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The anti-viral facet of anti-rheumatic drugs: Lessons from COVID-19

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ABSTRACT

The outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has posed the world at a pandemic risk. Coronavirus-19 disease (COVID-19) is an infectious disease caused by SARS-CoV-2, which causes pneumonia, requires intensive care unit hospitalization in about 10% of cases and can lead to a fatal outcome. Several efforts are currently made to find a treatment for COVID-19 patients. So far, several anti-viral and immunosuppressive or immunomodulating drugs have demonstrated some efficacy on COVID-19 both in vitro and in animal models as well as in cases series. In COVID-19 patients a pro-inflammatory status with high levels of interleukin (IL)-1B, IL-1 receptor (RIA) and tumor necrosis factor (TNF)-α has been demonstrated. Moreover, high levels of IL-6 and TNF-α have been observed in patients requiring intensive-care-unit hospitalization. This provided rationale for the use of anti-rheumatic drugs as potential treatments for this severe viral infection. Other agents, such as hydroxychloroquine and chloroquine might have a direct anti-viral effect. The anti-viral aspect of immunosuppressants towards a variety of viruses has been known since long time and it is herein discussed in the view of searching for a potential treatment for SARS-CoV-2 infection.

1. Introduction

The outbreak of the 2019-coronavirus (SARS-CoV-2) has posed the world at a pandemic risk [[1]]. Coronavirus-19 disease (COVID-19) is an infectious disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), an enveloped non-segmented positive-sense RNA β-coronavirus belonging to the same family as severe acute respiratory syndrome (SARS) and Middle East Respiratory Syndrome (MERS) viruses, which causes pneumonia, requires intensive care unit hospitalization in about 10% of cases and can lead to a fatal outcome [2–6]. COVID-19 is characterized in severe cases by an acute respiratory distress syndrome (ARDS) and cytokine release syndrome and there is an immediate need for any agent before the development of a vaccine [2]. To date, there is no registered molecule for the treatment of COVID-19 patients. However, there are ongoing trials on the use of some anti-viral and immunosuppressive or immunomodulating drugs that have demonstrated efficacy on COVID-19 both in vitro and in animal models as well as in small cases series [7]. Certainly, previous experiences on viruses belonging to the same β-coronavirus family have formed the cornerstones of the current therapeutic strategy [8,9]. The emergency facing the scientific community in addressing the pandemic from COVID-19 provides the rationale for the use of drugs that have not yet been approved and with still preliminary scientific evidence. So far, therapeutic regimes include a combination of anti-viral drugs and supportive care.

Accumulating evidence suggests that SARS-CoV-2 infection is associated with a pro-inflammatory status characterized by high levels of different cytokines, including interleukin (IL)-1β, IL-1Rα, IL-2, IL-10, fibroblast growth factor (FGF), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), interferon-γ-inducible protein (IP10), monococyte chemoattractant protein (MCP1), macrophage inflammatory protein 1 alpha (MIP1A), platelet derived growth factor (PDGF), tumor necrosis factor (TNFα) and vascular endothelial growth factor (VEGF). Critically ill patients requiring admission to intensive care unit (ICU) display markedly high concentration of IL-2, IL-10, G-CSF, IP10, MCP1, MIP1A, TNFα and IL-6. Interestingly, levels of IL-6 also correlated with increased mortality. Moreover, in severe COVID-19, a reduction of natural killer cells, CD4+ and CD8+ T lymphocytes and IFNγ expression in CD4+ cells, has been observed. Levels of IL-6, IL-10 and TNFα inversely correlates with lymphocyte count, suggesting that the cytokine release syndrome may hamper the adaptive immune response against SARS-CoV-2 infection [10,11]. Moreover, high levels of ferritin were demonstrated in patients requiring ICU hospitalization [2]. This provided rationale for the use of...
Table 1
Ongoing Clinical Trials on rheumatologic drugs in COVID-19 (last updated on the 1st of April 2020).

| Compound/Title                                                                 | Study type [ID] | Status          | Condition                      |
|--------------------------------------------------------------------------------|-----------------|-----------------|-------------------------------|
| Hydroxychloroquine<sup>4</sup> Tocilizumab for the Treatment of Pneumonia Caused by 2019-nCoV (HCnCoV) | Intentional RCT P/A [NCT04317092] | Recruiting (phase 2) | COVID-19 pneumonia            |
| Profilax for Healthcare Professionals Using Hydroxychloroquine Plus Vitamin C, D, and Zinc for COVID-19 Diarrhea | Intentional RCT | Recruiting (phase 2) | COVID-19 pneumonia            |
| Randomized Controlled Clinical Trials of Lopinavir/Ritonavir or Hydroxychloroquine in Patients With Mild Coronavirus Disease (COVID-19) | Intentional RCT P/A [NCT043078693] | Recruiting (phase 2, 3) | symptomatic COVID-19          |
| Post-exposure Prophylaxis for SARS-Coronavirus-2: A Pragmatic Randomized Clinical Trial | Intentional RCT P/A [NCT04316377] | Recruiting (phase 3) | COVID-19                      |
| Norwegian Coronavirus Disease 2019 Study: An Open Labeled Randomized Controlled Pragmatic Trial to Evaluate the Antiviral Effect of Chloroquine in Adult Patients With SARS-CoV-2 Infection | Intentional RCT | Recruiting (phase 3) | COVID-19 pneumonia            |
| Evaluation of the Safety and Clinical Efficacy of Hydroxychloroquine Associated With Azithromycin in Patients With Pneumonia Caused by Infection by the SARS-CoV-2 Virus - Alliance COVID-19 Brasil II - Severely Ill Patients | Intentional RCT | Recruiting (phase 3) | established COVID-19          |
| Multi-centre, Adaptive, Randomized Trial of the Safety and Efficacy of Treatments of COVID-19 in Hospitalized Adults | Intentional RCT P/A [NCT04315948] | Recruiting (phase 3) | Non-severe COVID-19 and contacts |
| Treatment of Non-severe Confirmed Cases of COVID-19 and Chemoprophylaxis of Their Contacts as Prevention Strategy: A Cluster Randomized Clinical Trial (PEP CoV-2 Study) | Intentional RCT P/A [NCT04304053] | Recruiting (phase 3) | COVID-19                      |
| Baricitinib Combined With Antiviral Therapy in Symptomatic Patients Infected by COVID-19: an Open-label, Pilot Study<sup>2</sup> | Non Randomized Crossover Assignment [NCT04320277] | Recruiting (phase 3) | mild to moderate COVID-19      |
| Sarilumab An Adaptive Phase 2/3, Randomized, Double-Blind, Placebo-Controlled Study Assessing Efficacy and Safety of Sarilumab for Hospitalized Patients With COVID-19 | Intentional RCT P/A [NCT04315298] | Recruiting (phase 3) | Severe/critical COVID-19       |
| Cytosolic multiple Randomized Controlled Trials Open-label of Immune Modulatory Drugs and Other Treatments in COVID-19 Patients - Sarilumab Trial | Intentional RCT P/A [NCT04324073] | Recruiting (phase 3) | COVID-19 pneumonia            |
| Sarilumab/Tocilizumab Effectiveness of Interleukin-6 Receptor Inhibitors in the Management of Patients With Severe SARS-CoV-2 Pneumonia: An Open-label, Multicenter Sequential and Cluster Randomized Trial | Intentional RCT Sequential Assignment [NCT04322773] | Not yet recruiting (phase 2) | COVID-19 with respiratory failure |
| Tocilizumab A Randomized, Double-Blind, Placebo-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of Tocilizumab in Patients With Severe COVID-19 Pneumonia | Intentional RCT P/A [NCT04320615] | Not yet recruiting (phase 3) | COVID-19 pneumonia            |
| Favipiravir Combined With Tocilizumab in the Treatment of Coronavirus Disease 2019- A Multicenter, Randomized and Controlled Clinical Study | Intentional RCT P/A [NCT04310228] | Recruiting (phase 3) | Severe COVID-19               |
| A Retrospective Study of Evaluating Safety and Efficacy of Tocilizumab Compared to Continuous Renal Replacement Therapy in Controlling CRS Triggered by COVID-19 | Observational | Recruiting (phase 3) | COVID-19 pneumonia            |
| Multicenter Study on the Efficacy and Tolerability of Tocilizumab in the Treatment of Patients With COVID-19 Pneumonia Tocilizumab (RoActemra) as Early Treatment of Patients Affected by SARS-CoV2 Infection With Severe Multifocal Interstitial Pneumonia | Single Group Assignment [NCT04317092] | Not yet recruiting | COVID-19 pneumonia            |
| Anakinra/Tocilizumab A Prospective, Randomized, Factorial Design, Interventional Study to Compare the Safety and Efficacy of Combinations of Blockade of Interleukin-6 Pathway and Interleukin-1 Pathway | Intentional CT Factorial Assignment [NCT04330638] | Not yet recruiting (phase 4) | COVID-19 pneumonia            |
| Anakinra/Emapalumab Efficiency and Safety of Emapalumab and Anakinra in Reducing Hyperinflammation and Respiratory Distress in Patients With COVID-19 Infection | Intentional RCT P/A [NCT04324021] | Not yet recruiting (phase 2, 3) | COVID-19 with respiratory failure |
| Adalimumab A randomized, open-label, controlled trial for the efficacy and safety of Adalimumab Injection in the treatment of patients with severe novel COVID-19 | Intentional RCT P/A [NCT04324089] | Not yet recruiting | Severe/critical COVID-19       |
| IVlg A Randomized, Open-label, Controlled, Single-centre Study to Evaluate the Efficacy of Intravenous Immunoglobulin Therapy in Patients With Severe 2019-nCoV Pneumonia | Intentional RCT P/A [NCT04261426] | Recruiting (phase 2, 3) | COVID-19 pneumonia            |
| Immunoglobulin From Cured 2019-nCoV Pneumonia Patients An Exploratory Clinical Study on the Treatment of Acute Severe 2019-nCoV Pneumonia With Immunoglobulin From Cured 2019-nCoV Pneumonia Patients | Intentional CT Non Randomized | Phase N/A | COVID-19 pneumonia            |
| IFN Clinical study for combination of anti-viral drugs and type 1 interferon and inflammation inhibitor TFF2 in the treatment of novel coronavirus pneumonia | Intentional Sequential Assignment [ChiCTR2000030262] | Phase N/A | COVID-19 pneumonia            |

