COVID-19, CYTOKINES, AND INFLAMMATION

UNIVERSITAT ROVIRA I VIRGILI

Bosco Ruiz de Mendoza Araiz

FINAL DEGREE PROJECT

Supervised by Dr Maria Guirro Castellnou

Degree in Biochemistry and Molecular Biology

Tarragona 2022

Index

Abstract

SARS-CoV-2 firstly appeared in 2019 in Wuhan due to an increase of unusual pneumonias for unknown causes. A lot of research has been done to understand its behavior but, after two years, there some molecular mechanisms which are still unclear such as the role of cytokines. For this reason, this review pretends to summarize the role of cytokines and its immunopathology, as well as the possible treatment to avoid the cytokines storm (CS) with interferon administration. New therapeutic strategies should be investigated for the population at risk or for those who do not respond to current treatment.

Introduction

At the end of 2019, an outbreak caused by a new coronavirus set off alarms in Wuhan. The new virus was called SARS-CoV-2 and it was discovered due to an increase in unusual pneumonias of unknown cause in Hubei province, China [1,2]. Its high transmissibility led the world to a pandemic. Currently, the weekly update published by the WHO on April 27, 2022, dictates that the total number of cases globally is around 507,184,387 with a total of 6,219,657 deaths [2]. (Table 1). Over the last few years, it has been demonstrated that the symptoms caused by SARS-CoV-2 resembled those of the common cold, but in some cases the infection can become more severe and cause from pneumonia to severe acute respiratory syndrome.

WHO Region	New cases in last 7 days (%)	Change in new cases in last 7 days *	Cumulative cases (%)	New deaths in last 7 days (%)	Change in new deaths in last 7 days *	Cumulative deaths (%)
Europe	2 289 820 (50%)	-23%	213 043 360 (42%)	6 811 (45%)	-23%	1 980 000 (32%)
Western Pacific	1 487 880 (33%)	-28%	53 464 927 (11%)	2 246 (15%)	-33%	222 968 (4%)
Americas	550 015 (12%)	9%	152 533 748 (30%)	4 029 (27%)	-19%	2 719 562 (44%)
South-East Asia	161 639 (4%)	-6%	57 734 555 (11%)	1 580 (10%)	41%	783 530 (13%)
Africa	35 994 (1%)	32%	8 721 105 (2%)	185 (1%)	110%	171 564 (3%)
Eastern Mediterranean	22 878 (1%)	-30%	21 685 928 (4%)	283 (2%)	-34%	342 020 (5%)
Global	**4 548 226 (100%)**	**-21%**	**507 184 387 (100%)**	**15 134 (100%)**	**-20%**	**6 219 657 (100%)**

Table 1. Newly reported and cumulative COVID-19 confirmed cases and deaths, by WHO

SARS-Cov-2 is a positive-stranded RNA virus (+ssRNA), the spike glycoprotein-S facilitates the entry of the virus into its receptor in humans, ACE2 [3,4]. After the entry through ACE2, the body up-regulates the immune system trying to eliminate the pathogen, dendritic cells and mononuclear macrophages provoke an acute response by

releasing proinflammatory cytokines such as IL-6, IL-1 or IFN- γ. Cytokines cause the initiation of an adaptive response mediated by T cells and interferon promotes the elimination of the pathogen through the activity of Natural Killer (NK) cells. However, recent studies have asserted that a poor initial response to type I and III interferons is associated with a more severe disease course [5,6], the failure of the first response causes an increase in immune activity that is difficult to downregulate, again causing a state of hyperinflammation or a cytokine storm (CS) [5,7]. Different studies have confirmed that the levels of cytokines such as IL-2R, IL-6, IL-10 and IFN-γ increased significantly during severe COVID-19 [8,9]. Some of the key inflammation signaling pathways are the interleukin-6/Janus kinase/STAT pathway, the interferon pathway, tumor necrosis factor α-nuclear factor kappa pathway, the toll-like receptor pathway, etc. These pathways cause excessive secretion of proinflammatory cytokines[7-10].

Vaccination against the virus has been a great medical advance in the face of the pandemic thanks to the protection it offers against symptomatic disease. Different studies throughout the world corroborate the efficacy of vaccination; For example, a study carried out in England by the epidemiologist Jamie López Bernal states that the risk of hospital admission with a single dose of BNT162b2 (Pfizer) decreases by 43% and mortality was reduced by 51% [11].

During the following bibliographic search, it is intended to collect information about the mechanisms of response to infection, as well as the role played by the different cytokines and the different treatment strategies to avoid the cytokine storm.

Hypothesis and Objective

The hypothesis of this project is to discover the role of cytokine storm by the promotion and suppression in the immune system during COVID-19 disease.

To achieve this hypothesis, the principal objective is to analyze and collect information about the role of cytokines in COVID-19 and gather information about different therapeutic strategies to avoid the cytokine storm. So, refined bibliographical research will be carried out using PubMed database. The research is aimed to collect information about SARS-CoV-2 virus and the main role of inflammatory cytokine in the disease.

SARS-CoV-2 Structure and characteristics

Coronaviruses were first described in 1960 as single strand positive RNA virus with a large genome size equal to 26-32 Kilobase, 29.9 for the SARS-CoV-2. They took corona name due to the similarity of the virion to a crown when watched under electronic microscope. There are multiple similarities between coronaviruses in terms of gene organization or expression, 16 nonstructural proteins, a spike protein (S), the envelope (E), a membrane (M) and the nucleocapsid (N)[12]. (Figure 1). A 2020 study showed that the SARS-CoV-2 virus in addition to sharing 79% of its genome with SARS-CoV also had similarities in the expressed protein length except for the S protein[13]. The S protein in SARS-CoV-2 is 1,273 kilobases long which is different to most of Sarbecoviruses [13]. Furthermore, there is a four amino acid insertion (PRRA) in the junction of S1 and S2 subunits in the spike protein[14,1516].

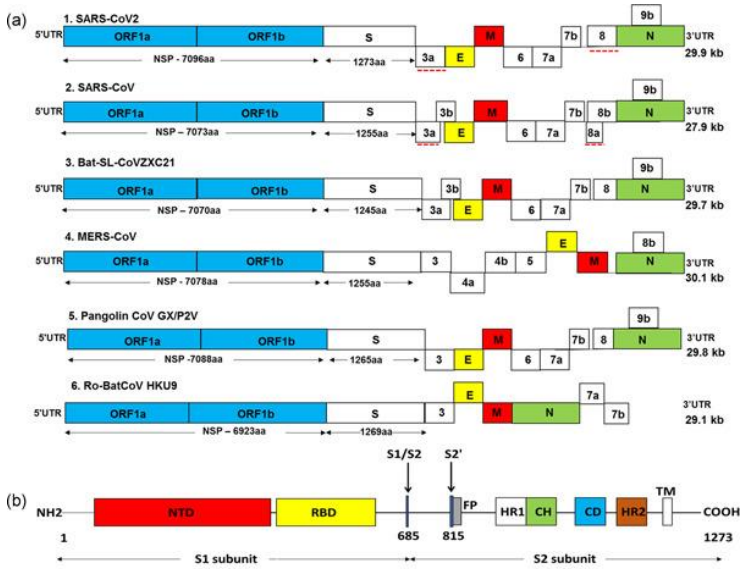

Figure 1 (a) Genome structure of SARS-CoV-2 and other coronaviruses. (b) SARS-CoV-2 spike (S) glycoprotein. Kadam, S. B., Sukhramani, G. S., Bishnoi, P., Pable, A. A. & Barvkar, V. T. ***SARS-CoV-2, the pandemic coronavirus: Molecular and structural insights****.* Journal of Basic Microbiology

Cell entry and infection

It has been demonstrated that metallocarboxyl peptidase angiotensin receptor 2 or ACE2 acts as a main receptor for the SARS-CoV-2's spike protein[17,18] . The S protein in the mature virion is formed by two non-covalently joined subunits, S1 subunit's function is to bind ACE2 and S2 anchors the S protein to the membrane, helping the cell fusion[19]. Once the S1 domain recognizes ACE2 a second step is crucial for the infection to be a success[20]: The transmembrane serin protease (TMPRSS2) cleaves the S1/S2 domain leading to the membrane fusion and infection[19,21]. However, in virus producer cells the S1/S2 domain is cleaved by furin[19]. If the virus is internalized by the endosomal entry the TMPRSS2 protease is not available, and Cathepsin L becomes the S2 cleaving protease[19,21,2223] (Figure 2). Once the fusion is done, a pore is created, and the viral RNA is introduced in the target cell. The coronaviruses start to replicate and express their large RNA sequence so the full sequence can be incorporated in the new formed viral particles. The ORF1a and ORF1b located at the 5' end codify for non-structural proteins such as replication complex proteins while the ORF located at the 3' end codify structural

proteins[23]. Some studies have showed that the new buddied virions travel to endoplasmic reticulum-Golgi system, so they get released by exocytosis[24]. However new studies evidence that the virions could be release via lysosomal traffic pathway[23,25].

