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Abbreviations: ACE2: angiotensin converting enzyme 2; AIDS: Acquired Immunodeficiency Syndrome; ARDS: Acute Respiratory Distress Syndrome; Covid-19: Coronavirus disease 2019; DAA: Direct Acting Antivirals; EBV: Epstein-Barr virus; HCV: Hepatitis C virus; HIV: Human Immunodeficiency virus; IAA: Indirect Acting Antivirals; IFN: interferon; IL: interleukin; ISG: Interferon-Stimulated Genes; IVig: Intravenous Immunoglobulin Therapy; MERS-CoV: Middle East respiratory syndrome-related coronavirus; RSV: Respiratory Syncytial Virus. SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus.

The majority of SARS-CoV-2-infected individuals remain paucisymptomatic, contrasting with a minority of infected individuals in danger of death. Here, we speculate that the robust disease resistance of most individuals is due to a swift production of type I interferon (IFNα/β), presumably sufficient to lower the viremia. A minority of infected individuals with a preexisting chronic inflammatory state fail to mount this early efficient response, leading to a delayed harmful inflammatory response. To improve the epidemiological scenario, we propose combining: i) the development of efficient antivirals administered early enough to assist in the production of endogenous IFNα/β; ii) potentiating early IFN responses; iii) administering anti-inflammatory treatments when needed, but not too early to interfere with endogenous antiviral responses.

Zoonotic RNA viruses provide an infinite reservoir of potentially dangerous emerging viruses, impossible to eradicate from the wild. SARS-CoV-2 mediates a respiratory infection difficult to deal with during an asymptomatic and contagious incubation period. An average **R0** (see Glossary) of ~3 (*i.*e., one infected person infecting an average of 3 other persons) ensures a vigorous spread of the SARS-CoV-2 associated disease **COVID-19**, in the absence of containment measures, enough to overwhelm most clinical/hospital settings witnessing a **fatality/case ratio** (FCR) ranging from 0.03 to 30% across patient aged <17 to >85 years old, respectively [1]. Past problematic pandemics comparable to the present SARS-CoV-2 crisis include the "1918" Influenza, the 1957-1958 Asian Influenza, and the 1968 Hong Kong Influenza. The "1918" Influenza (a H1N1 pandemic) had an estimated 2% fatality case rate which led to an astonishing number of more than 40 million fatality cases [2].

The fact that most SARS-CoV-2 infected individuals remain pauci- or asymptomatic suggests that an efficient immune response can be mounted against this virus. A first concern would therefore be to increase the probability of occurrence of this spontaneous response. When this first immune response is defective and the disease worsens, the major hurdle becomes the cytokine storm, which may lead to **ARDS (Acute Respiratory Distress Syndrome)**. Endeavors to prevent the tipping into this phase is another key issue. This Opinion article examines these different questions (Figure 1) - from virology to immunology. We analyze the scientific basis by which antivirals are being considered and tested in clinical settings to treat COVID-19. We mention two molecules capable of increasing the spontaneous antiviral response and propose several approaches that might be used to dampen the virus-induced cytokine storm. The conclusion will bear on how these virological and immunological approaches should be combined, for present and future pandemics.

Looking for efficient anti-COVID-19 antivirals

The birth of 'successful' antiviral therapies can be traced back to the fight against Human Immunodeficiency Virus (HIV), which became deeply influential for the Hepatitis C virus (HCV) field and provided a clear start for antiviral discoveries against non-chronic RNA viruses [3–8]. All antiviral molecules target at least one part of the virus life-cycle, (Figure 2), and are classified as early (entry) or late (replication) inhibitors.

Antiviral drugs having a viral target are called **Direct Acting Antivirals (DAAs)**, while those having an antiviral effect through host cell proteins are called Indirect Acting Antivirals (IAA). Since the mutation rate of cellular genes is lower than that of viruses, drug resistance is less likely to occur with IAAs [9]. Examples are the pegylated Type I Interferons (IFNs) against HCV, and the CCR5 antagonist maraviroc, targeting HIV-1 entry. None of them are first-line therapeutic choices for HCV or HIV-1-infected patients any longer, though. However, for COVID-19, the efficacy of Type I IFN needs to be evaluated, and a drug targeting SARS-CoV-2 entry is being tested. Indeed, baricitinib, targeting the janus kinase involved in endocytosis of angiotensin□converting enzyme 2 (**ACE2**), the SARS-CoV2 receptor, is an potential candidate [10] and has shown some activity against COVID-19 in a recent clinical trial (NCT04401579[I]). Accordingly, the TMPRSS2 protease, which also influences ACE2 endocytosis [11], might also constitute a potential target.

Based on HIV-1 and HCV research, an essential effort should be placed on DAAs, which generally target the most conserved viral enzymes: they remain efficacious within a given viral family, and might be tested as broad-spectrum antivirals, even for future outbreaks. There is, for example, a high probability that a nucleoside analog active against SARS-CoV might also work against SARS-CoV-2 and MERS-CoV, given that their RNA-dependent RNA polymerases are structurally and functionally conserved [12]. This may not apply for the Spike protein of SARS-CoV2, be it for vaccine or antiviral design, given that its receptor binding domain sequence differs significantly between coronavirus strains [12,13].

Discovery of efficient antivirals might be done by proper drug repositioning, i.e. using a drug that is already efficient against another disease, as illustrated by **favipiravir**. Favipiravir is a purine-base analog which is converted in the cell into a 5'-triphosphate nucleoside analog. Once incorporated into viral RNA, it selectively alters the genetic make-up of RNA viruses [14]. Favipiravir was initially designed and approved against Influenza virus [15]. However, a similar favipiravir mode-of-action has been recently described for SARS-CoV-2 [16]. Thus, it is now repositioned against SARS-CoV-2 and currently being tested in clinical trials for COVID-19 (NCT04358549[II], NCT04373733[III]). Nevertheless, its efficacy against COVID-19 is still unknown. **Remdesivir**, a purine nucleotide analog initially developed against Ebola virus, showed good and moderate efficacy *in vitro* and *in vivo*, respectively, and is currently the sole FDA-approved drug in the treatment of COVID-19 [17]. However, the remdesivir efficacy remains a controversial issue [18].

