the inhibition of IMPα/β1-mediated nuclear import of viral proteins, as demonstrated in other RNA viruses. The confirmation of this mechanism in the case of SARS-CoV-2, and the identification of the specific SARS-CoV-2 and/or affected host constituent(s), is relevant topics to be investigated in future studies in this field (Figure 4).

According to Patel et al. (2020), the application of 150 μg/kg of ivermectin to patients after mechanical ventilation application has potential to diminish hospitalization time and to improve survival rates in comparison to conventional treatments. Unfortunately, this report did not take into consideration comorbidities that could lead to these results. Thus, if ivermectin really provided these clinical findings, it would suggest that the study *in vitro* conducted by Caly et al. (2020) did not correlate to low amounts of the drug at the action site in humans. A clinical study with ivermectin must follow well-controlled clinical dose-response at low dose (dose approved) and at dose higher than that of placebo in COVID-19 patients (Schmith et al, 2020). Ivermectin doses up to 120 mg have only been applied to a small number of patients (Guzzo et al., 2002). Daily ivermectin applications at the approved dose (200 μg/kg) for longer periods (e.g., 14 days) have only been investigated in case of severe infections where non-approved subcutaneous formulation was applied (Turner et al., 2005). Thus, if higher doses are applied on a weekly basis, or other administration method is adopted, patients should be closely monitored. Therefore, an interesting solution would lie on investigating whether ivermectin-inhaled therapy is realistic. Unfortunately, only one non-clinical research on inhaled ivermectin has been reported and presented NOAEL (no-observed-adverse-effect level) equal to 380 mg/m^3 after 1 month of treatment (Ji et al., 2016). The literature lacks studies focused on investigating this administration route in humans. Experts must evaluate whether ivermectin has the ideal properties for inhalation treatments and whether it does not present any risk that might limit this application route. Therefore, Schmith et al. (2020) suggested that the outcomes observed in the study by Caly et al. (2020) stablished the opportunity for an interdisciplinary collaboration to help better understand the best likelihood of achievement in ivermectin-based therapy, before the application of a less-than-ideal dose in clinical studies.

Actually, clinical trials are already in Couse (ClinicalTrials.gov Identifier: NCT04381884-Argentina; NCT04390022-Spain; NCT04392713-Pakistan; NCT04373824-India).

Final remarks

Based on data analyzed in the current study, it is clear that despite tremendous efforts to access a new and effective antiviral drug against SARS-CoV-2 in clinical trials conducted during the Covid-19 pandemic, an efficient antiviral drug has not yet been found. The problem observed in clinical trials carried out to research potential therapies against COVID-19 hampers both the requirement and ability to generate high-quality prove, even in the middle of the Covid-19 pandemic. Although there are different mechanisms capable of inhibiting or killing SARS-CoV-2 virus, it is possible believing that the scientific community will be able to find a successful pharmaceutical capable of finishing this pandemic. Once we get to fully know the pathogenesis of Covid-19, we will be able to select a compound capable of presenting efficient results *in vivo*, rather than *in vitro*. This, it is due to the projection of the activity from culture cells is negligible to animal and then worse to human. Thus, it is necessary developing a well-stablished protocol for clinical trials in order to find a new and safe antiviral drug.

It is important emphasizing that, in march 2020, WHO made public plans to initiate a global "megatrial" named SOLIDARITY, based on a practice trial design, for to randomize attested cases into either basic care or 1 of 4 active therapy arms (chloroquines, lopinavir/ritonavir alone, or lopinavir/ ritonavir plus IFN-β) based on regional medicone availability (Kupferschmidt and Cohen, 2020). It was a really interesting proposal at that time; however, unfortunately, all priorities have changed from March to June 2020 and it is necessary re-evaluating all these possibilities.

Acknowledgments

The authors would like to thank the São Paulo Research Council (FAPESP grant 2014/11154-1; 2018/10052-1), the Brazilian National Council for Scientific and Technological Development (CNPq grant 552120/2011-1) and Hospital Municipal de Paulínia, Paulínia-SP.