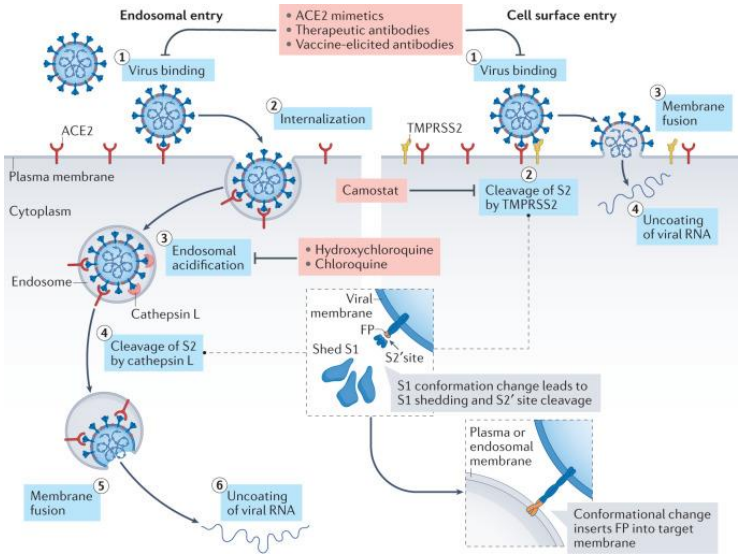

Figure 2 Jackson CB, Farzan M, Chen B, Choe H. ***Mechanisms of SARS-CoV-2 entry into cells****. Nat Rev Mol Cell Biol. 2022;23(1):3-20. doi:10.1038/s41580-021-00418-x*

Immunopathology of COVID-19: Cytokines and Pathways

When the SARS-CoV-2 infects airway epithelial cells or immune cells through the ACE2 causes the release of damage-associated molecular patterns (DAMP) and inflammatory cytokines thanks to the immune cells.

However, COVID-19 patients suffer an anormal immunity response with a high hyperinflammatory innate response and a stressed protective adaptative response[26]. The respiratory insufficiency caused by the exacerbated lung inflammation is a consequence of immune and cytokine disregulation[27]. Some studies have shown an increase in proinflammatory cytokines during severe COVID-19 such as interleukin-1 (IL-1), IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-17, IL-18; monocyte chemoattractant protein 1 (MCP-1)/CCL2 tumor necrosis factor-α (TNF-α) or IFN-γ[9,26–29].

Toll-like receptors and Type I interferon response

The type I interferons such as IFN-α or IFN-β are key factors for the antiviral immunity[27,30], besides regulating the adaptative response, they also regulate the innate response. Viral recognition promotes the INF production and NFκB pathway: The IFN-I production is controlled at transcription level, the IFN I gen is inducted thanks to a cytosolic pathway in which intracellular virus-associated molecular patterns are recognized[30]. When the SARS-CoV-2 infects the cell, the viral RNA is recognized by the pattern recognizing receptors (PRR) such as the toll-like receptors (TLR), retinoic acid-inducible gen 1 (RIG-1), melanoma differentiation-associated protein (MDA5), protein kinase C (PKC) or virus-associated molecular patterns[27,31–33]. The SARS-CoV-2 single strand RNA is recognized by the TLR7 while the TL3, RIG-1 and MDA5 recognize the double stranded RNA present in viral replication phase. However, different studies have showed that IFN I response decreases during COVID-19 disease[26,34] . (Figure 3). On the

one hand, some studies suggest that the SARS-CoV-2 inhibits the IFN response thanks to both structural and non-structural components of the virus such as nonstructural protein 1 (NSP1), NSP13, NSP14, the protein M, protein N, open-reading frame 3a (ORF3a) protein, ORF6 protein or S protein[26,27,35–40]. Furthermore, the fact ORF3b, ORF6, ORF8, and N proteins of SARS-CoV-2 dysregulate IFN response has been reported by different studies[38–40]. On the other hand, the decreased IFN response could be explained due to a decrease in the number of plasmacytoid dendritic cells during viral infection as they are the main IFN I producers[34,41].

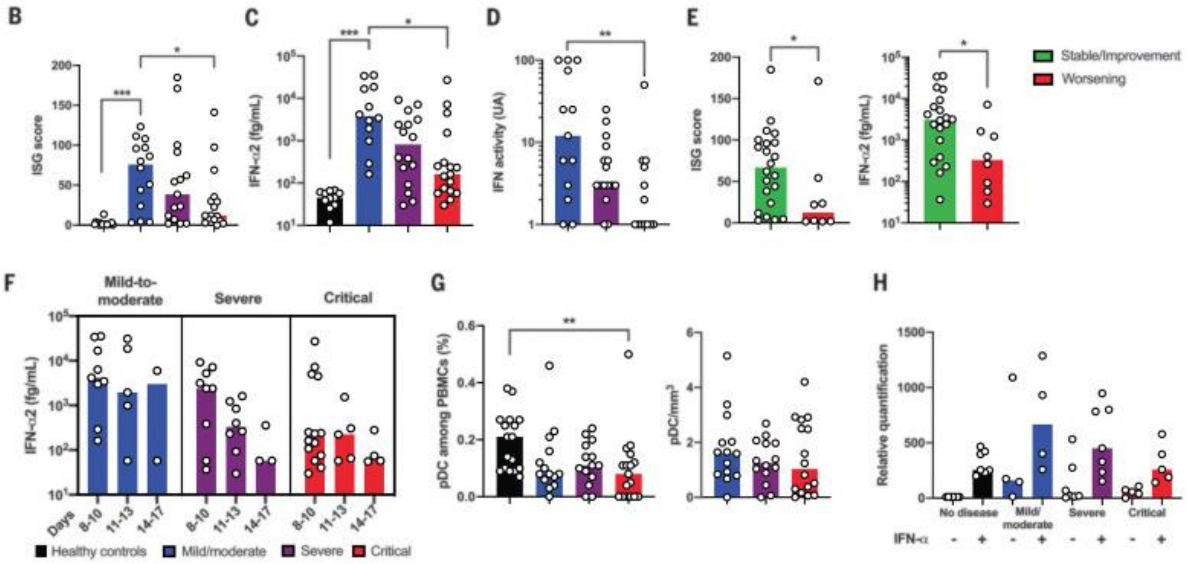

Figure 3 Hadjadj J, Yatim N, Barnabei L, et al. ***Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science****. 2020;369(6504):718-724. doi:10.1126/science.abc6027*

JAK/STAT signaling And Cytokines

The Janus kinase (JAK)-Signal transducer and activator of transcription (STAT) pathway is a gene regulation mechanism in which extracellular factors affect the genes expression thanks to the STAT activation family phosphorylation [42,43]. When a ligand, such as IL-6, is bound to a cytokine receptor where a receptor associated JAK is located, JAKs will start to phosphorylate each other creating a STAT binding site. STATs are cytoplasmatic transcription factors and they need to be phosphorylated and dimerized before translocating to the nucleus where they will activate inflammatory cytokines[34,42,44].

IL-6/JAK/STAT

As mentioned, IL-6 is a potent inflammatory cytokine capable of activating the JAK/STAT pathway. IL-6 is firstly produced in monocytes, dendritic cells and macrophages activates JAK/STAT pathway leading in a STAT3 phosphorilation[34,44]. A study demonstrated that patients with COVID-19 related pneumonia expressed more phosphorylated STAT3 in leukocytes compared with healthy controls[45].