Unfortunately, drug repositioning is sometimes attempted without scientific bases. In Box 1 are examples of DAAs, currently repositioned in clinical trials for COVID-19 without solid scientific basis. In Box 2, we discuss the case of hydroxychloroquine (HCQ), a highly controversial anti-viral molecule.

In summary, despite their strong need, few sound candidate antivirals have been identified. They include bariticinib, expected to block the entry of SARS-CoV-2 in ACE2-expressing cells, favipiravir and remdesivir which target viral replication.

The natural antiviral immune response and its reinforcement

All viruses trigger an antiviral response that relies on the immediate production of IFNβ in the host. The binding of IFNβ to its receptor IFNAR then triggers the production of IFNα. Both IFNβ and IFNα bind the receptor IFNAR, with different affinities [19]. Both IFNs trigger the expression of hundreds of ISG (Interferon-Stimulated Genes) [20,21]. All cell types are able to produce IFNα, but pDCs (plasmacytoid dendritic cells) can rapidly produce large amounts of this cytokine [22]. If the production of IFNα/β takes place immediately and is intense enough, the infection can be stopped. Although this remains to be demonstrated, this is probably what happens for SARS-CoV2-infected individuals who remain asymptomatic or paucisymptomatic, as in almost all children.

However, the virus-induced IFNα/β response may be weak, due to aging, comorbidities, and to anti-IFN mechanisms that most viruses have developed throughout millions of years of co-evolution with vertebrates [23,24]. In such situations, the virus replicates, and this triggers a second inflammatory/immune response, which may become explosive and potentially result in a cytokine storm and ARDS.

All **coronaviruses** (for review, see [25]) have developed multiple mechanisms for blocking IFNβ production or signaling in infected cells [26–28]. During the replication process of RNA viruses, double-stranded RNA (dsRNA) can be detected by receptors such as Toll like-receptor 3 (TLR3) or RIG-I-like (retinoic acid-inducible gene-I-like), and activate the IFNα/β response. However, coronaviruses hide their dsRNA replication/transcription intermediates within double-membrane vesicles that prevent detection by TLR3 [29,30] or RIG-1 [31,32]. Numerous nuclear shuttle protein (NSP) proteins (1,3,13 and 15), accessory open reading frames (ORF) proteins (3b,4ba and 6), M and N proteins from various coronavirus (MERS, SARS-CoV) have also been shown to prevent IFNα/β induction in human cell lines [3–8].

Another mechanism quite likely to occur but never reported so far, is the involvement of Transforming growth factor β (**TGFβ**) in coronavirus-induced inhibition of IFNα/β. Indeed, SARS-CoV can prevent the phosphorylation and nuclear translocation of IRF3, a key transcription factor for IFNβ induction, by a mechanism involving viral protease **PLpro** in human promonocyte cells [33]. PLpro can significantly increase the expression of TGFβ *in vitro* in the same cells [33].

Also, higher serum concentrations of TGFβ were measured in early stage SARS-CoV patients, compared to age-matched normal controls [34]. The same difference in serum TGFβ was observed between severe and mild SARS-CoV-2-infected patients [35]. Moreover, TGFβ can be an effective blocker of IRF3 phosphorylation, fully preventing its nuclear translocation and IFNβ signaling [36,37] in particular in myeloid cells, as shown in a mouse tumor model of breast cancer. In addition, in human THP-1 macrophages, TGFβ inhibits the production of pro-inflammatory cytokines, including IFNβ [38]. There is a wealth of data underlining the immunosuppressive action of TGFβ in cancer [36] as well as during viral infections [39], suggesting that relieving this suppression might increase the efficacy of the immune response.

For allowing an efficient IFNα/β production, one could aim not only at alleviating a TGFβ-dependent brake, but also at potentiating its production. One such possibility could hypothetically be offered by **1,8-cineole**, a small molecule capable of amplifying an immune response dependent on the IRF3/IFNβ pathway [40], as demonstrated in healthy human tissue maintained for several days in culture, in response to **poly (I: C)** stimulation. In this work, performed with biopsy slices of nasal mucosa isolated during nasal surgery, cineole accelerated the poly (I: C)-induced nuclear translocation of IRF3 [40]. What remains to be established is whether this potentiation of IRF3 activation translates, as expected, in a faster production of IFNα/β. Such a possibility must be examined, given that efficient ways of potentiating IFNα/β production are not available so far.

Altogether, a swift and vigorous IFNα/β increase in necessary to inhibit viral replication. We speculate that potentiating its production with 1,8-cineole or with the blockade of TGFβ signaling deserves to be rigorously tested. In addition, whether administering exogenous IFNα, either inhaled or injected subcutaneously when symptoms become clear, might be beneficial to the host, is another direction to be investigated, without forgetting the well-known adverse effects of IFNα/β injections [41].

Cytokine storm and ARDS

One main complication of unresolved viral infections can include a cytokine storm that occurs when many leucocytes, mainly macrophages, become over-activated and secrete pro-inflammatory cytokines. The system then triggers an uncontrolled positive feedback, with these cytokines activating more leukocytes. If not properly treated, it may rapidly result in ARDS, multi-organ failure and potentially death [42].

The role of macrophages in the cytokine storm is central [42]. The release by these cells of exuberant pro-inflammatory cytokines and chemokines can follow massive epithelial and endothelial cell apoptosis and vascular leakage caused by early and rapid viral replication [42]. A key process triggering the cytokine storm is pyroptosis, or

pro-inflammatory programmed cell death [43], affecting mostly macrophages but also lymphocytes; during pyroptosis, the **inflammasome** of murine macrophages and African green monkey kidney-derived Vero E6 cells have been respectively activated via viroporin 3a and E protein, two SARS-CoV proteins [44,45]. In addition, in SARS-CoV infected Chinese macaques, binding of SARS-CoV-IgG complexes to monocyte/macrophage Fc receptors promoted inflammasome activation, the subsequent production of a large amount of proinflammatory cytokines in the lungs, and frequent fatal acute lung injury [46].