REFERENCES

Ahn DG, Choi JK, Taylor DR, *et al.* Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Arch Virol* 2012; **157**: 2095-2104.

Antony F, Vashi Y, Morla S. Therapeutic potential of Nitazoxanide against Newcastle disease virus: A possible modulation of host cytokines. *Cytokine* 2020; **131**: 155115.

Baranovich T, Wong SS, Armstrong J, et al. T-705 (favipiravir) induces lethal mutagenesis in influenza A H1N1 viruses *in vitro*. *J Virol* 2013; **87**: 3741–51

Barnes PJ. Inhaled corticosteroids. *Pharmaceuticals* 2010; **3**: 514-540.

Beigel JH, Tomashek KM, Dodd ÇLE, *et al*. Remdesivir for the treatment of Covid-19. *Preliminary report* May 22, 2020. Doi: 10.1056/NEJMoa2007764.

Borba MGD, Val FFA, Sampaio VS, *et al*. Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: A randomized clinical trial. *JAMA Network Open* 2020; **3**: e208857.

Caly L, Druce JD, Catton MG, *et al*. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res* 2020; **178**: 1047872.

Chang D, Lin M, Wei L, *et al*. Epidemiologic and clinical characteristics of novel coronavirus infections involving 13 patients outside Wuhan, China. *JAMA* 2020; **323**: 1092-1093.

Cai Q, Yang M, Liu D, et al. Experimental treatment with favipiravir for COVID-19: An open-label control study. *Engineering* 2020; doi:10.1016/j.eng.2020.03.007.

Chen SX, Wang N, Zhu Y, *et al*. The antimalarial chloroquine suppresses LPS-induced NLRP3 inflammasome activation and confers protection against murine endotoxic. *Mediat Inflamm* 2017; **2017**: Article ID 6543237.

Chen C., Qi F, Shi K, *et al*. Thalidomide combined with low-dose glucocorticoid in the treatment of COVID-19 Pneumonia. *Preprints* 2020b; **2020:** 2020020395 .

Chen N, Zhou M, Dong X, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet* 2020a; **395**: 507-513.

da Silva JC Mariz HA, da Rocha Júnior LF, *et al*. Hydroxychloroquine decreases Th17-related cytokines in systemic lupus erythematosus and rheumatoid arthritis patients. *Clinics* 2013; **68**:766-771.

Dastan F, Tabarsi P, Marjani M, *et al*. Thalidomide against Coronavirus Disease 2019 (COVID-19): A medicine with a thousand faces. *Iran J Pharm Res* 2020; **19**: 1-2.

Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov Ther* 2020; **14:** 58–60.

Durán N, Fávaro WJ. Pyroptosis in cancer cells and its perspectives in nanomedicine: A mini review. *Inter J Med Rev* 2020a; Submitted.

Durán N, Fávaro WJ. Pyroptosis: Physiological roles in viral infection. *medRxiv Preprint* 2020b; Submitted.

Fulcher AJ, Jans DA. Regulation of nucleocytoplasmic trafficking of viral proteins: An integral role in pathogenesis? *Biochim Biophys Acta* 2011; **1813**: 2176-2190.

Geleris J, Sun Y, Platt J, *et al*. Observational study of hydroxychloroquine in hospitalized patients with Covid-19. *N Engl J Med* 2020; DOI: 10.1056/NEJMoa2012410

Gao Y, Yan L, Huang Y, *et al*. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science* 2020. Doi: 10.1126/science.abb7498.

Guedj J, Piorkowski G, Jacquot F, et al. Antiviral efficacy of favipiravir against Ebola virus: A translational study in cynomolgus macaques. *PLOS Med* 2018; **15**: e1002535.

Guzzo CA, Furtek CI, Porras AG, *et al*. Safety, tolerability, and pharmacokinetics of escalating high doses of ivermectin in healthy adult subjects. *J Clin Pharmacol*. 2020; **42**: 1122-1133.