(continued on next page)
Table 1 (continued)

| ID   | Study Type (ID)                                                                 | Title                                                                 | Condition                                      | Status       | Condition                                      | Phase |
|------|---------------------------------------------------------------------------------|----------------------------------------------------------------------|-----------------------------------------------|--------------|-----------------------------------------------|-------|
| NCT04325060 | Interventional RCT P/A [NCT04325060]                                            | Glucocorticoids (GCs) and Other Immunomodulating Agents in Patients With 2019-nCoV Severe Pneumonia | Severe/critical COVID-19                       | Recruiting (phase 3) | COVID-19 with respiratory failure             | 2,3   |

a) Only trials in recruiting status were included about the use of hydroxychloroquine.
b) Controls. All consecutive patients with mild to moderate COVID-19 infection, older than 18, admitted during the previous 2 weeks, who were treated with antiviral and/or hydroxychloroquine.

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Glucocorticoids (GCs) are among the most frequently used agent in rheumatologic clinical practice. The GCs have proved to be effective in the management of a broad spectrum of diseases such as RA, systemic lupus erythematosus (SLE), vasculitis both representing the main treatment or a bridging/supporting therapy to the immunosuppressant [17–20].

Glucocorticoids are lipophilic molecules able to activate cytoplasm receptors and then, translocating into the cellular nucleus, to change the expression of several genes, including those responsible for inflammatory response. GCs promote anti-inflammatory proteins transcription such as IL-10 and, simultaneously, impair the expression of pro-inflammatory proteins such as IL-1, IL-2, IL-6, TNF-α and IFN-γ. GCs can also inhibit leukocytes migration to the inflammation site [21–24].

Therefore, it is not surprising that GCs are burdened with numerous and well-known side effects, including the increased infection risk [25–28]. For instance, in RA patients the use of 7.5 mg or more of daily prednisone can increase the risk of infection requiring hospitalization up to six times than in the control group [29,30]. On the other hand, an improved RA disease activity achievable with glucocorticoids is associated with lower serious infection rates [31]. Moreover, these drugs appear to be independent predictors of infection-related hospitalization [32]. Although most of the GC-related reported infections are bacterial, viral infections have been reported too, primarily represented by HSV and HBV reactivation [25,33]. The most debating question is the possible usage of GCs during COVID-19 pandemic and, in case, which, at which disease stage, and at which dosage/administration route. Hydrocortisone showed to improve respiratory and mortality outcome in bacterial severe community-acquired pneumonia [34] and inconstant efficacy in septic shock [35,36]. Budesonide might reduce post viral-inflammatory-cytokine production by human bronchial epithelial cells in vitro [37], while discordant results are reported in viral influenza pneumonia.

Based on the current evidences, as reported by the WHO regarding COVID-19, GCs should not be routinely given for treatment of viral pneumonia outside of clinical trials [38,39].

Several studies reported that GCs administration in patients with severe influenza pneumonia was associated with a higher rate of mortality [40–44]. A meta-analysis conducted with a total of 6548 patients with influenza pneumonia (H7N9 or H1N1), found the use of systemic GCs (methylprednisolone with different dose ranges, when reported) associated with higher mortality rate (risk ratio [RR] 1.75, 95% confidence interval [CI] 1.30–2.36, \( Z = 3.71, P = 0.0002 \)), longer intensive care unit permanence and higher rate of secondary infection [44–46].

ID: NCT, ClinicalTrials.gov identifier, ChiCTR, Chinese Clinical Trial Registry; P/A, parallel assignment; RCT, Randomized Clinical Trial; N/A, not applicable; IVIg, intravenous immunoglobulins; IFN, interferon.
The use of systemic GCs, especially methylprednisolone, in MERS-CoV-infected patients, was found as one of the most significant factors that contributed to increased mortality, with an odd ratio of 3.85 [47]. Nonetheless, no information about dose and duration of the treatment were reported in this retrospective study. As reported in a recent Cochrane analysis, these data are predominantly based on observational studies and mostly of low quality [46,48]. In fact, Li et al. observed in a prospective trial, that the use of low to moderate dose of GCs on 2141 patient infected by H1N1 virus, 54.2% complicated by ARDS, significantly reduced both 30 and 60-day mortality (aHR 0.49 [95% CI 0.32–0.77] (aHR 0.51 [95% CI 0.33–0.78]) [49]. Analysing the outcome of MERS-CoV infected patients treated with GCs, Arabi et al. did not find a rise in death rates correlated to the use of the drug but found delayed lower respiratory tract clearance of MERS-CoV RNA [50]. Equally, the use of GCs seemed to protract SARS-CoV viral clearance [51–53].

Nonetheless, in reference to ARDS, at the moment there is very low evidence on the positive effect of the treatment in this critical condition [54]. There is not conclusive evidence that supports the use of GCs in the treatment of viral respiratory infection and their use remains controversial in COVID-19 [52]. An expert consensus form the Chinese Thoracic Society developed few basic principles for the use of GCs in COVID-19 patients [55,56]. There is evidence that in patients with confirmed ARDS, not infected with COVID-19, a benefit from dexamethasone is provided when used at a low dosage and for a limited period (10 days) in reducing the consequences of mortality [28] thus supporting the assistance of such therapy on elective indication.

As mentioned above, GCs inhibit immune reactions acting on migration and chemokines production. [21–24]; thus, this might lead to prolonged viremia and can impair viral clearance. In addition, it is important not to forget the other corticosteroid-related adverse effects, especially on the cardio-vascular system, which can be another precipitating factor in these critically ill patients that are often already comorbid.

3. Nonsteroidal anti-inflammatory drugs

The nonsteroidal anti-inflammatory drugs (NSAIDs) are available over the counter for several indications and are prescribed widely for symptomatic relief for pain, fever, and inflammation.

The inhibitory effect of NSAIDs on prostaglandin synthases, also known as cyclo-oxygenases (COX) 1 and 2, reduces the production of prostaglandins (PGs). Many NSAIDs blocks both COXs, such as ibuprofen, while NSAIDs selective for COX-2, including celecoxib and diclofenac, selectively target COX2 thought to be of most relevance to prostanooid formation in inflammation. Acetaminophen belongs to NSAIDs but its effects on COX activity remain controversial being a weak, reversible, isofrom-nonspecific COX inhibitor [57]. The COX-2 is an important mediator of inflammation in response to viral infection, and it contributes to viral replication, for CMV, HCV, and viruses belonging to the Flaviviridae family. Recently, it has been documented that compounds that significantly inhibit COX-2 enzymatic activities and prostaglandin E2 levels, associated with viral replication, showed therapeutic safety and efficacy in vitro and in vivo for Flaviviridae infection [58].

Evidence on the severe pneumonia related to the avian influenza A H7N9 virus documented that treatment with a combination of antiviral and COX2-selective NSAID was able to ameliorate pulmonary inflammation in mice [59]. Interestingly, the tumorigenic potential of COX-2 and PGE2 through eicosanoid receptors is described in several viral associated malignancies such as the Kaposis’s sarcoma-associated herpes virus and support COX-2 as a therapeutic target with an anti-neoplastic purpose [60]. Several years ago, authors have shown that the spike protein of SARS-CoV activated the COX-2 expression via ERK/ NF-kB and PI3K/JNK pathways [61]. These studies provided key findings on molecular mechanisms involved in the viral infection and the induction of inflammation by SARS-CoV. Few data on the inhibitory effects of ibuprofen on ACE2 were reported in animal models [62].