Twenty years ago, the concept of '**inflammaging'** was proposed. It helps to explain the weakness of the immune system in the elderly [47]. One specific aspect of it underlines the importance of Interleukin (IL)-6. The plasma concentrations of IL-6 are low or undetectable in most young individuals and begin increasing in healthy individuals at approximately 50–60 years of age [47]. In the elderly, the plasma concentration of IL-6 is elevated [48] but not that of Tumor Necrosis Factor (TNF)α or IL-1β [49,50]. We posit that Inflammaging might potentially contribute to explaining the predominant susceptibility of the elderly to COVID-19, at least in part [51]. Specifically, several aging-related characteristics have been correlated with most COVID-19 fatalities, generally consisting of individuals older than 70, with a median age of a COVID19-induced death of 80 in Italy [52]. These age-related features concern namely, i) the presence of subclinical systemic inflammation without overt disease, ii) a blunted acquired immune system and IFNα/β response, as shown by comparing young and old macaques infected with SARS-CoV [53] and iii) a dramatic reduction of ACE2 expression, demonstrated in old versus young rats relative to uninfected controls [54]. An aging-dependent reduction in anti-inflammatory ACE2 activity is likely to worsen SARS-CoV-2 infection outcomes [52]. These possibilities remain conjectural at this point, and the contribution of inflammaging to COVID-19 disease severity will have to be robustly assessed.

Altogether, an excessive and prolonged inflammatory response leading to ARDS may underlie the main danger for SARS-CoV-2-infected patients. A major factor favoring its occurrence may be inflammaging, accompanied by elevated and persistent serum pro-inflammatory IL-6 in aged individuals, and by a low expression of ACE2 in the lung of aged animals compared to healthy controls. A variety of approaches to treating a cytokine storm are discussed in Box 3.

Kinetics of the anti-viral immune response, a key issue

Figure 3 illustrates hypothetical kinetics of viral load and cytokine production in young versus aged individuals infected with SARS-CoV2. In this model, resistance to the virus would be conditioned by the ability to mount a fast IFNα/β response (top panel) despite the anti-IFN arsenal of the virus, thus allowing a status of low viremia. This speculation is based on a large amount of data on the key antiviral role of IFNα/β. In susceptible individuals, a slowly growing

IFNα/β response might be unable to control the viremia, presumably allowing a strong inflammatory response to follow (second panel).

The link between the kinetics of IFNα/β responses and viremia has been established with a mouse-adapted strain of SARS-CoV [55]. In mice infected with a lethal dose of SARS-CoV, an early IFNα/β response was clearly beneficial, whereas the inhibition of this early response combined with a late IFN response, was absolutely deleterious, as shown by lung immunopathology, vascular leakage, and suboptimal T cell responses [55]. The efficient response depended on the ability of plasmocytoid DCs to mount an initial IFNα/β production in these mice [55]. The lack of beneficial effect of IFNα when administered too late, at the ARDS stage, has also been reported in patients infected with MERS-CoV [56].

Similarly, humans that are unable to mount a robust IFNα/β response, e.g., due to STAT1 or TYK2 deficiencies, are overly sensitive to a virus such as HSV-1 [57]. At least 10% of 987 patients with life-threatening COVID-19 pneumonia have been reported to harbor neutralizing IgG auto-antibodies against type I IFNs at the onset of the critical disease. These auto-autoantibodies, which neutralize the ability of the corresponding type I IFNs to block SARS-CoV-2 infection in vitro, were totally absent in 663 individuals with asymptomatic or mild SARS-CoV-2 infection [58].

If an immune response remains sustained instead of being transient, it may progressively lose its efficacy and be replaced by a harmful inflammatory state. The loss of efficacy results in part from the '**exhaustion**' of T cells subjected to chronic stimulation [59]. The inflammatory state might be a consequence of excessive cell-death, due to large tissue damage [60]. Such a hypothesis is based on experimental models, and on human findings, as illustrated below.

EBV is an example of a viral infection that is properly controlled in most humans. First, EBV infection leads to a transient intense immune response that lowers viremia and is accompanied by signs of transient inflammation, including fever [61]. This response is usually not followed by persistent inflammation, but in a second phase, viremia is kept under control due to the dynamic process of expansion and contraction of memory $CD4^+$ and $CD8^+$ T cell populations [62,63]. Thus, the virus is not eradicated, but it is symbiotic with the host. However, in immunocompromised individuals who are unable to mount an intense antiviral response, this virus may become dangerous [64], and can be associated with several malignancies, e.g., endemic Burkitt's lymphoma, nasopharyngeal carcinoma, and Hodgkin's lymphoma [65].

An example of an inappropriate antiviral immune response is that occurring upon HIV-1 infection. In this case, the initial antiviral response is usually unable to lower viremia enough to avoid progressive weakening of the adaptive response (in part because activated $CD4^+$ T cells are directly targeted by this virus), and an ensuing chronic inflammatory state. According to our hypothesis, if the initial attack against HIV-1 infection were to be stronger, it might presumably dampen the development of this harmful inflammation. Accordingly, although not a direct

demonstration of this hypothesis, in a study of HIV-1-uninfected young women at high risk of infection who later became infected during follow-up, an early HIV-1-induced cytokine storm was minimized in the subgroup who was given antiretroviral therapy as soon as viral RNA could be detected, and not just after serological tests indicated positivity for the virus [66]. This suggests that an early efficient antiviral treatment might be a solid option for minimizing the cytokine storm.

Furthermore, the importance of a biphasic immune response is illustrated by monkeys infected with Simian Immunodeficiency Virus (SIV). The natural hosts, African Green Monkeys, develop a strong but transient IFNα/β response following infection, allowing them to control SIV without developing chronic pathology. By contrast, Rhesus Monkeys have shown a sustained IFNα/β response to SIV infection, and progressively develop chronic pathology that is similar to AIDS [67,68]. This suggests that a strong early rise in IFNα/β may be key for preventing a delayed, chronic, deleterious IFNα/β increase.