Henry BM, de Oliveira MHS, Benoit S, *et al*. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): A meta-analysis. *Clin Chem Lab Med* 2020*;* https://doi.org/10.1515/cclm-2020-0369.

Hickson SE, Margineantua D, Hockenbery DN, *et al*. Inhibition of vaccinia virus replication by nitazoxanide. *Virology* 2018; **518**: 398-405.

Holshue, M.L., DeBolt, C., Lindquist, S, *et al*. First case of 2019 novel coronavirus in the United States. *N Engl J Med* 2020; **382:** 929-936.

Jin Z, Smith LK, Rajwanshi VK, et al. The ambiguous base-pairing and high substrate efficiency of T-705 (Favipiravir) Ribofuranosyl 5'-triphosphate towards influenza A virus polymerase. *PLOS One* 2013; **8**: e68347.

Johnson MA, Jaudzems K, Wüthrich K. NMR Structure of the SARS-CoV Nonstructural Protein 7 in Solution at pH 6.5. *J Mol. Biol* 2010; **402**, 619-628.

Huang C, Wang Y. Li X, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020; **395**: 497-506.

Jakobsen MR, Bak RO, Andersen A, *et al*. IFI16 senses DNA forms of the lentiviral replication cycle and controls HIV-1 replication. *Proc Nat Acad Sci USA*. 2013; **110**: E4571-4580.

Ji L, Cen J, Lin S, *et al*. Study on the subacute inhalation toxicity of ivermectin TC in rats. *Chin J Comparative Med* 2016; **26**: 70-74.

Keller M, Sollberger G, Beer H-C. Thalidomide inhibits activation of caspase-1. *J Immunol* 2009; **183**: 5593-5599.

Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat Commun* 2019; **10**: 2342.

Kupferschmidt K, Cohen J. WHO launches global megatrial of the four most promising coronavirus treatments. *Science.* Published March 22, 2020. Accessed March 23, 2020. https://www. sciencemag.org/news/2020/03/who-launchesglobal-megatrial-four-most-promising-coronavirustreatments#. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments

Lam MF, Ooi GC, Lam B, *et al*. An indolent case of severe acute respiratory syndrome. *Am J Respir Crit Care Med* 2004; **169**:125-128

Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov* 2020; Doi:10.1038/d41573-020-00016-0

Liang F, Zhang F, Zhang L, *et al*. The advances in pyroptosis initiated by infammasome in infammatory and immune diseases. *Inflamm Res* 2020; **69**:159-166.
Lippi G, Plebani M. Laboratory abnormalities in patients with COVID-2019 infection. *Clin Chem Lab Med* 2020; https://doi.org/10.1515/cclm-2020-0198.

Ma YZ, Jiang JX, Gao Y, *et al*. Research progress of the relationship between pyroptosis and disease. *Am J Transl Res* 2018; **10:** 2213-2219.

Mali SN, Pratap AP, Thorat BR. The rise of new coronavirus infection (COVID-19): A recent update and potential therapeutic candidates. *Eurasian J Med Oncol* (EJMO) 2020; **4**: 35-41.

Matsuyama S, Kawase M, Nao N, *et al*. The inhaled corticosteroid ciclesonide blocks coronavirus RNA replication by targeting viral NSP15. *bioRxiv preprint* Doi: https://doi.org/10.1101/2020.03.11.987016.

Qin Y-Y, Zhou Y-H, Lu Y-Q, *et al*. Effectiveness of glucocorticoid therapy in patients with severe coronavirus disease 2019: Protocol of a randomized controlled trial. *Chin Med J* 2020; Doi: 10.1097/CM9.0000000000000791.

Patel A, Desai S. Ivermectin in COVID-19 Related Critical Illness.. 2020; *SSRN* 2020; https://ssrncom/abstract=3570270 or http://dxdoiorg/102139/ssrn3570270.