The IL-6 pathway begins with the union of IL-6 to its receptor IL-6R. On the one hand, the classic signaling pathway, mediated thanks to the membrane-bound IL-6 receptor (mIL-6R) and gp 130, generates the IL-6/IL-6R/gp130 complex that activates

JAK/STAT3 and MAPK signaling[44,46]. This activation promotes differentiation of $CD8^{+}$T, B cells, migration of neutrophils[47] in addition to the differentiation of T CD4 helpers into Th17 cells which produce the inflammatory cytokine IL-17[46,48]. Furthermore, IL-6 showed an inhibition TGF-β-induced Treg differentiation[48–50]. On the other hand, in cells without mIL-6R, like endothelial cells, the trans-signaling mechanism activates gp130 by soluble sIL-6R, that results on a JAK-STAT signal. The IL-6/ sIL-6R complex can make endothelial cells produce IL-6, IL-8 and MCP-1 in addition to an increase of intercellular adhesion molecule-1 expression, which leads to leukocyte recruitment[50].

Moreover, when SARS-CoV-2 infects the cell binding to ACE2, the viral components provoke the secretion of IL-6 and NFκB from immune cells. The fact the virus binds ACE2 leads in a ACE2 downregulation[51]. The activation of AT1aR due to an excess of Ang II makes the mIL-6R to detach due to ADAM10 and ADAM17, resulting in a sIL-6R conversion. The suppressor of cytokine signaling 3 (SOCS3) can negatively feedback proinflammatory cytokine pathways but it is suppressed during IL-6 trans mechanism[52] which suggests that SARS-CoV-2 can promote angiotensin II expression by downregulating ACE2.

IL-2 and IL-2//IL-2R/JAK/STAT5

It has been demonstrated that IL-2 not only plays a key role in regulatory T cell, CD4+, CD8+ and, NK cells life through JAK/STAT5 pathway but is also capable of inhibiting the generation of proinflammatory T helper 17 cells[53,54]. Some studies have analyzed that IL-2 levels were increased in severe COVID-19 disease[55]. A clinical trial found that critical COVID-19 patients had low levels of IL-2 in plasma and low expression of IL-2R in peripheral blood mononuclear cells[56] in contrast to those with the severe COVID-19. So, this means that a decrease in IL-2 plasma levels could be a warning of patient's deterioration. So, the reduction of IL-2, IL-2R, JAK1 and STAT5 could be a consequence of the lymphopenia during cytokine storm[56].

IFN-γ and IFN-γ/JAK/STAT pathway

IFN-γ is a Type II interferon produced by CD4+ T helper cell type 1 (Th1) lymphocytes, CD8+ cytotoxic lymphocytes, NK cells, antigen-presenting cells, B cells, etc. In terms of infection defense, the secretion of IFN-γ by NK cells plays an important role activating macrophages [57]. The IFN-γ is an important inflammation response participant and its secretion is mediated by APC cytokines such as IL-12 or IL-18. MIP-1α chemokines attract NK cells to the inflammation area and the IL-12 makes them produce IFN-γ[57,58]. Furthermore, the IFN- γ can activate antiviral and adaptative immune response through JAK1/JAK2 what leads in STAT1-IFN-γ-activated site (GAS) cascades[34,59,60]. (Figure 4).

A recent study of the upregulated cytokines during SARS-CoV-2 infection found out that the inhibition of TNF-α and IFN-γ had a protective effect in SARS-CoV-2 related dead, sepsis, hemophagocytic lymphohistiocytosis (HLH), and cytokine shock[60].

HLH is a rapidly progressive systemic inflammatory disorder marked by a large cytokine production and an extremely high histiocyte and macrophage proliferation. Furthermore, there is a persistent activation of cytotoxic T lymphocytes and NK cells which results in a high but with an inefficient proinflammatory TNF-α and IFN-γ cytokine secretion [61–64].

Considering that COVID-19 patients have higher serum level of cytokines IFN-γ [65] and that IFN-γ produced by T $CD4^+$ cells was reduced during severe disease, the IFN produced in the severe diseases must come from macrophages[66].

A study showed that IFN-γ levels were negatively associated with the increase of fibrosis volume in COVID-19 at discharge. These data suggest that early intervention of anti-viral infection using IFN-γ could be substantial in the inhibition of fibrosis for better functional recovery[67].

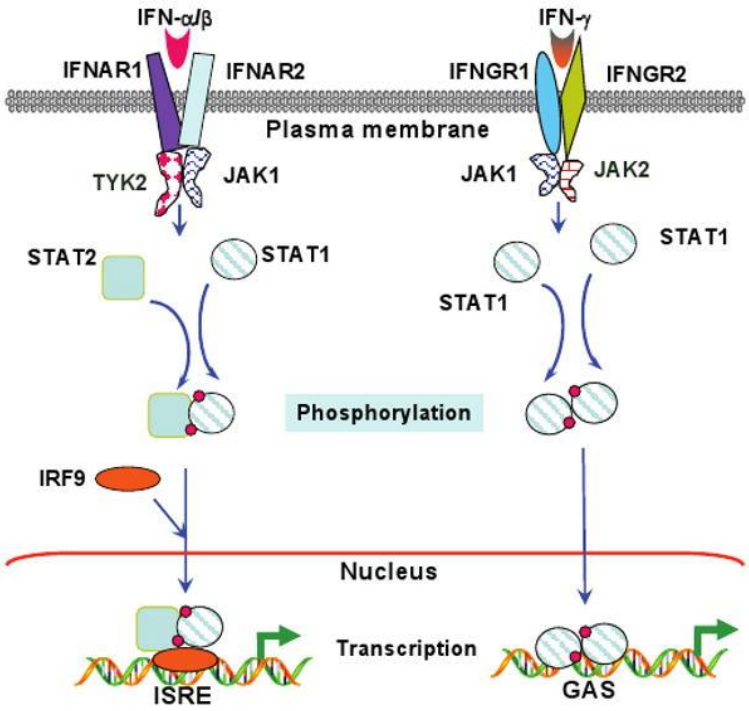

Figure 4 Li H, Gade P, Xiao W, Kalvakolanu DV. ***The interferon signaling network and transcription factor C/EBP-beta****. Cell Mol Immunol. 2007;4(6):407-418.*

IL-1

IL-1 is a pro-inflammatory cytokine which is produced by innate immune cells like monocytes and macrophages. One of the main functions is to promote the migration of immune cells but it also can promote the secretion of secondary inflammatory cytokines, th17 cell differentiation or activate the NF-κB pathway to stimulate the secretion of IL-1 itself[5]. The IL-1β, well known for its IL-1-systemic effect, is firstly expressed as pro-IL-1β and needs to be cleaved in specific sites for the secretion and biological function. The cleavage is performed by caspases inside the inflammasome complex. Canonical inflammasome sensor proteins (NLRP) can respond to specific signals such as pathogen or danger associates molecular patterns that indicate the infection. Once the signal is recognized by NLRP the inflammasome protein aggrupation is promoted which leads in the activation of the caspase that activates the IL-1β[68]. This canonical activation suggests that the NLRP3/IL-1β signaling pathway may be involved in hyperinflammation and cytokine storm. Furthermore, some publications reported that IL-1 receptor blockade with *anakinra* is associated with a reduced mortality in sepsis patients with macrophage activation syndrome[69]. *Anakinra* is a recombinant IL-1 receptor antagonist that has been

used the treatment of autoimmune diseases[5]. Nine patients with moderate to severe COVID-19 pneumonia, used anakinra and showed effectiveness improving clinical and biological markers[70].