Naproxen is an inhibitor of both COX-2 and of Influenza A virus nucleoprotein (NP) [63]. This drug may have the potential to present antiviral properties against SARS-CoV-2. A recent clinical trial showed that the combination of clarithromycin, naproxen and oseltamivir reduced mortality of patients hospitalized for H3N2 Influenza infection [64]. A phase 3 clinical trial is ongoing to explore the efficacy of addition of naproxen in the standard treatment of critical patients hospitalized for COVID-19 [65]. However, since the use of both NSAIDs and acetaminophen could be associated with a masking of the symptoms during COVID-19, and thus to a diagnostic delay and a prolonged illness, complications may be more common when NSAIDs are used—both respiratory and cardiovascular [66]. In patients with acute respiratory infection, NSAIDs have been widely used and evidence suggests that in these circumstances NSAIDs are associated with an increased risk of stroke and myocardial infarction [67–69]. Moreover, large randomized trials support that NSAIDs may cause more prolonged illness or complications when taken during respiratory tract infections [70,71]. Patients with COVID-19 have a higher risk of hospitalization, critical disease and mortality correlated with age and presence of comorbidities, particularly hypertension. Thus, besides further research evidence relating specifically to people with COVID-19 are awaited, regular symptomatic NSAIDs use should not be recommended as the first line option for managing COVID-19 [72,73].

4. Chloroquine and hydroxychloroquine

Chloroquine (CQ) and its derivative hydroxychloroquine (HCQ) are aminoquinolinic compounds currently registered for the treatment and prevention of malaria and for the treatment of a number of autoimmune diseases. CQ was developed in the 1930s and extensively used during World War II to prevent and treat malaria infection [74], but its use as an antimalarial is now very limited due to the widespread resistance of Plasmodium.

HCQ is currently part of first-line treatment for RA and SLE, due to its immunomodulatory effect. The mechanisms of action are still not fully elucidated, nonetheless some important aspects are well known. CQ is a weak base with a large distribution volume and accumulates at high concentrations in almost all body tissues, including the lungs [75]. After oral administration, it is efficiently absorbed and is present in the blood mainly as a protonated molecule. However, the circulating unprotonated portion can easily cross cell membranes and, due to its basic charge, tends to accumulate in acidic organelles such as lysosomes, Golgi vesicles and endosomes, where it binds to free protons, thus significantly increasing the pH. Thus, a large number of enzymes contained in these organelles, which optimally work in the acidic milieu, are inhibited [76,77]. This mechanism is presumably at the basis of the direct anti-parasitic, anti-viral, and immunomodulatory effects of the molecule. Although most of the evidence in this field regards CQ, there are some data showing similar effects by HCQ. Additionally, current data support that the mechanism of action of the two molecules is equivalent and HCQ appears to be significantly less toxic and has almost completely replaced the use of CQ in the treatment of rheumatic diseases [77,78].

Most of the data on the anti-viral activity of CQ derive from experiments performed on Human Immunodeficiency Virus (HIV), hepatitis A, and influenza viruses [76]. Some viruses, in fact, enter host cells via endocytosis and, following fusion of endosomes with lysosomes, cause the disruption of the host cells to release the nucleic acid and the enzymes required for replication. Similarly, some enveloped viruses require post-translational modifications of envelope glycoproteins by endosomal enzymes in order to complete the maturation process and shed from the host cell. Additionally, enzymatic activity is required in multiple intra-cytoplasmic signal cascade and post-translational modifications that lead to cytokine, such as TNFα and IL-6, and chemokine
After the outbreak of SARS in the early 2000s, studies have been performed to identify potential effective anti-viral treatments on SARS-CoV-2, including CQ. In fact, *coronaviridae* seem to require the above-mentioned steps to replicate. *In vitro* experiments showed that the CQ-treatment of infected Vero E6 cells – a cell lineage commonly used to assess viral replication *in vitro* – caused a significant increase in viral replication when the drug was added within the first 5 h post-infection, with more marked effects when administered before or within the first hour. Interestingly, the half maximal inhibitory concentration (IC50) doses range between 1 and 10 μM, that are easily and safely reachable in humans [79,80]. In addition to the mechanisms described above, CQ seems to inhibit the glycosylation process of angiotensin-converting enzyme (ACE)-2, which may have an effect on the affinity of SARS-CoV for its receptor, further contributing to the inhibition of virus cell entry [80]. Another possible effect through interfering with the bond between PICALM (phosphatidylinositol binding clathrin assembly protein) and clathrin impeding the endocytosis of the virus. Other *in vitro* data demonstrated that CQ, probably through its pH-dependent enzyme inhibitory effect, can significantly hamper phosphorylation of p38 mitogen-activated protein kinase (MAPK), thus, blocking replication of the human coronavirus HcoV-229E, similarly to a selective inhibitor of the same enzyme [81] (Fig. 1).

In February 2020, Wang et al. reported the first evidence of the *in vitro* efficacy of CQ on SARS-CoV-2 with an effective concentration (EC) 90 value of 6.90 μM, which can be achievable with clinically used doses [82]. These data have been subsequently confirmed by other authors who compared the *in vitro* anti-viral activity of CQ and HCQ. HCQ was demonstrated to be more potent than CQ with lower EC50 values, which were even lower for longer incubation time. This effect may be due to the slow and progressive accumulation of HCQ at higher concentrations into the cells [83]. Nevertheless, *in vitro* data are obtained in ideal experimental conditions and do not necessarily imply effects in vivo. Although very few in vivo data are currently available on SARS-CoV or SARS-CoV-2, some studies have been performed on animal models of coronavirus-related diseases, reporting conflicting results. An interesting study tested the anti-viral activity of CQ against HcoV-OC43 infection in new-born mice [84]. Female pregnant C57BL/6 mothers were administered different solutions of CQ and five-day-old pups were infected with HcoV-OC43 after birth. The highest survival rate was depicted in the group of pups breastfed by CQ-treated mothers with survival rates declining in a dose-dependent manner. On the contrary, the mortality rate in the untreated cohort and the cohort of pups that received CQ transplacentally was 100% [84]. Moreover, other studies have demonstrated the immunomodulatory effect of CQ on TNFα, IL-1β and IL-6 in coronavirus-related feline infectious peritonitis [85], while others have failed to show any significant effect [86]. It is possible that these effects may contribute to the clinical efficacy in COVID-19, also given the rationale behind the use of IL-6 inhibitors.

Taking into account the potential beneficial effect of CQ and HCQ in *in vitro* studies, clinical trials have been recently conducted to confirm these data in subjects affected by COVID-19 infection (Table 1). Preliminary results seem to confirm that CQ can improve COVID-19 associated pneumonia in terms of inhibition of pneumonia exacerbation, amelioration of lung imaging and shortening of disease course [87]. Based on this evidence, CQ and HCQ have been included in most treatment protocols in China and worldwide [88]. However, the risk of adverse events has been heavily underlined [89].

A recent systematic review included six articles and 23 ongoing clinical trials in China. CQ seems to be effective in limiting the replication of SARS-CoV-2 [90]. Gautret et al. reported twenty cases were treated for 6 days with HCQ at the dosage of 600 mg/day plus azithromycin that showed a significant reduction of the viral carriage compared to controls. The authors suggested that azithromycin added to HCQ was significantly more efficient for virus elimination [91].

In rheumatology practice, CQ and HCQ are among the most prescribed medications in patients with RA and connective tissue diseases and are very well tolerated. The potential adverse events, including retinopathy, cardiotoxicity and myelotoxicity, are rare and cumulative dose dependent. The risk of retinal toxicity in patients treated for 10 years with HCQ for SLE was shown to be around 7.5% and higher in patients treated for longer periods [92]. In the COVID-19 associated acute infection, CQ and HCQ are used for a very short time (5–20 days according to expert consensus) [88,93], with a probably negligible risk of adverse events. Nevertheless, acute adverse events, such as hypersensitivity and gastrointestinal intolerance, require attention, especially in critically ill patients that may develop similar clinical manifestations due to COVID-19. Additionally, CQ and HCQ can be safely used during pregnancy [94]. Nonetheless, the ideal dose regimen has not been established yet and most guidelines suggest the same protocols used in rheumatology, i.e. 500 mg BID of CQ or 200 mg BID of HCQ, including those from China and Italy [88,93]. Although clinical trials in vivo are needed to find the best dosing regimen, a physiologically-based pharmacokinetic model suggested a loading dose of HCQ 400 mg BID on the first day, followed by 200 mg BID in the next four days, which should allow to maintain an appropriate plasma concentration up to ten days [83].