Peti EW, Johnson MA, Herrmann T, *et al*. Structural genomics of the severe acute respiratory syndrome coronavirus: Nuclear magnetic resonance structure of the protein nsP7. *J Virol* 2005; **79**: 12905-12913.

Rosa SGV, Santos WC. Clinical trials on drug repositioning for COVID-19 treatment. *Rev Panam Salud Publica*. 2020; **44**:e40. https://doi.org/10.26633/RPSP.2020.40

Rosenberg ES, Dufort EM, Udo T, *et al*. Association of treatment with hydroxychloroquine or azithromycin with in-hospital mortality in patients with COVID-19 in New York State. *JAMA* 2020; Doi:10.1001/jama.2020.8630

Rossignol JF. Nitazoxanide, a new drug candidate for the treatment of Middle East respiratory syndrome coronavirus. *J Infect Public Heal* 2016; **9**: 227-230.

Sanders JM, Monogue ML, Tomasz Z, *et al*. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): A review. *JAMA* 2020; **323**:1824-1836.

Schmith VD, Zhou J, Lohmer LRL. The Approved dose of ivermectin alone is not the ideal dose for the treatment of COVID-19. *medRxiv Preprint* doi: https://doi.org/10.1101/2020.04.21.20073262

Siegel D, Hui HC, Doerffler E, *et al*. Discovery and synthesis of a phosphoramidate prodrug of a pyrrolo[2,1-f][triazin-4-amino] adenine Cnucleoside (GS-5734) for the treatment of Ebola and emerging viruses. *J Med Chem* 2017; **60**:1648-1661.

Sheahan TP, Sims AC, Leist SR, *et al*. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERSCoV. *Nat Commun* 2020; **11**: 222.

Shiraki K, Daikoku T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. *Pharmacol Ther* 2020; **209**:107512.

Sollberger G, Strittmatter GE, Garstkiewicz M, *et al*. Caspase-1: the inflammasome and beyond. *Innate Immun* 2014; **20**:115-125.

Subissi L, Posthuma CC, Collet A, *et al*. One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. *Proc Nat. Acad Sci USA* 2014; **111**: E3900-E3909.

Tabata C, Tabata R, Takahashi Y, et al. Thalidomide prevents cigarette smoke extract-induced lung damage in mice. *Inter Immunopharmacol* 2015; 25: 511-517.

Tay MZ, Poh CM, Rénia L, *et al*. The trinity of COVID-19: immunity, inflammation and intervention. *Nat Rev Innmunol* 2020; https://doi.org/10.1038/s41577-020-0311-8.

Te Velthuis AJW, Arnold JJ, Cameron CE, *et al*. The RNA polymerase activity of SARS-coronavirus nsp12 is primer dependent. *Nucleic Acids Res* 2010; **38**: 203-214 (2010).

Thi HTH, Hong S. Inflammasome as a therapeutic target for cancer prevention and treatment review. *J Cancer Prev* 2017; **22**: 62-63.

Turner SA, Maclean JD, Fleckenstein L, *et al*. Parenteral administration of ivermectin in a patient with disseminated strongyloidiasis. *Am J Trop Med Hyg* 2005; **73**: 911-914.

Wang M, Cao R, L. Zhang L, *et al*. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* 2020; **30**: 269-271.

Wang M, Cao, R, Zhang L, *et al*. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) *in vitro*. *Cell Res* 2020a; **30**: 269-271

Wang Y, Jiang W, He Q, *et al*. A retrospective cohort study of methylprednisolone therapy in severe patients with COVID-19 pneumonia. *Signal Transduc Target Ther* 2020b; **5**: 57

Wang Y, Zhang D, Du, G, *et al*. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020c; **395**: 1569-1578.

Warren T, Jordan R, Lo M, *et al*. Nucleotide prodrug GS-5734 is a broad-spectrum filovirus inhibitor that provides complete therapeutic protection against the development of Ebola virus disease (EVD) in infected non-human primates. *Open Forum Infect Dis 2* (Suppl. 1). 2015; LB-2. https://doi.org/10.1093/ofid/ofv130.02.