In another example, bats can be persistently infected with many viruses but rarely display clinical symptoms [69]. A viral infection usually triggers a rapid and significant - but transient - IFNα/β response, without evidence of subsequent persistent inflammation [70]. *in vitro*, macrophages obtained from bats or mice respond differently to stimulation from a TLR3 ligand [70]. Compared to murine macrophages, bat macrophages initially produce more IFNα/β, and more IL-10 [70]. If the same is true in vivo, a concept that is potentially ignored, it might lead to an optimal, swift, intense, and transient antiviral response. Of note, bats have developed an efficient antioxidant arsenal [71]. Thus, one might speculate that these different characteristics could help virus-infected bats to better control viremia compared with other mammals and, without developing chronic inflammation.

Altogether, a better understanding of the kinetics of antiviral immune responses against different viruses (highlighting successful outcomes) may better inform the development of new putative antiviral treatments.

Summary of rational treatments against COVID-19

In our view, a rational treatment against SARS-Cov-2 should take into account the disease kinetics summarized in Figure 1 and 3. We argue that some of the most important points include: i) a SARS-CoV2 infection that starts with an asymptomatic but contagious phase, in which ii) Both the kinetics and amplitude of the IFNα/β response are likely to be different in susceptible and non-susceptible individuals; iii) There is a preexisting, basal amount of inflammatory cytokines in susceptible patients (potentially due to inflammaging, or to co-morbidities such as obesity or diabetes). iv) The roughly two-week delay between infection and the surge of inflammatory cytokines might correspond to the delay necessary to produce immunoglobulins. (Figure 3)

Importance of early diagnostics and DAAs

To reduce or impair viral replication, a safe DAA might constitute an interesting option-- prophylactically centered around index cases and administered as early as possible following infection. Indeed, a drug capable of curtailing viremia at the beginning of the infection -- even modestly -- might save a series of complications (e.g. cytokine storms or hemorrhagic fevers, depending on the virus) and lower the case-fatality ratio [72,73]. To do this, the whole chain of diagnostics must be brought to a higher level of understanding and efficiency, e.g. by providing education about being tested (in the general population via family doctors) and offering accurate/cost-effective tests. We posit that only if accurate diagnostics are optimized in the management of infected patients, can morbidity and mortality be reduced in a viral pandemic. Thus, we argue that the identification and detection of early markers of infection, as well as normalized sampling for qPCR protocols need to become a standard routine.

Amplifying the IFNα/β-dependent spontaneous antiviral response.

The natural antiviral response, IFNα-dependent, is decisive for blocking virus propagation in the infected tissue. We propose that a first option might be to treat virally-infected individuals with IFNα/β when a paucisymptomatic disease begins to worsen. Associated with ribavirin, subcutaneous IFNα injections have increased the probability of clearance of HCV [74] and the survival of MERS-infected patients [75]. Moreover, compared to controls, a two-fold reduction in 28-day mortality of severe COVID-19 patients receiving intravenous IFNα injections was recently reported [76]. A second option may be to unleash the endogenous IFNα/β response that has been blunted by the virus. Specifically, since TGFβ is a potent immunosuppressor, and as SARS-CoV can trigger a PLpro-dependent increase of TGFβ in human promonocytic cells [33], we argue that there may be a strong rationale for unblocking the IFNα/β response with inhibitors of the TGFβ pathway, although this possibility remains to be rigorously investigated. Accordingly, several TGFβ inhibitors have been tested in ongoing clinical trials: e.g., along with chemotherapy in combination with galunisertib in patients with carcinosarcoma of the uterus or ovary (NCT03206177[VII]), or with TEW-7197, in patients with pancreatic ductal adenocarcinoma (NCT03666832[VIII]). Concerning PLpro, a careful and significant amount of work will be necessary to examine if the *in vitro* antiviral efficacy of PLpro inhibitors [77,78] can translate *in vivo*, first in animal models, and only then, in possible future clinical trials.

An anti-TGFβ approach might also be important for another reason: a chronic inflammatory condition is likely to activate anti-inflammatory mechanisms, including increased TGFβ concentrations that are susceptible to block IFNα/β signaling. This hypothesis is consistent with the finding that increased serum TGFβ concentrations have been measured in patients with asthma [79] or diabetes [80].

We hypothesize that 1,8 cineole might be another molecule of interest that could be tested with the goal of increasing the antiviral IFNα/β response in virally-infected patients. In *ex vivo* cultivated human nasal mucosa [40], it inhibits NF-

κB activation, a key pathway in inflammation, in human mucosa and in human cell lines [40,81]. NF-κB inhibition might contribute to explain the *in vivo* anti-inflammatory properties of 1,8 cineole in murine models of inflammation [82], and influenza virus-induced murine pneumonia [83], as well as in a double-blind placebo-controlled trial of asthma treatments [84]. If proven useful, this molecule (already available as oral capsule), might be potentially administered when the first clear symptoms appear, but this possibility also remains to be robustly tested.

Blocking the cytokine storm

We suggest that the best approach to preventing the occurrence of a cytokine storm in virally-infected individuals is via an efficient early antiviral treatment. A similar observation was made in the case of hyperacute HIV-1 infection [66]. We propose that the main treatments to be considered upon severe exacerbation of patient symptoms include: i) administering anti-inflammatory molecules: the use of corticosteroid dexamethasone can reduce the mortality of patients with severe COVID-19 [85]. Colchicine is already being used to safely treat certain chronic inflammatory diseases. Another possible approach might be the injection of anakinra, which is a natural antagonist to the receptor for inflammatory molecule IL-1. In a retrospective cohort study, anakinra administration reduced the need for invasive mechanical ventilation, as well as mortality among patients with severe forms of COVID-19, relative to controls [86] ([IX]). ii) Administering neutralizing antibodies against pro-inflammatory cytokines: these days, the most widely-tested antibody is anti-IL6R, but anti-TNFα and anti-IL-1β might certainly deserve consideration. iii) Administering high-dose IVIg as a potentially promising treatment. Note that the rationale for this approach –i.e. as an anti-inflammatory agent – is entirely distinct from that of administering serum from convalescent patients, in which improving the antiviral response of the patient is the major goal; unfortunately, this approach does not seem to be efficient against SARS-CoV-2 [87]. And iv) We argue that maintaining an antiviral treatment when the inflammatory symptoms increase might be important for limiting virus-induced inflammation.