To date, anti-malarials are one of the medications with the largest – albeit limited – scientific background warranting their use in COVID-19 treatment protocols. Soon, a significant amount of data should be available from clinical trials, in order to verify whether the theoretical strong effect of the drug has a real impact on survival and recovery of COVID-19 patients. Until then, due to the excellent safety profile and vast experience, their use remains a pillar of current treatment protocols.

5. Immunosuppressants

The other concern for the rheumatologist is that immunocompromised patients may be at higher risk of developing COVID-19, while turning the speech around immunosuppressant drugs may represent unpredictable weapons in this scenario. Indeed, Coronavirus have not shown to cause a more severe disease in immunosuppressed patients. For this family of viruses, the host innate immune response appears the main driver of lung tissue damage during infection. A review of the mortality and morbidity reports published on SARS-CoV and MERS-CoV, no fatality was reported in patients undergoing transplantation, chemotherapy or other immunosuppressive treatments, at any age. Thus, in this second part of the review, we seek for any immunomodulatory agent that at any potential at least in *in vitro* studies of anti-viral activity, thus excluding others such as azathioprine [95].

6. Leflunomide

Leflunomide [*N-(4-trifluoromethylphenyl)-methylisoxazol-4-carboxamide*; HWA 486] (LEF) is a member of the mononitrile amide family of compounds and a marketed therapeutic agent for the treatment of RA, autoimmune disorders, and renal transplantation. Its active form, teriflunomide, approved for the treatment of multiple sclerosis [96], named also A77 1726 [*N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxy crotoamide*] exhibits two main mechanisms of action: inhibition of protein tyrosine kinase activity [97] and inhibition of T-cell proliferation. Indeed, it interferes with the de novo pathway of pyrimidine synthesis through inhibition of dihydroorotate dehydrogenase, a key enzyme in the biosynthesis of pyrimidine nucleotide triphosphates (pNTP) [98]. However, additional mechanisms of activity may remain to be discovered yet.

Numerous studies have already demonstrated several anti-viral effects of LEF [99]. The most important is against cytomegalovirus (CMV) infection in solid organ transplant recipients. The American Society of
Transplantation and the Transplantation Society International CMV Consensus Group reported LEF as a possible alternative or experimental therapy in case of extensive resistance to standard anti-viral treatment [100]. Even though randomized controlled trial are lacking, numerous case reports described the efficacy of LEF in treating drug-resistant CMV systemic and ocular infections, or in preventing the recurrence of viruria in transplant patients [101]. In one case report and in a retrospective study, LEF (100 mg for 5 days followed by a maintenance dose of 20 mg every 12 h) resulted to be effective in clearing low-dose viremia and long-term CMV suppression in lung transplantation. It must be noted that this small cohort was already taking other anti-viral agents [102]. Three case reports described that LEF could be able to induce a persistent clearance of CMV UL97 and UL54 (genes of resistance against classical anti-viral therapy) in serum and clinical
resolution in transplant patients complicated by active CMV retinitis resistant to treatment with systemic and intra-vitreal foscarin, ganciclovir and oral valganciclovir [103,104]. Other case reports in hemopoietic stem cell transplant recipients described the use of LEF, alone or in combination with other anti-viral drugs as a salvage therapy for recalcitrant CMV infection, with variable results [105]. In addition, LEF was reported in the treatment of acyclovir-resistant perianal Herpes simplex type 2 (HSV-2) infection in HIV patients [106].

The anti-viral mechanism against CMV is an “off target” mechanism and also applies against β-HSV-1. In fact, even restoring pyrimidine nucleotide triphosphates to normal levels, viral replication is persistently impaired. LEF reduces the in vitro spreading of CMV and HSV-1 infection by interfering with virion cytoplasmatic assembly in a dose-dependent manner [107,108]. This action appears independent of the levels of pyNTP and persists also in CMV strain D16 usually resistant to the action of ganciclovir, cidofovir and foscarinet [109]. In particular, the drug induces failure to acquire tegument and external membrane in the cytoplasm of infected cells. It has been proposed that LEF may interfere with tegument assembly by inhibiting protein phosphorylation of the phosphoproteins [107]. Thus, its anti-viral activity, specifically against CMV and HSV-1, occurs at a late stage of viral assembly, in contrast with traditional anti-CMV agents that inhibit viral DNA replication [110]. Viral protein synthesis does not seem to be affected, while two different studies reported discordant results on the viral DNA production Data from in vivo experiments document that LEF is able in reducing viral load in nude rats [107,108]. LEF can significantly reduce CMV-induced apoptosis of the infected cells, interfering with the spreading of the virus [111]. Bilger et al. showed that teriflunomide can have a role against Epstein-Barr virus (EBV) [112]. Drug concentrations lower than those normally used for treatment of RA can prevent EBV-transformed human B cell proliferation thanks to an “on target” mechanism on B cells and an “off target” action that alter EBV latency protein expression. In fact, an increased expression of EBV nuclear antigen-2 (EBNA2) and latent membrane protein-1 (LMP1), the latter protein expression, is demonstrated for MMF [131]. Cho et al. demonstrated that MMF, blocking IMPDH, could demonstrate for MMF [131].

Nevertheless, LEF inhibits, through MEK, MAP kinase pathway and protein tyrosine kinase, thus resulting in the reduction of CD28 and ICOS activity; this leads to an increased viral load [113].

Reducing pyrimidine pool, teriflunomide can inhibit the replication of Junin virus, the agent of Argentine hemorrhagic fever and an enveloped negative-sense ssRNA arenavirus, in a dose-dependent manner. The combination with ribavirin appeared to have a stronger anti-viral activity than monotherapy [114].

The same mechanism of action seems to explain the anti-viral activity of teriflunomide on the foot-and-mouth disease virus, an RNA virus member of the Picornaviridae family, usually affecting livestock breeding [115].

Martin et al. reported preliminary effect on several RNA viruses such us Newcastle disease virus, Rabies virus and Influenza A virus [116]. BK polyomavirus is a DNA virus member of polyomaviridae family and causative agent of infectious hemorrhagic cystitis in transplant recipients and of polyomavirus-associated nephropathy (PVAN), a potentially dreadful complication after kidney transplantation. For several years, LEF has been used as second-line therapy in refractory infection after reducing maintenance immunosuppression. Small case series and a prospective open-label trial analyzed the effect of LEF, used alone or in combination with other drugs (tocrolimus/everolimus, fluorquinolones), in PVAN; they described a partial success in viremia clearance or better renal outcome [117–119]. Nonetheless, as reported by the American Society of Transplantation Infectious disease, true efficacy of these approaches has not been proved so far because of the lack of randomized controlled trials. Additionally, a meta-analysis, albeit with various limitations, failed to document significant benefits of these adjunctive therapies [120,121].

No direct anti-viral effect on the Dengue virus was reported by LEF, despite the drug seem able to reduce dendritic cell migration and virus-induced cytokine releasing induced in vitro [122].

Enterovirus 71 (EV-A71) is a neurotropic virus that can cause severe complications such as encephalitis. As it happens in viral infections, such as Dengue fever and most recently COVID-19, a prominent role in the progression of tissue damage and of the severity of the disease appears to be secondary to the resulting cytokine release syndrome. Similarly, in EV-A71 infection, IL-6 seems to be closely correlated with the clinical severity of the disease [123,124]. Hung et al. observed that teriflunomide can significantly reduce EV-A71 yield in infected cells in vitro and is able to induce a 40–50% reduction of IL-6 levels, compared to controls [125].

Interestingly, LEF showed robust efficacy in vitro and in vivo even against respiratory syncytial virus (RSV), an RNA virus. Significant dose-dependent reduction of RSV induced syncytia formation in human Hep-2 has been reported, and when the drug was used on infected cotton rats (30 mg/kg/day), it reduced by > 3 log the pulmonary viral load compared to controls, similarly to what Chong et al. reported for CMV infected rats [126].

The anti-viral effect of high concentrations of the drug was comparable to that of ribavirin, though LEF showed greater anti-viral efficacy than ribavirin at lower concentration [127].

Dose-limiting side-effects of LEF therapy are liver damage, lung disease, immunosuppression, diarrhea, rash, and reversible alopecia [128]. In vivo, high dose of LEF appears to be a potent inhibitor of T cells but also a partial inhibitor of B cell responses and can totally suppress humoral immune response against various antigens [129]. The mechanisms by which LEF can hamper the immune response to viral infections in humans are not known. Although anti-viral effects of LEF against various agents gave been demonstrated in humans, very few data are available on the optimal dosage, treatment duration and target blood levels of teriflunomide for an effective anti-viral activity, and its usage in COVID-19 treatment are doubtful.

7. Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an immunosuppressive agent commonly used for allograft rejections in different organs transplants and in the treatment of autoimmune diseases.

MMF and its active form mycophenolic acid (MA) inhibit inosine monophosphate dehydrogenase (IMPDH) and block the conversion of inosine monophosphate (IMP) to guanosine monophosphate (GMP), hampering lymphocyte proliferation [130]. This action is shared by ribavirin and it is almost the only anti-viral mechanism that has been demonstrated for MMF [131].

Different studies have shown anti-viral properties of MMF against various members of Orthomyxoviridae family, especially against influenza viruses. These are single stranded ribonucleic acid negative-sense (−) ssRNA viruses. MMF and MA have anti-viral activity against influenza viruses A (IAV); MA also against influenza viruses B (IBV).

MA can prevent virus-induced cell death by IVB B/411 and by the pandemic IAV(H1N1)pdm09 virus H1/415. It interferes with the early stage of the viral replication, before the protein synthesis [132,133]. Other experiments demonstrated MA anti-viral activity against eight different clinical isolates of A(H1N1), A(H3N2), A(H7N9) and influenza B viruses (IC50 < 1 μM) [133].

Cho et al. demonstrated that MMF, blocking IMPDH, could
completely inhibit A/Vietnam/1194/2004 (H5N1) virus mRNA replication and protein expression in Madin-Darby Canine Kidney (MDCK) cells and in infected mice. All infected lab rat treated survived, and the drug led to substantially reduced lung viral titers. Additionally, MMF treatment reduced the IL-1β, interferon (IFN)-β, IL-6, and IP-10 mRNA expression levels in MDCK infected cells [132].

In conclusion results have been obtained against several other strains of influenza virus [134] and there are discordant evidences regarding the action of the drug against Zika virus (ZKV) [135,136]. ZKV is not the only Flavivirus against to MMF was tested. In one study the drug could significantly reduce cytopathic effect in West Nile-virus-infected Vero cells [137] and, in another one, MMF demonstrated also a significantly anti-viral activity against Japanese encephalitis virus in vitro and in vivo, protecting the infected mice with an IC50 of 3.1 µg/ml and a therapeutic index of 16 [138]. Other Flavivirus susceptible to MMF are dengue virus type 2, Yellow fever virus 17D, Modoc virus, and Montana myotis leukencephalitis virus [139-141]. Some studies report anti-viral effect of the drug against other several different RNA viruses including Chikungunya [142,143], smallpox virus, foot-and-mouth disease virus (FMDV) [144], peste des petits ruminants virus (PPRV) [145], Junin virus [146], norovirus [147], the arenavirus causative agent Lassa’s hemorrhagic fever [148], the reovirus [149,150] and others [151,152].

The use of MA It has been also reported that the use of MA on rotavirus-infected-Caco2 cells, could induce a 99% inhibition of viral RNA production at the clinically relevant concentration (10 mg/ml), with a persisting effect in time [153].

The anti-viral effect against all these numerous viruses mentioned above appears to be due to the reduction of GMP cell pool, whereas and it can be reversible with the addiction of guanosine in a dose dependent manner [154,155]. Similarly, MMF inhibits viral RNA, mRNA protein synthesis, and it almost completely blocks virus entry and cytopathic effect of human parainfluenza virus type 2 (HPIV-2), but these restrictions are recovered by guanosine and 5-(4-nitrobenzyl)-6-thioinosine administration [156]. The only evidence of the presence of another antiviral mechanism of MMF derives from a study on hepatitis C virus (HCV). It seems that the drug is able to inhibit in vitro and in vivo the virus replication by augmentation of IFN-stimulated gene expression [157], suppressing HIV-1 cell autonomy, decreasing autophagosome formation, and increasing p62 level expression in vitro [158]. MERS-CoV caused several fatal infections in the Middle East and traveler-associated cases in Europe and Africa with over 35% fatality since its emergence in 2012 [159]. A clinical retrospective analysis on 51 patients infected by MERS-CoV showed that treatment with MMF was one of the predictors of increased survival [160]. MA together with disulfiram and/or 6-thioguanine seemed to synergistically inhibit MERS-CoV papain-like protease Plpr [161]. Chan et al. found that a concentration ≤0.063 µg/ml of MMF inhibited cytopathetic effects, reduced the viral load and achieved a 100% plaque reduction in Vero cells infected. The combination of MA and IFN-1β showed synergistic activity [162]. Despite these evidences, the same author observed that MMF and MA were ineffective in coronavirus-infected-animal models. In fact, in one study all MERS-CoV-infected marmosets treated with MMF developed severe and/or fatal disease with higher mean viral loads than the untreated animals, and higher mortality than the ones treated with lopinavir/ritonavir and IFN-1β [163]. Barnard et al. found the drug unable in preventing viral replication in the lungs of SARS-infected BALB/c mice, although it did not significantly increase it, as ribavirin did [164]; the same inefficacy against SARS-CoV replication has been previously reported [165].

Interestingly, MMF could act as a synergistic drug to potentiate the anti-viral effects of other drugs, through the depletion of the endogenous GTP pools [166]. MA increases the anti-HBV activity of penciclovir, lobucavir, 37-fluorodeoxyguanosine, dianinopurine dioxolane [167,168]. Similar results have been obtained with the combination of MMF with other classical anti-viral drugs, such as abacavir, didanosine and tenofovir, treating HSV-1, HSV-2, CMV and others [169-176]. All these evidences could allow the clinician to use of MMF as a support-drug of other anti-viral drug, in specifically clinical contest, even thou the lack of in vivo clinical trial. This is a strong limitation, considering that frequently in vivo activity does not correspond to a satisfactory action in vivo. Nevertheless, MMF is a strong immunosuppressive agent, it reduces the immunity response to vaccination in transplant patients [177], and it is associated with increased risk of viral infections from human herpesvirus 6, herpes zoster (HZ) and CMV [178,179], although data remain inconclusive [180,181].

8. Methotrexate

Methotrexate (4-amino-10-methylfolic acid, MTX), an analogue and antagonist of folic acid, is the first-line disease-modifying anti-rheumatic drugs (DMARDs) in the treatment several rheumatic autoimmune diseases including RA, adult-onset Still’s disease (AOSD), and Psoriatic Arthritis (PsA) [182]. Its effectiveness is related to its ability to inhibit key enzymes involved in the biosynthesis of purines and pyrimidines by interfering with dihydrofolate reductase (DHFR) pathway. MTX thus limits the viability of highly replicating cells. Moreover, MTX-induced reactive oxygen species generation modulates different cell functions, such as suppression of cytokine production and cell proliferation decreasing TNF-α, IL-1β, and adhesion molecules (E-selectin and VCAM-1) [183].

Interestingly, DHFR inhibitors have also been investigated to treat infectious diseases [184].

Current evidence supports MTX as a potential treatment of viruses mediated arthritis, specifically Parvovirus B19, HBV, HCV, and HIV [185]. Studies documented that pro-inflammatory mediators were markedly decreased after MTX treatment in virus-induced arthritis [186]. As a remark, recent studies suggest that hepatitis B reactivation or on accelerated liver disease are rather infrequent manifestations during long-term MTX treatment [187].

In vitro findings reported an anti-viral effect for MTX against ZKV through inhibition of DHFR pathway with mechanisms similar to other published results in related Flaviviruses [184].

It is notable that authors documented the efficacy of intravitreal MTX as treatment option for patients with necrotizing retinitis EBV-positive not responding to conventional anti-viral therapy [188]. MTX has a high risk of reactivation demonstrated for several viruses, including the abovementioned HBV and EBV, thus its usage as an anti-viral drug and as therapeutic weapon against COVID-19 may not be suitable.