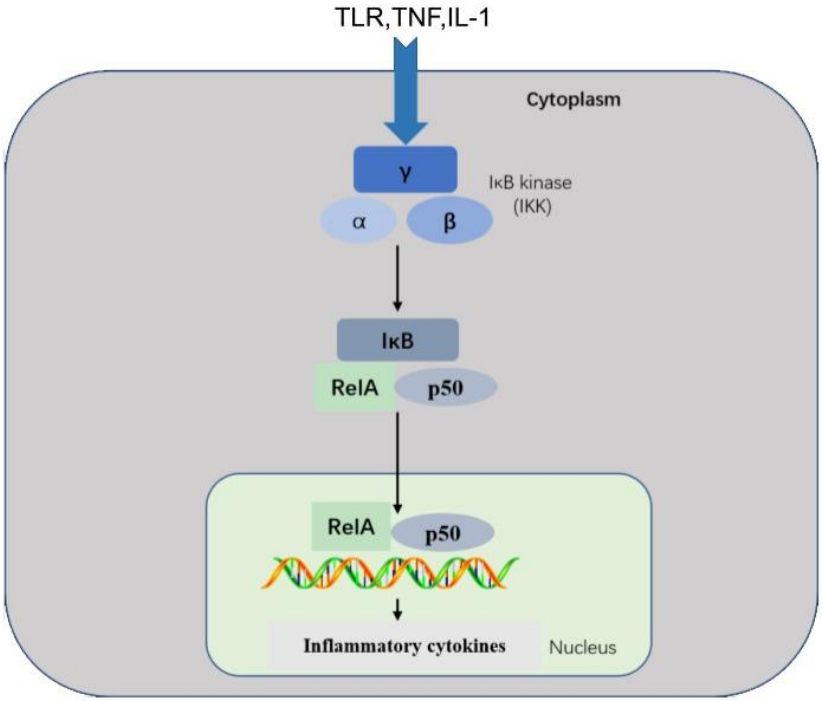

Figure 5 Chen L, Deng H, Cui H, et al. ***Inflammatory responses and inflammation-associated diseases in organs. Oncotarget****. 2017;9(6):7204-7218. Published 2017 Dec 14. doi:10.18632/oncotarget.23208*

TNFα/NF-κB Pathway

The nuclear factor kappa-light-chain-enhancer of activated B cells family (NF-kB family) consist of five related proteins: p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB and c-Rel (Rel) [71]. They form a transcription factor which, through the NF-kB pathway, induces pro-inflammatory signaling due to its role in inflammatory cytokines, chemokines, and adhesion molecule related gene expression[72]. Moreover, expression of several inflammatory mediators, for instance, cox-2, inducible nitric oxide synthase (iNOS), TNF-α, and ILs are regulated through NF-κB [73]. Cytokine signals can induce the phosphorylation, ubiquitination, and degradation of IκB provoking the nuclear translocation of NF-κB[71] where can bind to different promoter regions of several genes and activate around 400 inflammation related genes[74]. (Figure 6). One of the main NF-κB pathway inducers is the TNF-α cytokine[72]. TNF-α is a proinflammatory cytokine that activates the NF-κB canonic pathway through the TNFR1 receptor [72,75,76].

The relationship between TNF-α levels and the COVID-19 pathogenesis is still not clear: A bibliographic research analyzed the correlation between TNF-α, D vitamin and COVID-19 severity and mortality[77]. Despite the elevation of TNF-α due to the SARS-CoV-2 infection, the differences between the cytokine levels in different patient groups (mild, severe, and critical) is not clear. Merza et al. reported that the difference in TNF-α levels where not significantly higher in severe COVID-19 patients compared with non-

severe patients[78]. In contrast, a meta-analysis by Mulchandani showed that the difference between severe and not severe patients TNF-α levels where significantly diferent[79]. The TNF-α increment was supported by other studies [66,80,81]. The role of the TNF-α during the COVID-19 disease needs more observation.

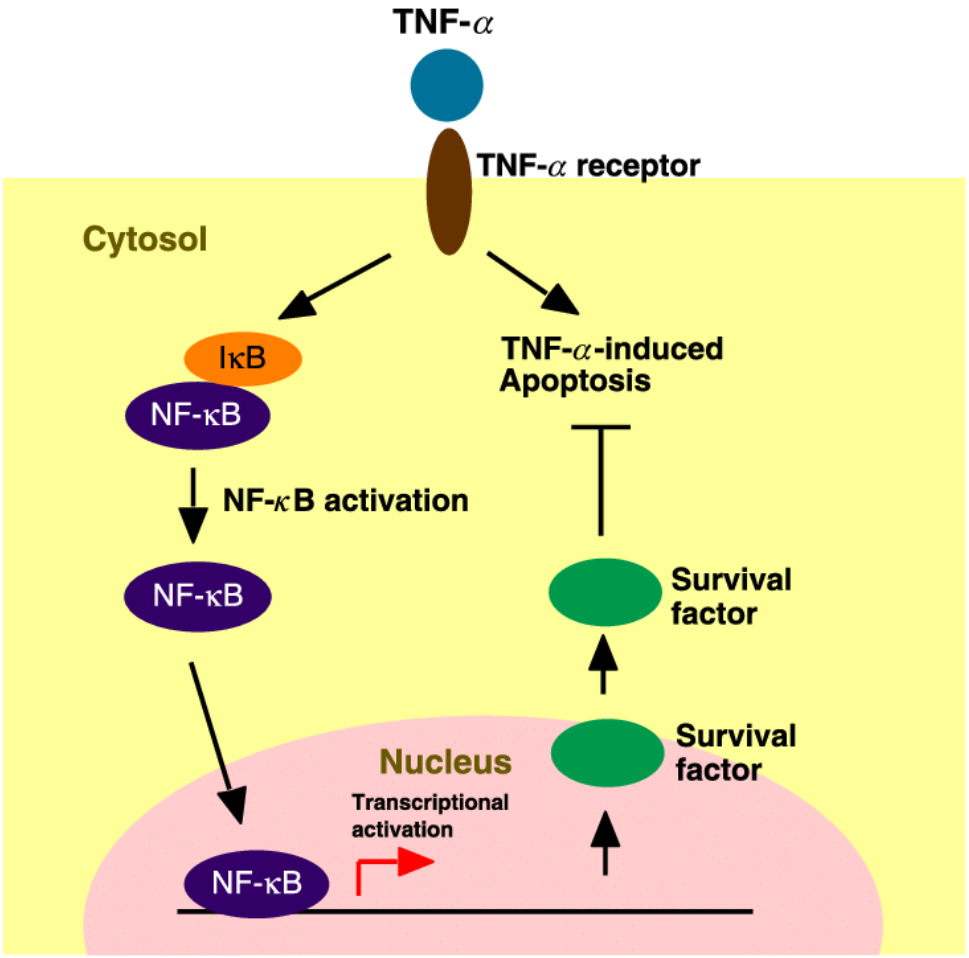

Figure 6 Hayashi, T. & Faustman, D. ***A role for NF-κB and the proteasome in autoimmunity****. Archivum Immunologiae et Therapiae Experimentalis 48, 353–365 (2000).*

IL-10

IL-10 is a protective anti-inflammatory cytokine that keeps the host protected from exuberant immune responses. It is expressed by many adaptative immune cells like Th1, Th2, TH17 and innate response cells such as DC, macrophages, mast cells, NK cells, eosinophils, and neutrophils[82,83]. The inhibition function starts with the binding of IL-10 to its receptor IL-10R which activates the JAK1-TYK2-STAT3 cascade resulting on a STAT3 anti-inflammatory response[84]. (Figure 7). The response occurs due to a STAT3 transcription reprogramming that includes the formation of transcriptional repressors, chromatin modifiers, and post-transcriptional or post-translational regulators. This inhibition can inhibit responses such as those induced by PPR[82,84].

It has been demonstrated that during severe COVID-19, IL-10 levels where significantly elevated[66]. Furthermore, some studies showed the importance of IL-10 and IL-6 as disease severity predictors[85]. The high levels of IL-10 during severe disease may be a

product of the attempt to inhibit the hyperactivity of the immune system. However, in front of a hyperinflammatory response and a cytokine storm, the IL-10 cannot function properly[34].