Concluding Remarks

In this Opinion, we have provided arguments in favor of the presented hypothesis to explain the paradox between the low morbidity of SARS-CoV-2 observed in a majority of individuals, and the high morbidity observed in a minority of the global population. The key arguments for our reasoning are the importance of IFNα/β and its release kinetics, the presumed deleterious effects of inflammaging and other inflammatory conditions, and the importance of early antiviral treatments, which underscores the continued efforts in the discovery of efficient candidate antivirals (see Outstanding questions). Thus, we argue that research efforts should not be placed exclusively on vaccination, but also on antiviral discovery. HIV-1 or HCV infections constitute examples of vaccination attempts that have not been successful in the past, contrary to progress in antiviral discovery research. Anticipating other pandemics due to emerging viruses in the

future, we posit that if efficient and early diagnostics are made available, a combination of antivirals and immunomodulators with appropriate treatment kinetics might significantly lessen the burden of an epidemic/pandemic.

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Resources

[I] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT04401579

[II] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT04358549

[III] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT04373733

[IV] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT04322565

[V] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT04326790

[VI] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT04322682

[VII] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT03206177

[VIII] This trial is listed in https://clinicaltrials.gov/ct2/show/NCT03666832

[IX] This retrospective cohort is registered on the French National Institute of Health Data platform (MR4810020420)

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Box 1. Illegitimate drug repositioning

The first illegitimate drug repositioning for COVID-19 is the neuraminidase inhibitor oseltamivir, initially used against Influenza viruses. This inhibitor was designed using the crystal structure of the Influenza A N9 neuraminidase [88]. It is the first successful example of structure-based drug design against a virus pathogen. However, since there is no neuraminidase in SARS-CoVs, one wonders what is motivating the repositioning of this drug against COVID-19. Perhaps public ignorance and confusion is sustaining this illegitimate path. The second example is that of ritonavir or lopinavir. These drugs are HIV protease inhibitors -- tested as early as 2004, and found to have an 'apparent favorable effect' in the original publication [89]. Later, the effects of these drugs were to be non-existent [90]; yet, they are still being tested in numerous clinical trials that have mobilize research efforts based on a result that is already known to be disappointing (https://clinicaltrials.gov/ct2/results?cond=COVID-19). The HIV protease is an aspartate protease, whose structure, substrate specificity, and mechanism are totally different from that of the Cys-His main protease 3CLPro of the SARS viruses [91].

Box 2. The case of Hydroxychloroquine (HCQ)

HCQ has a clinical recognized efficiency as an antimalarial agent [92,93] and as an anti-inflammatory/immunosuppressor drug -- useful in certain inflammatory diseases [94]. In addition, certain HCQ antiviral effects against HIV-1 were demonstrated *in vitro* 30 years ago [95]. However, its *in vitro* activity does not translate to comparable concentrations *in vivo*. This is the case for several viruses in mouse, ferret, hamster and guinea pig models, for influenza [96], Nipah [97] and Ebola [98], and in humans for chikungunya [99] and dengue [100].

The direct antiviral effects of HCQ observed *in vitro* are likely linked to the alkalinization of acid compartments of infected cells. This can interfere with the entry of the virus into the cell (since endocytosis is slowed down by such alkalinization), and/or at a later stage of viral replication [98]. However, *in vivo*, any potential antiviral effects of HCQ (if an effective concentration is large enough), if at all, are likely to be masked by its immunosuppressive properties, although this remains to be tested. This might explain why HCQ, while efficient *in vitro* against the Vero cell line infected with SARS-Cov-2, is totally inefficient in preventing infection, or in treating SARS-CoV-2-infected macaques [101]. HCQ has also been reported to be an efficient putative treatment against COVID-19 in a few clinical trials without control groups [102] – findings that so far, have not been confirmed in trials with control groups [103–106]. Therefore, any use of HCQ as a putative treatment/aid of COVID-19 patients remains completely unsubstantiated.

Box 3. Treating the cytokine storm

The main treatments used to limit the consequences of cytokine storms include administering anti-inflammatory molecules such as glucocorticoids, antibodies neutralizing pro-inflammatory cytokines, and high-dose intravenous immunoglobulin therapy (IVIg).

Anti-inflammatory molecules. Since inflammation is an essential component in the establishment of an effective anti-viral immune response, anti-inflammatory drugs may constitute an aggravating factor in the initial stages of viral infection [107]. It is thus reasonable to propose that their use should be strictly restricted to the late stage of the cytokine storm. During the pandemics of SARS-CoV and MERS-CoV outbreaks, corticosteroids were not routinely recommended. However, in the case of SARS-CoV2, the use of dexamethasone significantly reduced mortality of patients with severe COVID-19, and is now considered as an FDA- and EMA- (European Medicines Agency) approved treatment for COVID-19 [85]. An interesting alternative to corticosteroids might be colchicine, a safe and low-cost drug, which can inhibit the inflammasome [108], and is already used to treat several inflammatory diseases such as atherosclerosis [109], as well as having shown some anti-viral effects against *Flaviviridae* [110]. Accordingly, colchicine is currently being tested in several clinical trials related to COVID-19 and results are eagerly awaited (NCT04322565[IV], NCT04326790[V], NCT04322682[VI]), the latter foreseeing the inclusion of 6000 participants.

Neutralizing antibodies against pro-inflammatory cytokines. Anti-IL6, anti-TNFα and anti-IL-1 antibodies have been successfully used to treat several autoimmune inflammatory diseases such rheumatoid arthritis, inflammatory bowel disease, ankylosing spondylitis, and gout [111]. The serum concentration of these cytokines can be found to be abnormally high in severe COVID-19 patients, and are therefore being considered as potential therapeutic targets [112–114]. Moreover, the cytokine storm that can be triggered in certain cancer patients upon adoptive transfer of chimeric antigen receptor (CAR)T cells can be controlled in certain situations with anti-IL6/IL6R neutralizing antibodies [115,116]. Preliminary studies have described that treatment anti-IL-6R might reduce the risk of invasive mechanical ventilation or death in patients with severe COVID-19 pneumonia [117].