9. Colchicine

One of the oldest know drugs so far in the field of rheumatology is Colchicine. This is an inexpensive, orally administered, potent anti-inflammatory medication that was initially extracted from the autumn crocus and has been used for centuries [189]. Its mechanisms of actions are through the inhibition of tubulin polymerization and microtubule generation and, possibly, effects on cellular adhesion molecules, inflammatory chemokines, and the inflammasome [190,191]. Colchicine is currently indicated for the treatment of gout, familial Mediterranean fever, and pericarditis [192-194]. Indeed, this agent is the main treatment of several rheumatic conditions [195] in which the activation of the inflammasome and the release of pro-inflammatory cytokines are the key pathogenic events. It alters the organization of actin cytoskeleton by binding to tubulin monomers and inhibits polymer formation [196]. Colchicine drug has shown anti-inflammatory effects, more pronounced on the IL-1 and IL-6 axis. It is highly concentrated in neutrophils and macrophages prolonging its action [197]. Its ability to bind tubulin achieves multiple cellular actions, including inhibition of
the assembly of the nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) inflammasome [198]. In addition to its effect on neutrophils [199,200], colchicine impairs the release of IL-1β into neutrophil extracellular traps [201]. Many of the actions have been demonstrated in patients with coronary disease [202,203]. Colchicine blocks the NLRP3 inflammasome, a cytosolic complex responsible for the production of IL-1β and IL-18. In patients with acute coronary syndromes, in which IL-1β, IL-18, and downstream IL-6 that are key inflammatory cytokines, colchicine administration was able to significantly reduce transcapillary gradients of all 3 cytokines in by 40%-88% [204]. A similar reduction in IL-6 but also in IL-8 or TNF-α levels can be observed in mucocutaneous Behcet’s disease patients [205]. Moreover, colchicine has anti-viral properties against Flaviviridae and against the recombinant demethylating strain of mouse hepatitis virus RSA59 as demonstrated reducing virus replication since microtubules are crucial in cell entry and blocking neuronal transport [206,207]. It inhibits RSV replication reducing IL-6 and TNF-α levels [208]. Some studies have suggested that colchicine and colchicine derivatives may have some influence HIV viral load [209]. Certain subsets of the 2003-SARS coronavirus replication machinery have been shown to move in the cell in a manner that corresponds with microtubule-associated transport, inducing the formation of double-membrane vesicles in infected cells. SARS-CoV-2 is a similar virus to SARS-CoV and microtubule disruption may influence viral replication [210]. It was also documented that SARS-CoV and its accessory protein are potent activators of pro-IL-1β gene transcription and protein maturation, and thus are able to activate the NLRP3 inflammasome and that influenza virus M2 or encephalomyocarditis virus 2B proteins stimulate IL-1β secretion following activation of the NLRP3 inflammasome [211], thus making colchicine a promising treatment for these conditions.

Indeed, acute respiratory syndrome related to COVID-19-infection is associated with a wide release of pro-inflammatory cytokines, including interleukin IL-1 and IL-6 [212]. Specifically, the binding of COVID-19 to the toll like receptors leads to the lung inflammation, fever and fibrosis. Thus, it might be useful to obtain a suppression of pro-inflammatory IL-1 family members and IL-6 to reach a therapeutic effect in viral infections. Moreover, a dysregulated activation and inflammatory activity of myeloid cells is one of the main pathogenic events characterizing the infection by coronaviruses [213,214]. Colchicine is a well-known inhibitor of the pro-inflammatory mechanisms induced by neutrophils thus it was proposed as an adjuvant treatment for RSV bronchiolitis [15]. Thus, colchicine has broad anti-inflammatory effects, anti-viral properties summarized in Fig. 2, and it is not hampered by an immuno-suppressant effect. It is cheap, which may be of great usefulness in the eventful case of an outbreak in countries that may not sustain expensive biologic treatments. Colchicine has a narrow therapeutic index; gastrointestinal disturbance may be seen in up to 10% of patients possibly limiting its usage in COVID-19 due to the relatively frequent occurrence of diarrhea in these patients. Nonetheless, potential intravenous administration may reduce this side effect and increase its bioavailability. It could be suggested that colchicine may be trialed in an appropriate COVID-19 patient population to reduce both viral entry and inflammatory status. In this view, an open-label, phase 2 study enrolling patients with COVID-19 disease to evaluate the efficacy and safety of colchicine has been recently promoted by the Italian Society of Rheumatology (SIR), the Italian Society of Infectious and Tropical Diseases (SIMIT) and by the Italian Thoracic Society (AIPO) and has been approved by the Italian Drug Agency (AIFA).

Encouraging results are provided by the case reported by Gandolfini et al. who treated a patient with signs of systemic inflammation (plasmatic IL-6: 108.2 pg/ml; normal range 0–10 pg/ml), who received colchicine (1 mg on day 8, and 0.5 mg/day thereafter) with benefit due to worsening pf respiratory functions [215]. So far, the patient is stable, reinforcing that this is a suitable option in patients with COVID-19 disease.

10. Anti-interleukin 1

As known, the innate immune response of the host against infections plays a crucial role in inducing pro-inflammatory host factors, leading to tissue damage. IL-1 is a pleiotropic cytokine with a key role in inflammation, immunity, and hemopoiesis. The major of IL-1 agonistic molecules are IL-1α and IL-1β, which bind to IL-1R type I (IL-1R1) inducing similar biologic functions. The precursor of IL-1α (ProIL-1α) is constitutively expressed in several tissue cells and its expression increases during stress or inflammation, whereas IL-1β is produced mainly by myeloid cells upon inflammatory stimulation and is active as a secreted molecule [216]. IL-1β is up-regulated during respiratory viral infections. Interestingly, community-acquired pneumonia (CAP) observed in children below 5 years of age in India has been related to a phenotype-genotype association with pro-inflammatory cytokines. Specifically, IL-1RA gene polymorphism and cytokine levels were linked with an adverse outcome in severe CAP [217].

Authors reported that IL-1β mediates virus-induced M2 muscarinic receptor dysfunction and airway hyper-reactivity during parainfluenza virus infection [218]. Data from the literature reported that IL-1β and IL-17A are key mediators of neutrophilic inflammation in influenza-induced exacerbations of chronic lung inflammation [219]. Using a mouse model of disease, authors demonstrated the crucial role of IL-1β in driving neutrophilic recruitment and inflammation during the whole phase of viral infection. Blocking of IL-17A or IL-1 resulted to be potential targets for therapeutic treatment of viral exacerbations of chronic lung inflammation [219]. Authors described that inflammation some complex/IL-1β are an essential signaling pathway, which is over activated and directly causes the severe liver disease during viral infection [220].

Anakinra (ANK), a human IL-1R antagonist, has been approved for the treatment of RA, AOSD, and other severe autoimmune and auto-inflammatory disorders including tumor necrosis factor receptor-associated periodic syndrome (TRAPS) [221]. The objective of IL-1Rα-based therapy is to occupy enough IL-1 receptors with IL-1Ra to block IL-1 cell signaling [222]. Rilonacept and Canakinumab are other molecules currently used in clinical practice to treat autoinflammatory diseases. Rilonacept is a long-acting soluble decoy receptor that binds to both IL-1α and IL-1β and prevents their interaction with the IL-1 cell surface receptor [223]. It was approved in 2008 for the treatment of adults and children 12 years of age and older with cryopyrin-associated periodic syndromes (CAPS). Canakinumab is a fully human anti-IL-1β antibody that is used in the therapy of AOSD and other autoinflammatory conditions. Evidence documented that the control of the inflammation achieved with this drug, targeting the IL-1β innate immunity pathway, can reduce inflammatory tissue damage as it is demonstrated in cardiovascular disorders such as atherosclerosis and diabetes [224]. Currently, no evidence of anti-viral properties of these drugs exists but, as widely described, SARS-CoV is capable of inducing a storm of pro-inflammatory cytokines [225]. Several studies suggest that cells infected by SARS-CoV produce elevated levels of IL-1 β which strongly contribute to the immuno-mediated damage to the lungs and other organs, resulting in acute lung injury (ALI) and, subsequently, multi-organ dysfunction. Therefore, the application of IL-1 β antagonists may reduce the severity and mortality of SARS-CoV-2 infection [226].

11. Tumor necrosis factor α inhibitors

TNF-α is a vital cytokine mediating host immune response [227]. Inhibition of TNF leads to a significant decrease of the inflammatory response, and this approach is used in therapy of autoimmune conditions, most effectively in RA, juvenile idiopathic arthritis, PsA, Psoriasis (PsO), Spondyloarthritsis (SpA) and inflammatory bowel disease [228].

Five TNF inhibitors (TNFI) have been developed and introduced in clinical medicine: a chimeric monoclonal antibody, infliximab, a
soluble recombinant form of the TNF cellular receptor etanercept, two fully human monoclonal antibodies (adalimumab and golimumab) and a humanized Fab fragment of anti-TNF linked to polyethylene glycol, certolizumab pegol [229]. These TNFi show differences in molecular design, route of administration, and potency in clearing TNF-α [229].

TNF gene expression can be induced primarily in cells of the monocyte-macrophage lineage by a variety of inducers, including lipopolysaccharides (LPS) and viruses [230].

The role of specific TNF receptor (TNFR) family members in antiviral immunity depends on the stage of the immune response being different in accordance with the virus type and its virulence [231]. Depending on conditions, TNFR induces cell survival/apoptosis, and programmed necrosis which may play a role in controlling viral infection [232]. The risks of bacterial and mycobacterial infection are increased in patients receiving biological DMARDs therapy, particularly TNFi [233]. These drugs are relevant immunosuppressants leading to reactivation of latent infections such as hepatitis B and tuberculosis [233]. For instance, hepatitis B and C assessment is required prior to start a TNFi and in patients with a positive HBsAg, infliximab has the most reported cases associated with HBV reactivation [234].