Warren TK, Jordan R, Lo MK, *et al*. Therapeutic efficacy of the small molecule GS-5734 against Ebola virus in rhesus monkeys. *Nature* 2016; **531:** 381-385.

Yamasnith E, Saleh-arong FA, Aviruntan P, *et al*. Efficacy and safety of ivermectin against dengue infection: a phase III, randomized, double-blind, placebo-controlled trial, In: *The 34th Annual Meeting The Royal College of Physicians of Thailand*, Internal Medicine and One Health. 2018, Pattaya, Chonburi, Thailand.

Yang, M. Cell pyroptosis, a potential pathogenic mechanism of 2019-nCoV Infection *SSRN,* 2020 ;https://ssrn.com/abstract=3527420; http://dx.doi.org/10.2139/ssrn.3527420.

Yang Y, Peng F, Wang R, *et al*. The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. *J Autoimmun* 2020a; **109**: 102434.

Yang SNY, Atkinson C, Wang C, *et al*. The broad spectrum antiviral ivermectin targets the host nuclear transport importin α/β1 heterodimer. *Antiviral Res* 2020b; **177**: 104760.

Yin W, Mao C, Luan X., *et al*. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science* 2020; Doi: 10.1126/science.abc1560.

Yoon JJ, Toots M, Lee S, et al. Orally efficacious broad-spectrum ribonucleoside analog inhibitor of influenza and respiratory syncytial viruses. *Antimicrob Agents Chemother* 2018; **62**: e00766–18.

Zeng C, Wang R, Tan H. Role of pyroptosis in cardiovascular diseases and its therapeutic implications. *Inter J Biol Sci* 2019; **15**:1345-1357.

Ziebuhr J. The coronavirus replicase. *Curr Top Microbiol Immunol* 2005; **287**: 57-94.

Zhai Y, Sun F, Li X, *et al*. Insights into SARS-CoV transcription and replication from the structure of the nsp7-nsp8 hexadecamer. *Nat Struct Mol Biol* 2005; **12**: 980-986.

Zhang R, Wang X, Ni L, *et al*. COVID-19: Melatonin as a potential adjuvant treatment. *Life Sci*. 2020a; **250**: 117583.

Zhao C, Zhao W. NLRP3 Inflammasome:A key player in antiviral responses. *Front Immunol* 2020; **11**:211 (and reference therein).

FIGURES

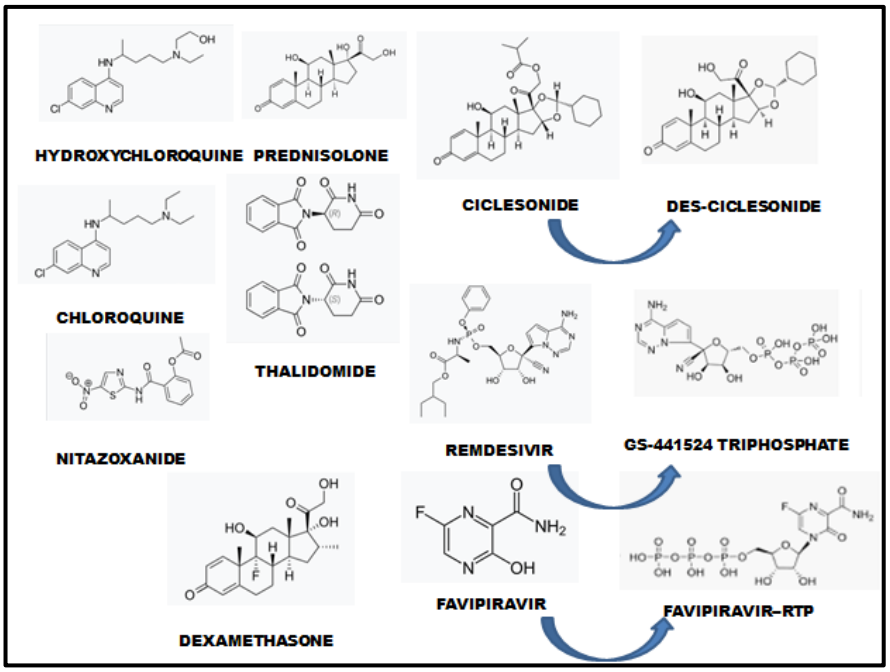

Figure 1. Potential antiviral pharmaceutics.