High-Dose Intravenous Immunoglobulin Therapy (IVIg). High-dose IVIg therapy has successfully been used for treating inflammatory autoimmune diseases such as thrombocytopenia purpura [118] or Kawasaki's disease[119]. Such therapy in humans has achieved good tolerance and variable clinical benefits in Respiratory Syncytial Virus (RSV) [120], SARS-CoV [121] and dengue virus infections [122]. Its most likely mechanism of action includes inhibition of myeloid cells mediated by the FcγRIIB receptor (CD32b), since the effect of IVIg was reported to be completely lost in FcγR–deficient mice [123]. Consequently, IVIg has begun its use for severe cases of COVID-19, in some cases, successfully [124].

These three ways of treating cytokine storms (anti-inflammatory molecules, neutralizing monoclonal antibodies and IVIg) – among others –await further consideration in future studies.

Figure Legends

Figure 1. Hypothetical model of the two phases of COVID-19 disease, and the steps at which various treatments are likely to be efficient (blue rectangles). During the initial phase, infection of epithelial cells by SARS-CoV-2 induces a weak production of IFNα/β by these cells, and the initiation of a limited antiviral immune response, leading to apoptosis of infected cells, the production of pro-inflammatory molecules, and the recruitment of immune cells. At this time, the viral load might be reduced by antivirals combined with an anti-viral IFNα/β response enhanced by immunomodulator treatments (e.g. potentially TGFβ β blockade). Later, in some patients, an excessive inflammatory/immune response might give rise to a cytokine storm and/or ARDS. This deleterious hyperinflammatory immune response might be dampened by anti-inflammatory/immunosuppressive treatments.

Figure 2. Coronavirus life-cycle and target sites of potential antiviral agents. The Spike (S) protein binds to its main receptor, the cellular Angiotensin-Converting Enzyme 2 (ACE2), and the virion enters through endocytosis

and/or direct fusion of cell and viral membranes. The S protein is cleaved by various cellular proteases, e.g., TMPRSS2, into two subunits, S1 and S2 ("priming process"), and at a S2' site upstream to the fusion peptide [13]. The viral genome is translated into two polyproteins which are cleaved by two viral proteases, chymotrypsin-like 3CLpro and papain-like PLpro, to generate a large replication and transcription complex orchestrating genome replication and synthesis of messenger RNAs. New viral genomes recruit viral structural proteins to generate new virions released by exocytosis [6]. Red: potential inhibitors pointing towards their demonstrated targets. A question mark indicates that the target is putative, as discussed in the text. CQ: Chloroquine; HCQ: Hydroxychloroquine.

Figure 3. Kinetics of SARS-CoV2 viral load following infection, in parallel with the IFNα/β response, and the evolution of inflammatory cytokines. Top: illustrates the case of most individuals in the population that remain asymptomatic or paucisymptomatic. In these individuals, efficient anti-viral immune responses -- characterized by a significant production of IFNα/β and a limited production of inflammatory cytokines-- can lead to virus eradication [1]. Bottom: illustrates the case of patients more severely affected by the virus. These patients show ineffective/delayed production of IFNα/β, uncontrolled viral load and subsequent overproduction of inflammatory cytokines (cytokine storm) [1]. In the latter case, we propose that antivirals should be administered to patients as soon as possible, and maintained, whereas immunomodulators should be given when the disease worsens because of harmful inflammation.

Glossary

ACE2: Converts angiotensin I to angiotensin. Expressed in lung, heart, intestine and kidney. ACE2 is the cellular receptor of SARS-CoV and SARS-CoV-2.

Acute Respiratory Distress Syndrome (ARDS): clinical manifestations of severe lung damage and respiratory failure.

Basic Reproduction Number (R0): indicator of the contagiousness of infectious agents. One infected person infects an average of R0 other persons.

Cineole: 1,8 cineole (eucalyptol) is a small monoterpene (152 Da), highly lipophilic, present in the essential oil of eucalyptus or rosemary, with well-established anti-bacterial effects.

Coronaviruses: enveloped, positive-stranded RNA viruses associated with respiratory and enteric diseases of a broad range of vertebrate hosts. Amongst seven human coronaviruses, only SARS-CoV, MERS, and SARS-CoV-2 are highly pathogenic.

COVID-19: Coronavirus viral Disease 2019, associated with SARS-CoV-2 infection.

Cytokine storm: or cytokine release syndrome (CRS); excessive and uncontrolled release of pro-inflammatory cytokines mediating systemic inflammation, multiple organ failure, with high inflammatory parameters.

Dengue virus: RNA virus of the *Flaviviridae* family, responsible for a mosquito-borne tropical disease (dengue fever). In some cases, the disease may evolve towards dengue hemorrhagic fever, or dengue shock syndrome.

Direct Acting Antivirals (DAAs): antiviral molecules targeting viral proteins.

Drug repurposing: a drug recognized for its efficacy against a first disease is repurposed when it is used for treating another disease.

Exhaustion: state of T cells that have lost most of their efficacy and effector function, after prolonged, chronic stimulation.

Fatality/case ratio (FCR): proportion of disease-induced deaths compared to the total number of people diagnosed with the disease.

Favipiravir: purine-base analog showing broad-spectrum antiviral activity against influenza virus infections.

IFNβ: natural and potent antiviral molecule secreted by most cells of the organism. Binds to its receptor IFNAR, triggering the expression of IFNα, which binds to the same receptor with a lower affinity than IFNβ.

Indirect Acting Antivirals (IAA): molecules targeting host cell components required for virus replication, thus producing an indirect antiviral effect.

Inflammaging: used to describe changes observed in the elderly, appearing as a chronic low-grade inflammation associated with a weakening of immune system efficacy.