Receptors and ligands of the TNF family play key roles in controlling lymphocyte activation and survival during an immune response. TNF family ligands are being explored as adjuvants for viral vaccines, and agonistic antibodies to TNFR family members are being investigated for immunotherapy of chronic viral infection alone and in combination with checkpoint blockade.

Recent evidence demonstrated that TNFα has potent anti-rotavirus effects achieved by NFκB-regulated genes via the activation of classical NF-κB signaling and independent of type I IFN production. Moreover, the use of infliximab totally abrogates the anti-rotavirus effect of TNF-α [235]. TNFi have potent activity in several diseases marked by excessive production of pro-inflammatory cytokine. That effect may prove useful in viral infections highly characterized by immune activation and inflammation such as HIV-1 infections. HIV-1 infection results in the depletion of CD4+ /CD8+ T cells with abnormalities in the cytokine network in the infected individuals. HIV-1 uses the TNFα signaling for expanding its reservoir. Several HIV-1 proteins mimic and regulate the TNF-α signaling pathway. Thus, TNFi has been considered as an additional therapeutic option for HIV-1 infection [236–238]. Evidence shows that TNF-α is a key mediator of virus-induced M₂ muscarinic receptor dysfunction and airway hyper-responsiveness in influenza infected-animals [239]. Interestingly, in this model etanercept has been shown to be able in preventing virus-induced airway hyperresponsiveness and M₂ receptor dysfunction, without changing lung viral titres. Nonetheless, all these data do not support enough the use of these molecules as anti-viral drugs. The only potential beneficial mechanism in COVID-19 patients could be reduction of TNF levels that appears to be increased especially in the critical stages. Moreover, a possible role in COVID-19 is the interaction with the ACE2 receptor. Indeed, SARS-CoV-related severe respiratory distress sees associated with the ability of the spike protein of SARS-CoV to down-regulate ACE2 expression [240]. In addition, SARS-CoV modulates with its spike protein the TNF-α-converting enzyme (TACE)-activity by via the cytoplasmic domain of ACE2 leading to both the viral entry and the only potential beneficial mechanism in tissue damage through TNF-α production [241]. Since the downregulation of ACE2 by SARS-CoV spike protein was proposed as an etiological event in the severe lung injury, authors previously suggested that TACE antagonists can block SARS-CoV infection and attenuate the severe clinical outcome [242]. Other studies confirmed the role of TNFα in promoting pathogenesis in SARS-CoV infection.
through its receptors and suggested that inhibition of TNFα signaling may represent viable chance for effective therapies [243]. Accordingly, evidence reports markedly higher serum levels of TNF-α together with IL2R, IL-6, IL-10, in severe than in moderate cases of COVID-19 [244]. A phase 4 randomized, open-label, controlled trial for the efficacy and safety of Adalimumab especially in those patients with particularly elevated TNF levels in the critical stages with severe COVID-19 is ongoing in Shanghai with the main outcome of Time to Clinical Improvement [245].

12. Tocilizumab

IL-6 is a cytokine produced by innate immune cells, mostly monocytes and macrophages, and also by mesenchymal, endothelial cells and fibroblasts, after the recognition of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [246]. IL-6 stimulates acute-phase immune response and hematopoiesis and it is involved in the complex pathogenesis of autoimmune disease like RA, Giant Cell Arteritis (GCA), Systemic Juvenile Idiopathic Arthritis (SJIA), and of cytokine release syndrome (CRS). CRS is a possible consequence of the administration of chimeric antigen receptor engineered T cells (CAR-T) immunotherapy, recently approved for the treatment of B-cell leukemias and lymphomas [246–249]. Tocilizumab, a humanized monoclonal antibody directed against IL-6 receptor, is capable to reduce and control the effects of the abnormal production of IL-6 and is approved by FDA and EMA for the treatment of RA and these diseases [250]. CRS related to CAR-T therapy can be caused directly by the infused CAR-T cells or through the activation of other immune cells and usually manifests with a clinical presentation that can range from a smolder condition to a multiple organ failure [251]. Since COVID-19 has some clinical similarities eventually progressing in the 15% of cases in ARDS, acute cardiac injury and acute kidney disease requiring ICU monitoring and a key role seems played by IL-6 that is markedly elevated along with TNF-α, IFN-γ, IL-2, IL-10, IL-8 and granulocyte macrophage–colony-stimulating factor (GM-CSF), the use of Tocilizumab (IV 8 mg/kg i.v.) was proposed [252–254].

Indeed, retrospective analysis of 452 patients admitted in the Tongji hospital for COVID-19 showed that inflammatory cytokine levels in patients with severe infection compared with non-severe, were significantly elevated, including IL-6, IL-2R, IL-8, IL-10, and TNF-α [214]. Analogous findings were reported by Lei et al. in a cohort of 29 Chinese patients: the levels of IL-2R and IL-6 in peripheral blood of patients with COVID-19 pneumonia were significantly increased and related to the severity of the disease [252].

Even though tocilizumab does not seem to have any direct anti-viral effect, the elevated serum levels of IL-6 in severe COVID and the evidence of a cytokine pattern similar to CRS led to the hypothesis of the use of this drug as treatment of severe respiratory infections whose manifestations could be correlated to the abnormal innate immune response stimulated by IL-6. A randomized controlled clinical trial is actually underway in China and another has been started in Italy, as abovementioned. Tocilizumab has already been used in the past weeks as an off-label drug in the absence of a specific therapy for the treatment of severe manifestations of COVID (ICU and not-ICU patients) in Italy during the epidemic outbreak that is leading to an emergency situation with more than 4000 victims [12,13]. Notably, another anti-IL6 drug is currently available for the treatment of RA, Sarilumab, a human monoclonal antibody against the IL-6 receptor. Tocilizumab is available both IV as well as subcutaneously, while Sarilumab only subcutaneously, possibly limiting its usage due to the slower effect in an acute scenario as that of ARDS or pre-ARDS from SARS-CoV-2 infection.

13. IVIg

Intravenous (IV) immunoglobulins (Ig), therapeutic preparations of pooled normal serum derived poly-specific IgG are widely used for the treatment of several immune-mediated conditions and rheumatologic diseases [255]. The immunomodulating properties of IVIg seem to be related to a complex and not yet understood direct action both on innate immune components (complement, monocytes, macrophages, natural killer, cells) and on adaptive immune cells such as CD4 T-cells and B-cells [255].

Far before the use of IVIg as immunomodulating therapy, serum Ig were identified as one of the cardinal constituents of the adaptive immune system against microorganisms (especially viruses, bacteria). The bond of Ig to viral superficial antigen is capable to prevent virus cell penetration, induce phagocytosis and clearance of viral structure by macrophages and neutrophils and finally to activate NK cells to eliminate infected cells. Enriched Ig preparation extracted from human placenta and then from human serum can be considered the first antiviral drug and the first example of the use of immunotherapy against an infection [256].

Intramuscular Ig were first used to prevent HAV and measles in the ‘40s later in the ‘80s of intravenous preparation with reduced anaphylaxis risk, specific Ig preparations developed from donors with high titers of desired antibodies (the so called hyperimmune preparations) were used to prevent and treat a variety of viral infections (HAV, rabies, CMV, variella zoster virus (VZV), Measles etc.) [257].

As derivatives from blood of healthy donors, IVIg contains a panel of polyclonal Ig directed against a wide variety of common microorganisms. In an interesting work Krause et al. evaluated the presence of specific antimicrobial activity in 5 commercial kit of IVIg showing that all of these preparations have significant activities against HSV, CMV, VZV, EBV, measles, mumps, rubella, parvovirus B19 [258]. For these reasons, IVIg are employed as a substitute treatment for patients with hypogammaglobulinemia (primary or secondary deficiency) and childhood acquired immunodeficiency syndrome (AIDS) to prevent common opportunistic viral and bacterial infection [259,260]. Many examples in literature were reported about the use of IVIg as prevention or treatment for opportunistic infections in immunocompromised patient secondary to immunosuppressive therapy, AIDS and bone marrow transplantation (BMT).

CMV is a common herpes virus that infects around 90% of the general population. In transplant patients, IVIg administered with acyclovir and ganciclovir seem to be capable of preventing CMV related complications [261]. A retrospective study by Ljungman et al. showed no additional benefit of IVIg in combination to the standard anti-viral therapy compared to anti-viral therapy alone, in the treatment of CMV gastrointestinal disease in BMT [262]. Some case series showed that IVIg could be a possible treatment strategy for active CMV infections in immunocompromised patients [263].