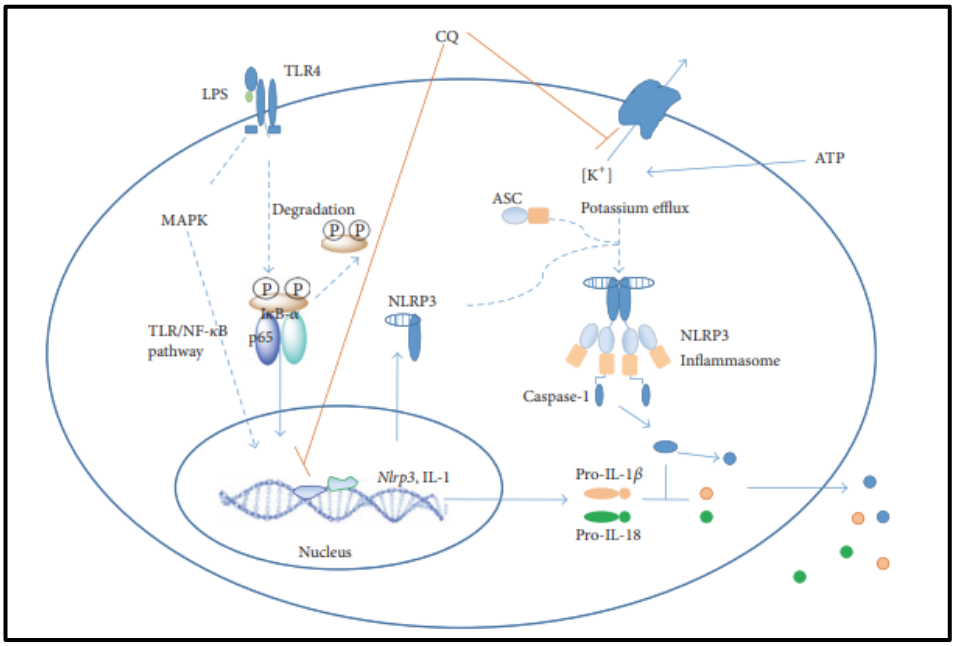

Figure 2. The proposed mechanism of Chloroquine for the inhibition of NLRP3 inflammasome activation (extracted from ref. Chen et al., 2017 and under Creative Commons Attribution License approved by Hindawi).