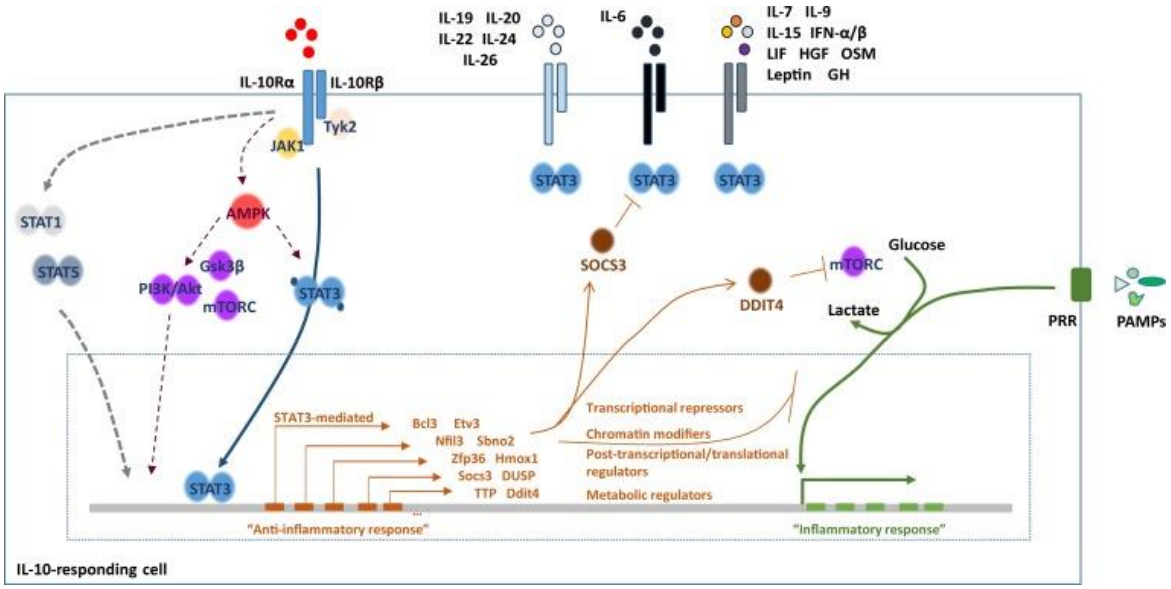

Figure 7 Saraiva M, Vieira P, O'Garra A. ***Biology and therapeutic potential of interleukin-10****. J Exp Med. 2020;217(1):e20190418. doi:10.1084/jem.20190418*

IL-12

The interleukin 12 is a key cytokine in terms of intracellular infection defense due to its ability to stimulate not only innate response cells but also adaptative cells[86]. The IL-12 secretion by dendritic cells promotes the proliferation of Th1, th17 or NK cells and consequently promote the IFN-γ production in these cells[87]. It has been demonstrated that IL-12 levels were not significantly higher in COVID-19 patients compared with healthy controls[55]. In contrast a recent study has reported that IL-10 and IL-12p50 levels can predict severity in COVID-19 in patients with comorbidities[88]. Despite the probability of progression to severe disease in hypertensive patients with obesity and diabetes was 0.1% (in absence of increased IL-10 and IL-12 (p70) levels), severe disease probability increased to 81.5% with levels of these two cytokines exceeding the 90% sensitivity thresholds[88].

Moreover, a recent study has reported that patients with COVID-19 disease can present neurological manifestations and the study showed that inflammatory neurological disease was associated to an increase of some interleukins such as IL-12[89].

IL-17

The IL-17 is a proinflammatory cytokine mainly produced by Th17 cells, NK cells and type 3 innate lymphoid cells (ILC3)[90]. The IL-17 can promote the secretion of granulocyte colony-stimulating factor, chemokines that recruit neutrophils or pro-inflammatory cytokines such as IL-6 and IL-17 itself[91]. In addition, it can also prevent the apoptosis in acute respiratory distress syndrome (ARDS)[92].

Clinical trials reported that during severe COVID-19 hospitalization the IL-17, granulocyte colony stimulating factor (G-CSF), MCP-1 among others were elevated in

patients[93]. The breach of the epithelial barrier, the tissue damage and an increased immune activation increases the magnitude of the IL-17 response[91] during the infection. In fact, the Treg/Th17 diminished during severe COVID-19 due to a descension in Treg cells[93].Furthermore, the Treg/th17 disbalance is associated with systemic inflammation and acute respiratory distress syndrome (ARDS)[93–95]. The IL-17 has a key role in Cytokine Storm due to its ability, among others, to recruit neutrophils[34].

Granulocyte-macrophage colony-stimulating factor

The Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is colony stimulating growth factor that stimulates the proliferation of neutrophilic granulocytes and macrophages in the bone marrow[96] produced by endothelial, epithelial, fibroblasts and T lymphocytes cells, among others. GM-CSFR signal pathway starts with the trans-phosphorylation of Janus Kinase 2 (JAK2)[97]. In addition to promote cell differentiation, it has been shown that GM-CSF can activate NF-κB directly through IKK2 binding or indirectly through JAK2 activation[97].

During Cytokine storm the GM-CSF stimulate the migration of proinflammatory cytokine secreting cells what worsens the inflammation[98]. The GM-CSF increases during mild and severe COVID-19 [55,66].

Immunopathology of COVID-19: Cytokine Storm

The cytokines are key mediators in terms of immune response because of their ability to mediate cellular survival, inflammation, coagulation, among other functions. However, an imbalance in the immune response that cause an excessive secretion of cytokines can be harmful due to the hyperinflammation state named CS[99]. CS is characterized by elevated levels of inflammatory cytokines, in particular IL-6, and a NK/CD8 cell cytolytic function decrease. The cytolytic cells cannot lyse infected cells what results in a pro-inflammatory perpetuated cascade[100].

Cytokine Storm and Cells (Dendritic Cells, endothelial cells and macrophages)

During the immune response different cells participate in cytokine secretions: Dendritic cells (DC) present antigens to T cells during infection. Hermans et al. reported that cytotoxic T cells can eliminate charged DC as a negative feedback mechanism[101] without been affected by IFN-γ. Patients with HLH are not able to eliminate DC due to the malfunction of cytolytic cells, provoking a persistent antigen presentation and consequently, a cytokine overproduction. HLH is a very rare disease, however, severe COVID-19 patients can fulfill the HLH diagnosing criteria. An increase in HLH patients have been notified suggesting that SARS-CoV-2 could produce type 2 HLH. More attention needs to be paid in severe COVID-19 associated HLH[102,103].

Endothelial cells can also contribute to the hyperinflammatory state through endothelial activation (EC). EC refers to the interaction between cells of the immune system (macrophages and leukocytes) and endothelial cells. Car-T therapy patients who died of cytokine storm syndrome showed that endothelial cells were contributing on the IL-6 secretion[104] allowing to suggest that during COVID-19 CS endothelial cells play a key role contributing on the inflammatory molecule secretions[105,106].

Different studies have shown that macrophages produce inflammatory cytokines such as TNFα or IL-6 during inflammatory diseases[100]. During COVID-19 macrophages are uncontrollably activated what leads into a macrophage activation syndrome (MAS) responsible of ARDS[100,107]. Moreover, in patients with severe COVID-19 disease, the percentage of mononuclear phagocytes in bronchoalveolar lavage fluid was 80%. In contrast, those patients with mild COVID-19 had 60% of mononuclear phagocytes[108].

Mechanism of Cytokine Storm in COVID-19

The cytokine storm provoked by Sars-CoV-2 differs of CS in other pathologies. One of the most remarkable clinic reports demonstrated a destruction of lymphoid tissue and the malfunction of different organs[109]. When SARS-CoV-2 infects an alveolar epithelial cell or an endothelial cell through the ACE2, the virus starts its cycle, replicating and consequently destroying the cells. The stress or destruction of the cells create signals that stimulate the immune response by activating macrophages, mastocytes, DC, NK, humoral mechanisms like the complement system as well as the secretion of inflammatory cytokines[110]. The infection promotes a reduced expression of ACE2 while the ACE expression is not affected[51,111] causing an ACE/ACE2 imbalance. After MERS-CoV and SARS-CoV infections different studies reported that the catalytic activity of ACE2 protects lungs from acute lung injury[111–114]. Consequently, the reduction of the ACE2 protective function contribute the hyperinflammation state and necrosis[113]. In addition, the Ang II upregulates due to the ACE2 repression and overstimulates pulmonary angiotensin II type-1 receptor (AT1R)[52,115]. As mentioned, the overactivation of AT1R promotes an sIL-6R augment after detaching mIL-6R by ADAM10 and ADAM17. sIL-6R is also produced in cells without mIL-6R, like endothelial cells. sIL-6R/IL-6 complex activates the JAK/STAT proinflammatory trans-signaling pathways which lacks SOCS3[52]. (Figure 8). The absence of the negative feedback leads to an amplification cycle of IL-6 secretion that, simultaneously will promote the secretion of IL-6 interleukin itself or IFN-γ among others[52,115,116]. Furthermore, Ang II can bind the NF-κB and promote the transcription of more inflammatory cytokines[52,117]. The ACE2 function in the transformation of Ang II into Ang (1-7) is crucial: Ang (1-7) has been reported to oppose the harmful effects of Ang II/AT1R axis by binding to G-coupled receptor MAS or to AT2R[118].