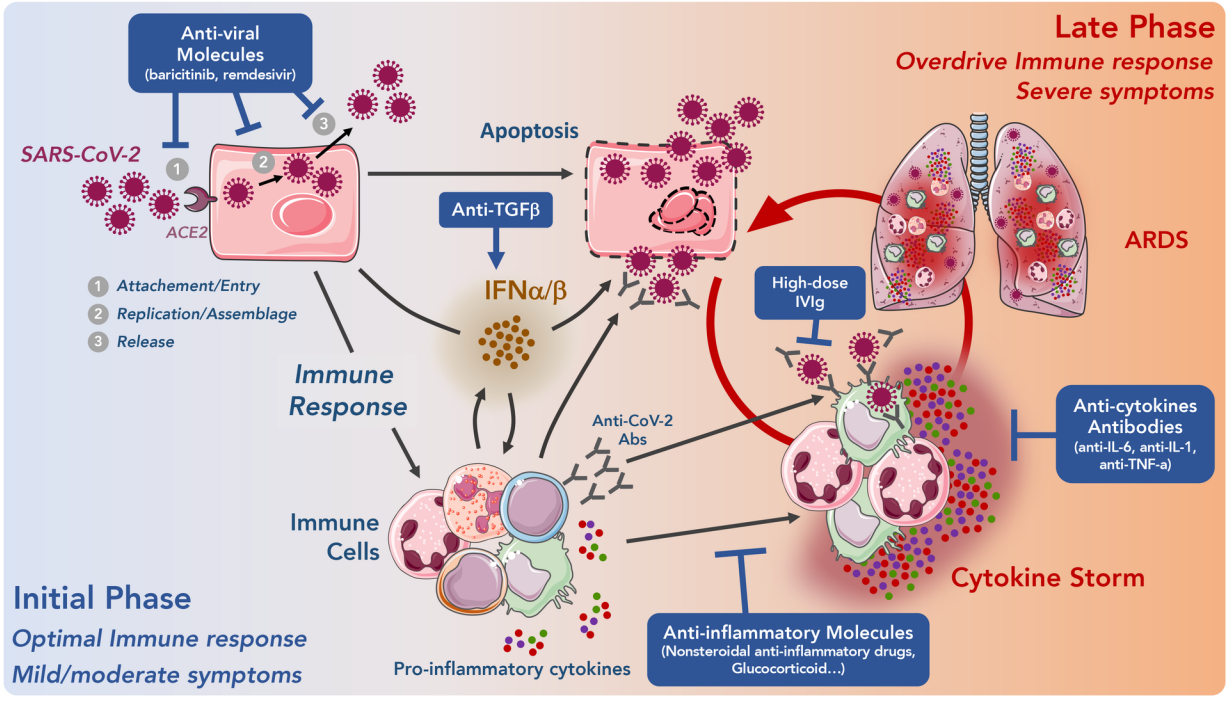
inflammasome: cytoplasmic complex regulating caspase activation, converting interleukins from inactive to active forms.

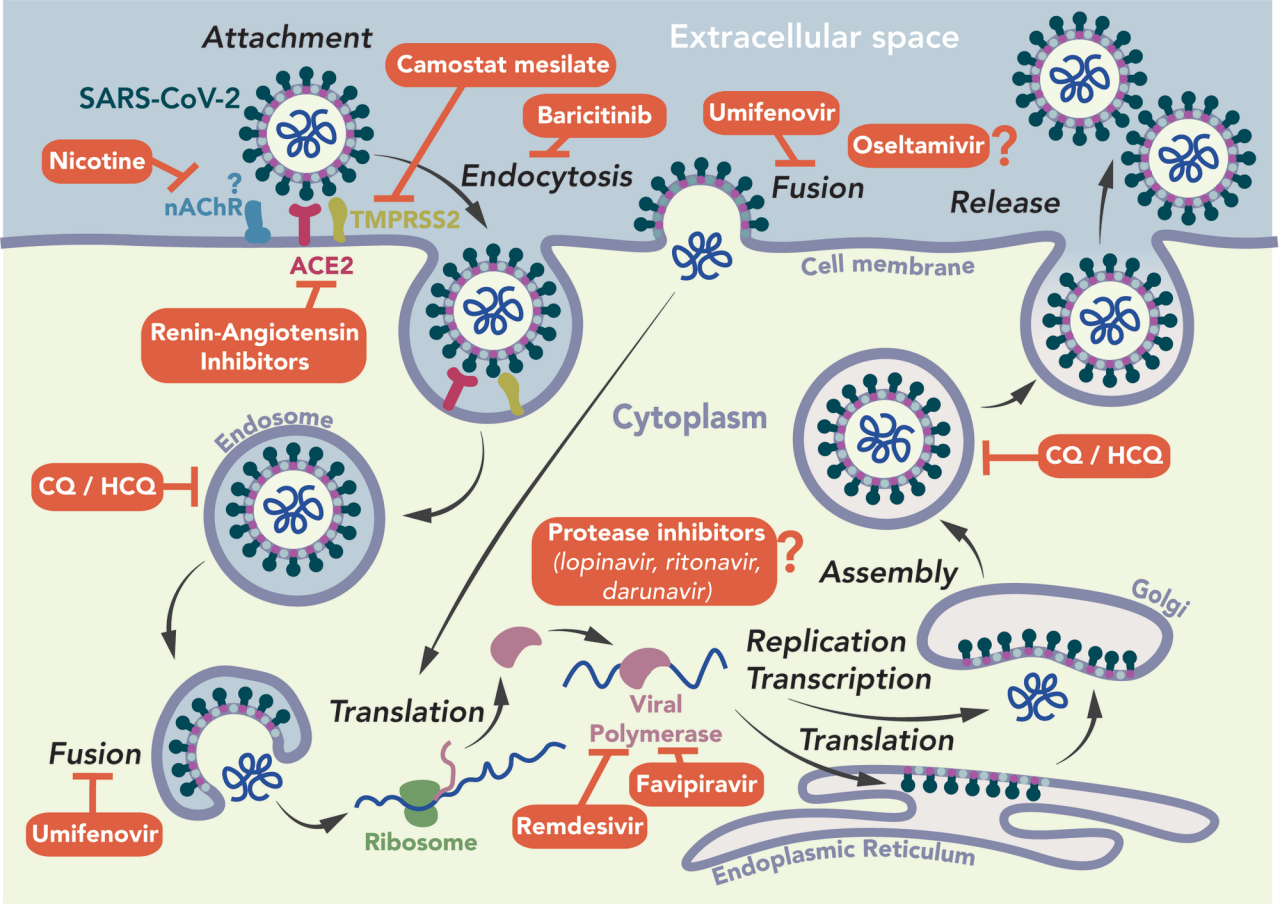
PLpro: papain-like protease, a deubiquitinating CoV enzyme, also regulating several host cell genes, including *TGFB.*