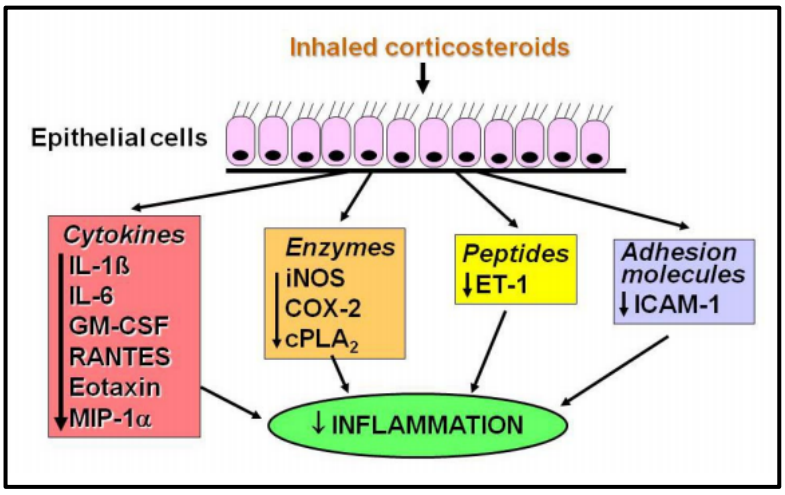

Figure 3. Inhaled corticosteroids may inhibit the transcription of several inflammatory genes in airway epithelial cells and thus reduce inflammation in the airway wall. GM-CSF = granulocyte-macrophage colony stimulating factor; IL-1 = interleukin-1; RANTES = regulated on activation, normal T cell expressed and secreted; Eotaxin = potent and selective chemoattractant for eosinophils; MIP-1α = macrophage inflammatory protein; iNOS = inducible nitric oxide synthase; COX-2 = inducible cyclooxygenase; cPLA2 = cytoplasmic phospholipase A2; ET = endothelin; ICAM = intercellular adhesion molecule (extracted from ref. Barnes, 2010; authorized by MDPI (Basel, Switzerland- under Creative Commons Attribution license) .

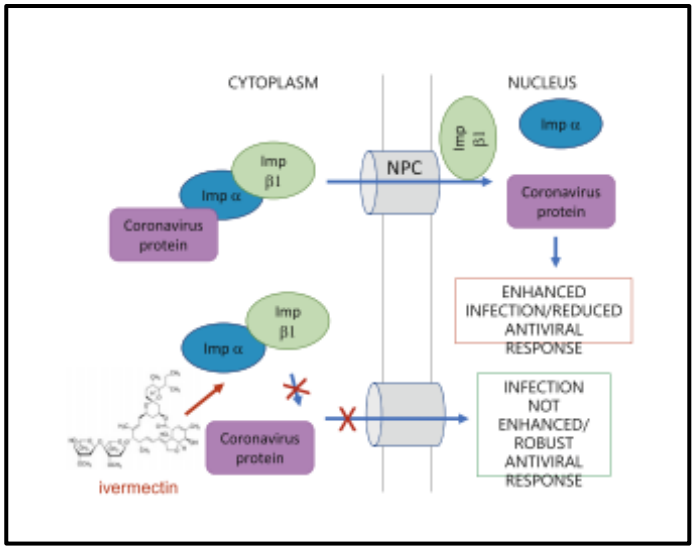

Figure 4. Ivermectin is a potent inhibitor of the SARS-CoV-2 clinical isolate Australia/VIC01/2020. Vero/hSLAM cells were in infected with SARS-CoV-2 clinical isolate Australia/VIC01/2020 (MOI = 0.1) for 2 h prior to addition of vehicle (DMSO) or Ivermectin at the indicated concentrations. Samples were taken at 0–3 days post infection for quantitation of viral load using real-time PCR of cell associated virus (A) or supernatant (B). IC50 values were determined in subsequent experiments at 48 h post infection using the indicated concentrations of Ivermectin (treated at 2 h post infection as per A/B). Triplicate real-time PCR analysis was performed on cell associated virus (C/E) or supernatant (D/F) using probes against either the SARS-CoV-2 E (C/D) or RdRp (E/F) genes. Results represent mean ± SD (n = 3). 3 parameter dose response curves were fitted using GraphPad prism to determine IC50 values (indicated). G. Schematic of ivermectin's proposed antiviral action on coronavirus. IMPα/β1 binds to the coronavirus cargo protein in the cytoplasm (top) and translocates it through the nuclear pore complex (NPC) into the nucleus where the complex falls apart and the viral cargo can reduce the host cell's antiviral response, leading to enhanced infection. Ivermectin binds to and destabilises the Impα/β1 heterodimer thereby preventing Impα/β1 from binding to the viral protein (bottom) and preventing it from entering the nucleus. This likely results in reduced inhibition of the antiviral responses, leading to a normal, more efficient antiviral response (extracted from ref. Caly et al., 2020; authorized by Elsevier B.V.).