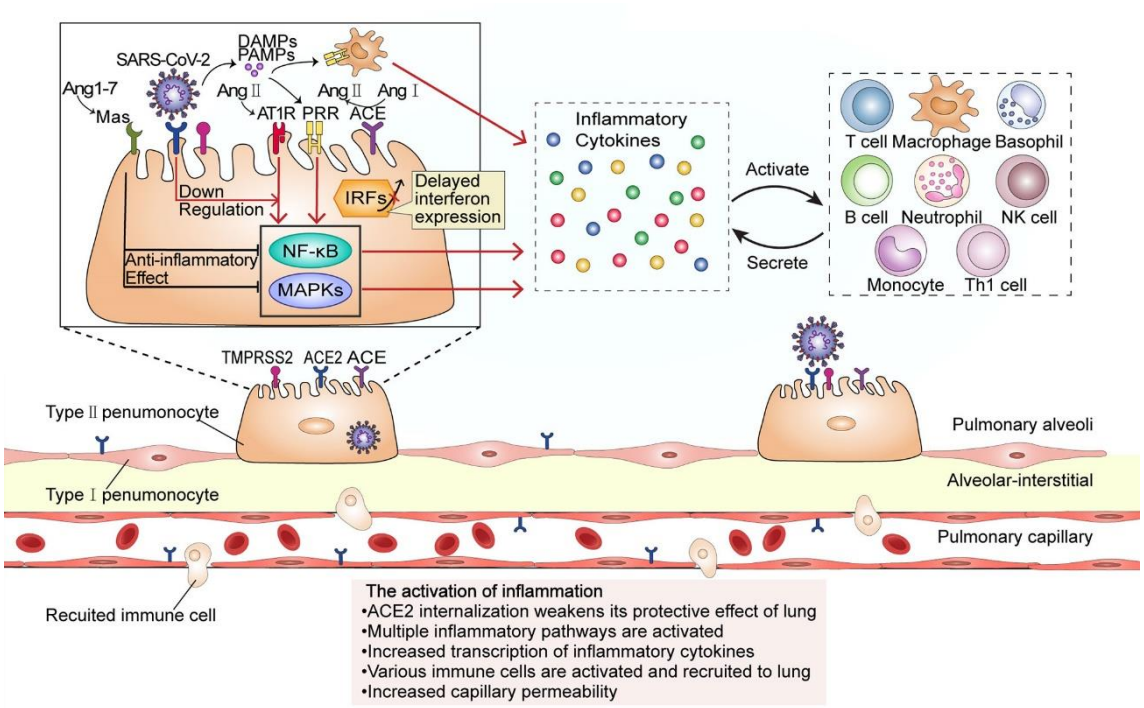

Figure 8 5Chen R, Lan Z, Ye J, et al. ***Cytokine Storm: The Primary Determinant for the Pathophysiological Evolution of COVID-19 Deterioration****. Front Immunol. 2021;12:589095. Published 2021 Apr 28. doi:10.3389/fimmu.2021.589095*

Additionally, while the SARS-CoV-2 infect and destroy cells, PAMPs and DAMPs start to appear in the damaged areas. The PAMPs and DAMPs are recognized by PRRs such as TLRs[119]. The SARS-CoV-2 single strand RNA is recognized by the TLR7 while the TL3, RIG-1 and MDA5 recognize the double stranded RNA, present in viral replication phase[26] what promotes the INF I secretion and NFκB pathway[120]. The TLR ligands promote a fast phosphorylation of IκB proteins and the polyubiquitination and degradation by the 26S proteasome, allowing NFκB factor's nuclear translocation and promoting the secretion of inflammatory cytokines like TNF-α, IL-6 and chemokines[120]. In addition, IL-6, firstly produced in monocytes, dendritic cells and macrophages, activates JAK/STAT pathway leading in a STAT3 phosphorilation[34,44] what results on a differentiation of T CD4 helpers into Th17 cells that produce IL-17[46,48]. So, the increased secretion of IL-6 and its cyclic amplification is a key trigger in cytokine storm. Indeed, clinical trials have reported an increased IL-6 in severe COVID-19 patients [55,66]. The cyclic inflammation environments added to secretion of chemokines keep attracting lymphocytes, macrophages, neutrophils, NK and basophils to damaged and infected tissues, keeping the inflammatory state upregulated with more cytokine secretions[121]. In this inflammatory environment, the differentiation of the regulatory T cells (Treg) that control the immune homeostasis is decreased. In fact, an excess of IL-6 can inhibit TGF-β-induced Treg differentiation[53,122].

As mentioned, IFN have a key role in the antiviral immunity[27,30] because besides stimulating cells and NFκB, they can block virus replication at many levels [123]. However, low levels of Type I interferon were found in severe COVID-19 patients [55,66]. Some studies suggest that the Sars-CoV-2 inhibits the IFN response thanks to both structural

and non-structural components of the virus such as (NSP1), NSP13, NSP14, the protein M, protein N, open-reading frame 3a (ORF3a) protein, ORF6 protein or S protein[26,27,35–40]. Other studies demonstrated that a decreased IFN response could be explained due to a reduction in the number of the main IFN I producers, pDC[34,41]. In fact, the inefficient or retarded IFN response is associated with hyperinflammation driven by NF-κB and lower viral clearance[124]. Moreover, upregulated gens in patients with severe disease belong to NF-κB. The NF-κB malfunction may come from an excessive PAMPs and DAPMS activation according to some studies[124] and it was observed that nucleic acid containing DAMP/PAMPs were elevated in ICU COVID-19 patients[125].

The hyperinflammation also promotes the secretion of anti-inflammatory cytokines such as IL-10 to attempt to control the response. IL-10 levels were elevated in severe COVID-19 patients[55,66]. The high levels of IL-10 during severe disease may be a product of the attempt to inhibit the hyperactivity of the immune system. However, during cytokine storm, the IL-10 cannot function properly[34]. It has been remarked the importance of IL-10 and IL-6 as disease severity predictors[85].

Leukopenia, Lymphopenia and Neutrophilia.

Referring to cell counts, in 85 fatal disease cases lymphopenia, thrombopenia monocytes, eosinophils, and low basophils frequencies but an elevation on the neutrophil count was reported[126]. Before the patient deterioration due to the CS exposure, CD4+ T cells differentiate into T-helper 1 (Th1) cells and produce GM-CSF. GM-CSF activate CD14+ and CD16+ inflammatory monocytes and recruit macrophages, which produce more IL-6[115]. Huang, Chaolin et al. also reported that blood counts of patients on admission showed leucopenia in 10 [25%] of 40 patients and lymphopenia in 26 [63%] patients[127]. The neutrophilia is suggested to be a direct consequence of cytokine storm and hyperinflammatory atmosphere[128]. An article hypothesized that lymphopenia could be a result of lymphocyte infiltrations or sequestration in lung or other tissues; Sars-CoV-2 direct infection of lymphocytes or an excess production of IL-6. In fact, the over-secretion of inflammatory cytokines and chemokines can chemoattract monocytes and neutrophiles to infected tissues and provoke the infiltration of inflammatory cells[109]. However, surviving patients restored gradually the cell accounts, meaning that there is a dysregulation of the adaptative response during severe COVID-19[115,129].

Cytokine Storm (CS) Treatment

CS during severe COVID-19 is relatively common and an anti-inflammatory therapy during the disease is the key to avoid and prevent tissue damage. There is a large variety of anti-inflammatory agents such as glucocorticoids, immunosuppressors or inflammatory cytokine target antagonists [130]. Although cytokine blocking therapies have been demonstrated to be a success in diseases like HLH, they cannot be used as a general therapy. Cytokine blocking is a good therapeutic option when a specific cytokine has a key role in the CS[5].

Pathway inhibitors

JAK Inhibitor (JAKi)

Proinflammatory cytokine secretion has been associated with JAK/STAT pathway in previous sections, meaning that the JAK-STAT inhibition could play a beneficious role avoiding hyperinflammatory state.[131]. The JAKi are biological agents that bind to the ATP catalytic sites[130]. Different JAKi can be used during severe disease:

Baricitinib is a JAK1 and JAK2 inhibitor firstly proposed in 2020 as a therapeutic option in acute respiratory disease[132]. Baricitinib has previously been approved in other inflammatory diseases such as rheumatoid arthritis (RA)[133].