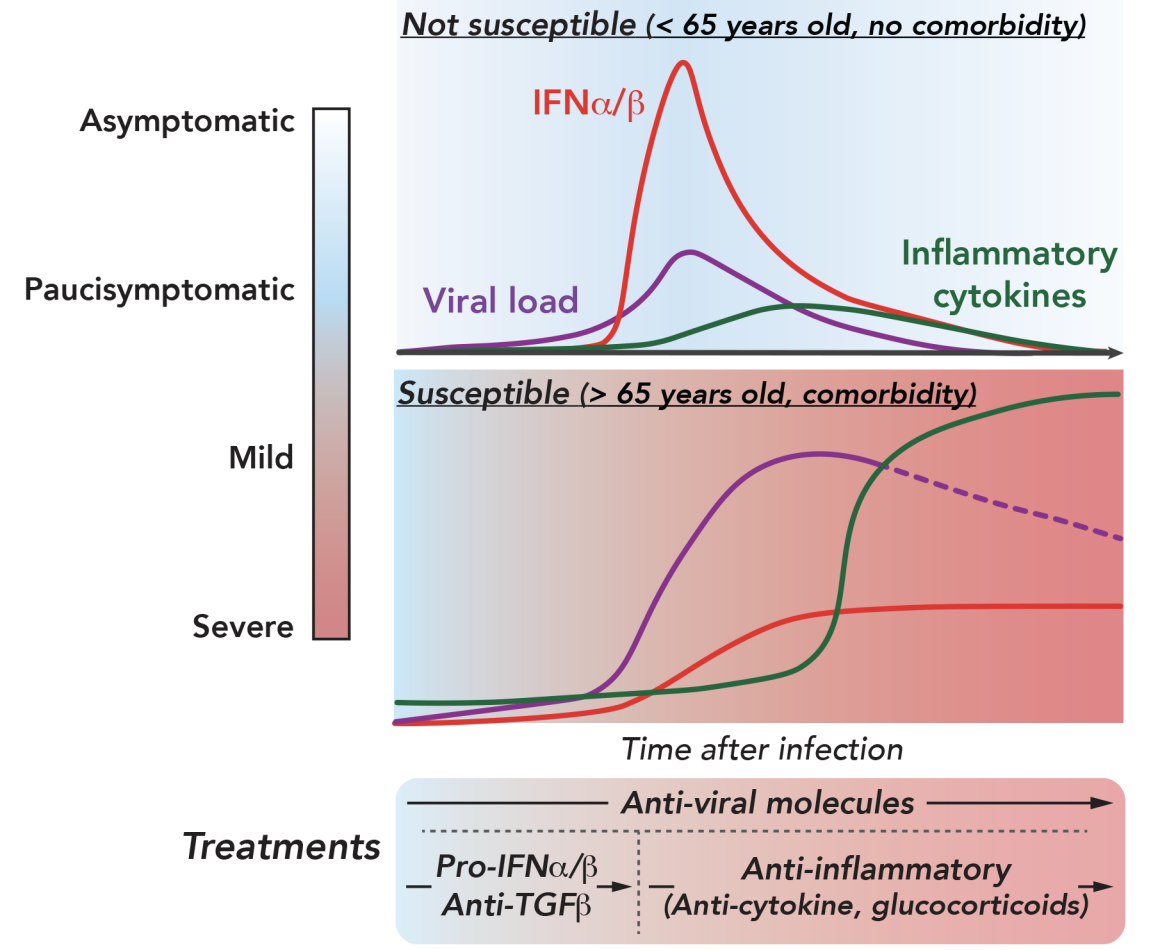
Poly (I: C): TLR3 ligand mimicking the presence of viral RNA in the cell.

Remdesivir: broad-spectrum antiviral nucleotide adenosine analog prodrug; active against CoVs.

TGFβ: multifunctional cytokine secreted by various cell types, in particular macrophages; key for tissue repair and fibrosis, with immunosuppressive properties. Potent blocker of IFNβ induction.


Late Phase
Overdrive Immune response
Severe symptoms
Anti-viral Molecules
(baricitinib, remdesivir)
SARS-CoV-2
ACE2
1 Attachement/Entry
2 Replication/Assemblage
3 Release
Apoptosis
Anti-TGFβ
IFNα/β
Immune Response
Anti-CoV-2 Abs
Immune Cells
Pro-inflammatory cytokines
High-dose IVIg
ARDS
Anti-cytokines Antibodies
(anti-IL-6, anti-IL-1, anti-TNF-a)
Cytokine Storm
Anti-inflammatory Molecules
(Nonsteroidal anti-inflammatory drugs, Glucocorticoid…)
Initial Phase
Optimal Immune response
Mild/moderate symptoms


Attachment
Extracellular space
SARS-CoV-2
Camostat mesilate
Baricitinib
Umifenovir
Oseltamivir ?
Nicotine
nAChR ?
TMPRSS2
Endocytosis
Fusion
Release
ACE2
Cell membrane
Renin-Angiotensin Inhibitors
Cytoplasm
Endosome
CQ / HCQ
CQ / HCQ
Protease inhibitors (lopinavir, ritonavir, darunavir) ?
Assembly
Golgi
Replication Transcription
Translation
Viral Polymerase
Translation
Fusion
Favipiravir
Umifenovir
Ribosome
Remdesivir
Endoplasmic Reticulum


Not susceptible (< 65 years old, no comorbidity)
IFNα/β
Viral load
Inflammatory cytokines
Asymptomatic
Paucisymptomatic
Mild
Severe
Susceptible (> 65 years old, comorbidity)
Time after infection
Treatments
Anti-viral molecules
Pro-IFNα/β
Anti-TGFβ
Anti-inflammatory
(Anti-cytokine, glucocorticoids)