Common respiratory viruses like human metapneumovirus (hMPV), adenovirus (ADV) and RSV emerged as important etiological players in serious respiratory infections in immunocompromised patients following BMT and renal transplantation. IVIg demonstrated direct anti-viral activity in vitro against hMPV and RSV [264]. Many case reports and case series highlighted the efficacy of a regimen which included ribavirin or cidofovir and IVIg with complete resolution of symptoms in pneumonia caused by the abovementioned viruses [265–268].

IVIg were also used as prophylaxis for VZV infection in newborns exposed to the virus after birth [269] and were effective for the treatment of a disseminated VZV infection [270].

IVIg seem able to reduce the recurrence of genital manifestations of HSV 2 [271] and were shown to reduce the burden of BK polyomavirus in a proportion of transplant patients [272,273].

West Nile (WN) fever is caused by a flavivirus and provokes encephalitis and meningitis. In a mouse model, Commercial IVIg from Israel, in which the disease is endemic, showed efficacy to prevent the development of such disease if administrated during the viremic phase (i.e., administrated 1 day before and after the inoculation of the virus). The efficacy was directly proportional to the dose administrated. Interestingly, IVIg derived from USA donors, in which the disease is not
endemic, showed no protective effect [274].

On the other way, Ig specific serum antibodies could be also associated with an increased risk of infection by promoting viral entry in susceptible cells through a mechanism of antibody-dependent enhancement (ADE), firstly described for Flaviviridae, HIV and Ebola viruses, which involves specific cellular receptors like Fc receptor (FcR) or complement receptor type 3 (CR3) [275,276]. This process has been recently proposed by Fu et al. as a possible mechanism of lung damage in SARS-CoV pneumonia [277]. In particular, the viral surface antigen S-host antibodies (anti-Spike IgG) complex promotes the FcR-mediated internalization of the virus in macrophages (ADE) and activates intracellular signaling of FcR. This interaction results in the up-regulation and release of the pro-inflammatory cytokines responsible for severe lung disease. Moreover, it was observed that the appearance of anti-viral IgG coincided in 80% of patients with the onset of acute respiratory disease [278]. The same study identified IVlg as an agent able to saturate FcR and possibly prevent the lung damage in SARS-CoV infection [279]. Despite a systematic review of SARS treatment showed inconclusive results in a retrospective analysis of IVlg treated patients, a possible therapeutic role of IVlg in COVID-19 could be hypothesized [280].

14. Janus kinases inhibitors

Janus kinases (JAKs) are intracellular signaling components that function downstream of many cytokines [281]. Four members are included in the JAK family: JAK1, JAK2, JAK3, and non-receptor tyrosine-protein kinase (TYK2). All JAKs play a key role in immune response, and JAK1/2 are also involved in hematopoiesis [282]. JAK-1 and JAK-2 (JAK 1/2) kinase are found in the cell cytoplasm and are associated with key cytokine receptors and activating kinases. Upon activation, JAK 1/2 induce downstream activation of signal transducer and activator of transcription (STAT) protein, with transduction of the signal and the final transcription of several genes involved in immune cells proliferation, differentiation, activation/inhibition, and survival/apoptosis [283].

In the immune response to pathogens including viruses, the signaling occurs via the JAK-STAT pathway, including type I (regulated by JAK1-JAK2 complexes) and type II IFNs (mediated via JAK1-JAK2 complexes) [284]. The IFNs are a primary defense against pathogens because of their strong anti-viral activities. As known, IFN-α is an important therapeutic option for the successful treatment of HBV and HCV and have effects against hepatitis E infection and for chronic delta hepatitis [285].

JAK1 is required for both type I (α and β) and type II (γ) IFN signaling pathways, while JAK3 is crucial for lymphocyte development and function [281]. Viral infections induce the expression of pro-inflammatory cytokines such as IL-1, IL-6, TNF, IL-8, as well as chemokines [282]. Generally, IFN-α/β would be considerably upregulated by viral infections: studies found that distinct anti-viral activities correlate with virus-specific expression levels of Interferon-stimulated genes (ISGs) subsets. For instance, some ISGs have strong anti-viral effects while others promote viral replication in vitro [283]. Macrophages produce significant levels of IFN-α/β, IL-1β, TNF-α, chemokines, and IL-18 [284]. Dendritic cells (DCs), especially plasmacytoid DCs, respond to virus infections dose dependently by inducing high expression of IFN-α/β or on the contrary by a rapid pDC apoptosis. Moreover, IFN-α downregulates IFN-γ production by NK and CD8+ T cells through IL-10 production, leading to a rapid induction of an adaptive immune response [285].

JAK inhibitors (JAKI) are small molecules targeting the intracellular cytokine signaling [286]. There are a number of JAKI currently both used and under investigation for use in several autoimmune systemic diseases such as RA, PsO, PsA, some of which are also approved or under investigation in other indications [287]. Tofacitinib has specificity for JAK3 and JAK1 over JAK2 and baricitinib mainly inhibits JAK1 and JAK2 [288,289] while upadacitinib that will be soon marketed demonstrated a 74- and 58-fold greater selectivity for JAK1 over JAK2 and JAK3, respectively [290].

JAKI have anti-viral potential since may lower the pro-inflammatory response mediated by viruses. For instance, baricitinib induces a dose dependent inhibition of IL-6 [291]. Intriguingly, baricitinib inhibits the AP2-associated protein kinase 1 (A2K1) that is a regulator of endocytosis that the SARS-CoV-2 uses to infect lung cells through binding with ACE2. This may possibly interrupt the passage of the virus into cells and also the intracellular assembly of virus particles. Baricitinib, at therapeutic dosing (either as 2 mg or 4 mg once daily) is sufficient to inhibit A2K1, thus it has been suggested to be trialed in an appropriated COVID population [6,292]. On the other hand, therapies targeting JAK complexes may interfere with normal anti-viral response including inhibition of IFN-γ activity and may potentially increase the risk of infection and/or reactivation of several viral infectious diseases [293–295], including a dose dependent risk for VZV observed for tofacitinib and baricitinib [296]. Thus, their usage should be carefully evaluated. Moreover, selective JAK1, such as fedratinib, a JAK2 targeted JAKI able to inhibit Th17-mediated response implicated in the disease cytokine release syndrome, may be more beneficial than others [297].

15. Granulocyte-monocyte colony stimulating factor

Granulocyte-monocyte colony stimulating factor (GM-CSF) is a fundamental cytokine and growth factor involved in the proliferation and maturation of myeloid precursors, such as granulocytes, monocytes and macrophages that express its receptor. In fact, it is widely used to reverse neutropenia induced by cytotoxic agents. However, there is extensive evidence that GM-CSF is able to prime macrophages to transcribe and secrete cytokines, such as IL-6 and TNF-α, responsible for common adverse events such as fever and myalgia [298].

Following the outbreak of COVID-19, Huang et al. demonstrated that patients display markedly increased plasma levels of GM-CSF compared to healthy population, suggesting GM-CSF may play a role in the context of CRS [2]. Additionally, pathologic studies demonstrated a significant macrophage infiltration in lungs of patients who died with SARS-CoV infection, which shares significant similarities with COVID-19 [299].

Recently, a more detailed analysis has been performed on T cells from peripheral blood of COVID-19 patients. Very interestingly, high levels of CD4+ cells expressing both GM-CSF and IL-6 were found in COVID-19 patients, along with GM-CSF–secreting CD14+CD16+, compared to healthy controls. Even more intriguingly, Th1 cells expressing both GM-CSF and IFN-γ were detected exclusively in ICU patients, thus suggesting a potential contribution of GM-CSF to CRS [300]. These data are further supported by the evidence that human pulmonary microvascular endothelial cells (HPMEC) are able to secrete GM-CSF following an inflammatory stimulus such as LPS, suggesting a potential important role in the pathogenesis of ARDS, in which increased levels of GM-CSF are present in BAL fluid [301]. Additionally, animal models have demonstrated that GM-CSF neutralization is effective in reducing the severity of neuroinflammation induced by CAR-T therapy, with no negative effect on its efficacy [302].

Multiple GM-CSF and GM-CSF receptor inhibitors (namilumab, mavrilimumab, otlimumab) are currently under investigation for the treatment of RA and preliminary data seem promising [303,304]. The blockade of this pathway may represent an additional interesting experimental approach to treat CRS in COVID-19 patients.

16. Concluding remarks

The fight against SARS-CoV-2 is one of the greatest challenges not only for medical science but that the entire human community had to
face in the last century. The search and discovery of any weapon that could possibly ameliorate this disease is eagerly awaited and a crack in the door has been opened by already existing drugs from the rheumatologist armamentarium. Table 2 summarizes the currently used agents with an anti-viral effect and their supposed mechanism of action. Moreover, we aimed at giving new ideas towards a rheumatologic approach at the treatment of the SARS-CoV-2 infection. Notwithstanding that this fight will be won only together with the all the professional figures collaborating every day in treating COVID-19 patients [305–307].
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