A study that treated a group of 20 patients with baricitinib demonstrated that a remarkable reduction in serum levels of IL-6, IL-1β and TNFα. Moreover, a rapid recovery of circulating T and B cell frequencies, and increased antibody production against the SARS-CoV-2 spike protein were also reported, which was clinically associated with a reduction in the need for oxygen therapy[134]. Fabrizio Cantini, Laura Niccoli et al. reported that baricitinib reduces cytokine release and SARS-Co-V2 entrance, COVID-19 mortality rate and ICU admissions of pneumonia. The use of the drug for 2 weeks had not adverse effects[135]. In addition, baricitinib can inhibit both AP2 associated protein kinase (AAK1) and cyclin G-protein associated kinase (GAK) which regulate the mechanism for clathrin-dependent viral endocytosis[7]. (Figure 9).

The WHO strongly recommends baricitinib or an IL-6 inhibitor in severe patients in combination of corticosteroids, it is suggested a daily 3mg oral administration of the drug until hospital discharge[136,137]. However, the evidence has limited efficacy against Omicron BA1 variant[136].

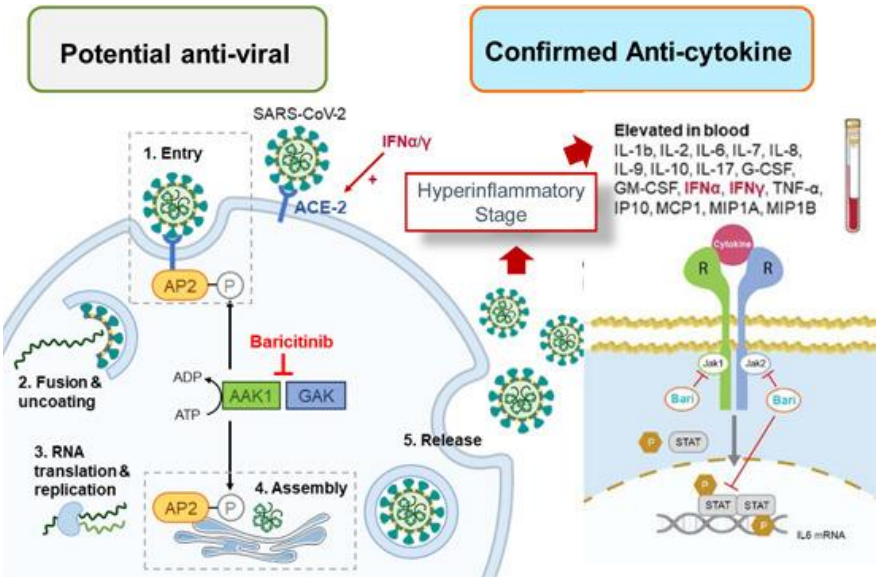

Figure 9 Stebbing J, Krishnan V, de Bono S, et al. ***Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients****. EMBO Mol Med. 2020;12(8):e12697. doi:10.15252/emmm.202012697*

Tofacitinib is a JAK1, JAK2, and JAK3 inhibitor that has been approved in RA. Its efficacy has been tested on decreasing CRP and ESR in RA but it was not able to inhibit AAK1[7]. A study reported that randomized, double-blind, placebo-controlled trial involving hospitalized patients with COVID-19 pneumonia, tofacitinib was superior to placebo in reducing the incidence of death or respiratory failure through day 28[138,139].Moreover, the study demonstrated that tofacitinib was not associated to secondary infections or thromboembolic events. The addition of standard care treatments like glucocorticoids to tofacitinib decrease the risk of clinical events among COVID-19 related pneumonia patients compared with the placebo [138,139].For that reason, the reports suggested that tofacitinib could replace baricitinib due to its ability on decreasing the risk of clinical events among COVID-19 related pneumonia patients compared with the placebo [138,139]. However, it is not able to inhibit AAK1[7] so its use should be limited to replace baracitinib.

Ruxolitinib is a JAK1 and JAK2 inhibitor mostly used in hematological patients with diseases like Myeloproliferative neoplasms (MN), Myelofibrosis (MF), or Polycythemia vera (PV)[131]. Despite being a promising compound during 2020, a small non-randomized series[140], Ruxolitinib did not get the primary endpoint in larger studies in phase III[141,142]. The first study, named RUXCOVID (n=432) (NCT04362137), could not demonstrate a decrease in death, respiratory failure, or ICU admission by day 29 compared to those patients with standard care[142]. The second study, named RUXCOVID-DEVENT, did not find statically significant improvement on mortality rate through day 29 comparing it to the placebo group.

Studies have reported that inhibitors like baricitinib, tofacitinib or ruxolitinib have similar affinities towards JAK but baricitinib seems to be the only compound with a good affinity towards AAK1[139,143]. The "Hospitalized Adults: Therapeutic Management | COVID-19 Treatment Guidelines"[137] (The panel) does not recommend using JAKi other than baricicinib or Tofacitinib. The ACTT-2 trial reported that baricitinib given in combination with remdesivir to severe patients with COVID-19 who require supplemental oxygen, but not mechanical ventilation, had positive effects in terms of recovery[144,145].

Remdesivir is a nucleotide adenosine analog that binds to the viral RNA-dependent RNA polymerase and inhibits viral replication by terminating RNA transcription prematurely[146]. Nevertheless, the WHO does not reccomend ruxolitinib and tofacitinib in patients with severe or critical COVID-19[147]. The WHO reported an incremental benefit for patients receiving baricitinib and IL-6 receptor blockers together, rather than either drug individually [147].

Bruton's Tyrosine Kinase Inhibitors (BTKi)

BTK is an B cell antigen receptor and cytokine receptor pathway molecule. BTKi such as Acalabrutinib, Ibrutinib or Zanubrutinib have been previously used to treat B cell malignancies like leukemia. However, the panel recommends against the use of BTKi [137]. The clinical trials do not allow to suggest clinical benefits using Acalabrutinib and Ibrutinib due to the study's small sample size and lack of a control group[148,149]. Moreover, there are not clinical reports of using Zanubrutinib as COVID-19 treatment[137].

Interleukin Inhibitors

IL-1 inhibitor

The studies have shown the key modulator in both systemic and local inflammation is IL-1. IL-1 can perpetuate the inflammation pathway activation by promoting its own secretion itself. Epithelial damage produces the release of IL-1α that recruit neutrophils and monocytes promoting IL-1β secretion in monocyte/macrophages. The SARS-CoV-2 will induce pro-IL-1β which in turn will induce more IL-1 recruiting and activating innate immune cells in a loop. (Figure 10). However, the loop of autoinflammation can be broken thanks to a recombinant IL-1R antagonist called Anakinra [150]. Studies have reported a safe way to block the IL-1 signaling with *Anakinra* leading on the block of the NF-κB-mediated upregulation of cytokines like IL- [7,151] . Kyriazopoulou, Evdoxia et al. demonstrated that the 22.3% of patients treated with anakinra and the 59.2% of the comparators progressed into severe respiratory failure. Moreover,30-day mortality was 11.5% and 22.3% respectively. Anakinra was associated with decrease in circulating interleukin IL−6 and sIL2-R[151].

Furthermore, a study tested a high dose of anakinra treatment and reported that at 21 days treatment with high-dose, anakinra was associated with reductions in serum C-reactive protein (CRP) and progressive improvements in respiratory function in 72% of the patients[152].

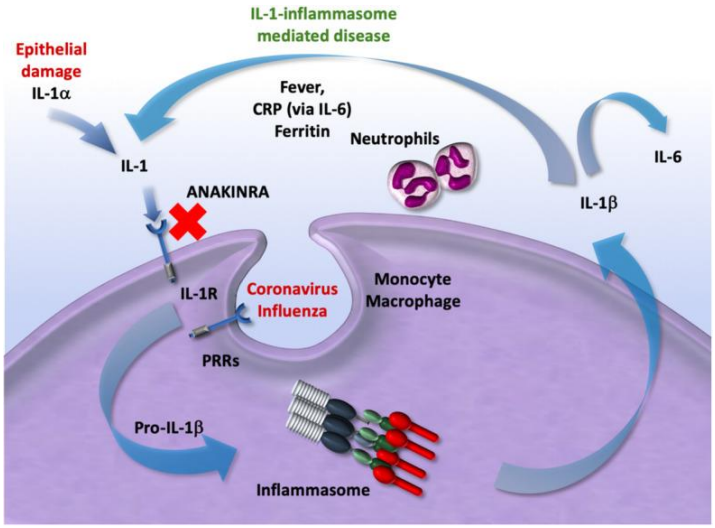

Figure 10 van de Veerdonk FL, Netea MG. ***Blocking IL-1 to prevent respiratory failure in COVID-19****. Crit Care. 2020;24(1):445. Published 2020 Jul 18. doi:10.1186/s13054-020-03166-0*

Blocking IL-1β secretion with IL-1β antagonist canakinumab is an option. A study demonstrated that canakinumab reduced systemic inflammation and improved oxygenation in inflamed patients without mechanic ventilation[153]. The results suggested that canakinumab could help in inflammation control in mild to severe patients. However, COVID-19 treatment guidelines do not recommend it for or against IL-1 blockers such as anakinra and canakinumab because there is insufficient evidence.

IL-17 inhibition

IL-17 is an inflammatory cytokine which has an important role in cytokine storm because it acts upstream in IL-6 and IL-1 pathway and promotes the neutrophil recruitment[7,91]. It has been demonstrated that blocking IL-17 and IL-17R inhibits the Th-17 response[154]. However, IL-17 blockers are just approved as a psoriasis treatment. Ecukinumab, Ixekizumab, and brodalumab are anti IL-17 inhibitors currently approved for the treatment of moderate-to-severe psoriasis[155]. Furthermore, a retrospective, observational, multicenter clinical trial from Italy (n=5206) of patients with psoriasis who have been prescribed with IL-17 inhibitors showed that only four patients were hospitalized, and no deaths occurred[156]. Despite the good-looking results, more investigation on CS treatments with IL-17 is needed to study the effects in healthy/comorbidity population.

IL-12 inhibition

IL-12 has been studied as a possible target on CS treating. Ustekinumab is a monoclonal antibody that binds to p50 subunit of IL-12[157] making it unrecognizable for the IL-12R. However, side effects like nasopharyngitis or respiratory infections have been described in patients treated with Ustekinumab[158].

TNF-α Inhibition

Different studies have reported the blocking of TNF-α could be a key treatment of CS due to its role in proinflammatory cytokine secretion[159,160]. Duret, Pierre-Marie et al. reported in 2020 a case in which an immunocompromised 60 years old (not vaccinated) man recovered from the COVID-19. The patient was treated with etanercept (TNF-α inhibitor) and methotrexate due to his spondylarthritis. The patient did not develop ARDS and had no UCI need[160].

Infliximab has been also tested as a possible TNF-α blocker in COVID-19 disease. A study reported that namilumab (GM-CSF inhibitor), but not infliximab, showed a reduction in inflammation in hospitalized patients with COVID-19 pneumonia[161].

The positive results obtained by these authors [160] suggested a positive effect on etanercept in immunosuppressed people, but more investigation is needed with larger population trials including healthy, vaccinated and comorbidity suffering volunteers to see the potential of the drug.

IL-6 Inhibition

As mentioned, IL-6 looks like one of the main CS triggers when binding to IL-6R. So, several IL-6 and IL-6R inhibiting drugs such as tocilizumab or siltuximab have been proposed[48,162].

Tocilizumab (TCZ) is a humanized anti-IL-6 receptor antibody that have been used in RA or CAR-T CS and now in COVID-CS[7]. The large, randomized trial "RECOVERY" (ClinicalTrials.gov NCT04381936) indicated that TCZ was effective in COVID-19 treatment in hospitalized patients who had hypoxia and evidence of inflammation (CRP ≥75 mg/L). Hypoxic and inflamed patients, treated with a combination of a systemic corticosteroid plus TCZ can reduce mortality by about one-third Moreover, TCZ

improved survival and the chances of discharge from hospital by 28 days[163]. Other studies had similar results, critically ill patients with Covid-19 in ICUs, treatment with TCZ and sarilumab improved outcomes, including survival[164] (REMAP-CAP ClinicalTrials.gov number, NCT02735707). However, some studies reported that TCZ treatment did not significantly ameliorate the clinical status or did not show lower mortality than placebo at 28 days[165]. The RECOVERY's strengths included randomized, large sample size, and included patients requiring various amounts of respiratory support in contrast to smaller trials that did not find significant decrease in mortality [165–171]. It also had four times more information than all the previous trials combined [165–171].

According to "Hospitalized Adults: Therapeutic Management | COVID-19 Treatment Guidelines" an IL-6 receptor inhibitor called Sarilumab can be used instead of TCZ[137,172]. As mentioned, the REMAP-CAP trial reported that in patients with severe disease, TCZ and sarilumab are improving similarly the survival.

The combination of sarilumab with dexamethasone reduced in-hospital mortality, shorter time to ICU discharge, and more organ support-free days[137].

The panel recommends the use of TCZ or Sarilumab and recommends against the use of siltuximab due to the limited data on the efficacy of the drug in COVID-19 patients, a study reported that respiratory failure requiring ventilatory support patients may benefit from siltuximab but more investigation in requiered[173].

IFN treatment

IFN are cytokines which play a key role in antiviral and inflammation responses. Most of interferon treatments were proposed in early 2020s before the use of remdesivir and corticosteroids.

The interferon β-1a has been approved by the FDA for Hepatitis B and C treatments it has been tested as a COVID-19 treatment. A double-blind, randomized, placebo-controlled, phase 3 trial tested interferon β-1a plus remdesivir compared with remdesivir alone in hospitalized patients. The clinical trial concluded that the combined tretament was not superior to remdesivir alone in hospitalized patients with COVID-19 pneumonia[174 137].

Interferon γ is the primary activator of macrophages. It has been found a significant reduction in CD4+ T cell count and consequently a reduction in IFN-γ expression in severe cases[66]. A study found out that, before the patient deterioration, the inhibition of TNF-α and IFN-γ had a protective effect in Sars-CoV-2 related dead, sepsis, HLH, and cytokine shock[60]. In contrast, some studies have reported positive results using IFN-γ treatments: a clinical trial reported that adding recombinant IFN-γ to the complex therapy in moderate COVID-19 pneumonia patients resulted in stabilization of vital signs, and reduced length of fever and hospital stay by 2 days. The results suggested a positive effect of recombinant IFN-γ in moderate COVID-19 patients[175] . The results were limited to a small number of patients. What is more, Nguyen, Lee S et al. observed that patients with COVID-19-related pneumonia had decreased MHC class II cell surface receptor (mHLA-DR) expression. The decrease in mHLA-DR expression is associated with the development of severe respiratory failure. IFN-γ treatment led to a fast increase in HLA-DR monocyte expression except in one patient who died from septic shock[176]. However,

the results were limited to six patients and the authors remark that the study just showed exploratory results. Moreover, an observational study concluded that nasal IFN-γ had a potential positive preventive effect on Acute Respiratory Viral Infection like COVID-19 in healthy volunteers[177].

The interferon λ (IFN-λ) reduces the mononuclear macrophage-mediated proinflammatory activity of IFN-αβ[178]. In addition, IFN-λ inhibits the recruitment of neutrophils to the site of infection and promote antiviral gene expression without over-stimulating the immune system. IFN-λ accelerated viral decline and increased the viral clearance by day 7, in patients with high viral load. IFN λ has potential to prevent clinical deterioration and shorten duration of viral shedding[179]. However, IFN-λ is not currently approved by the FDA for any use.

Most of the IFN trials have tested and compared the drug in combination to other agents, so, larger IFN alone testing is required to prove their efficacy.

Corticosteroids

SARS-CoV-2 infection can lead to a hyperinflammatory state, in which the anti-inflammatory properties of corticoids are effective therapeutic option[180]. The WHO strongly recommends the use of systemic corticosteroids in patients with severe or critical COVID-19, but they are not recommended against the use of them in patients with moderate disease[147]. The RECOVERY trial studied the effects of oral or intravenous corticoid dexamethasone administration in hospitalized COVID-19 patients. The results showed a decrease in 28-day mortality among those who were receiving invasive mechanical ventilation or oxygen. However, dexamethasone had no benefits in patients without respiratory support[181]. In addition, COVID-19 Dexamethasone (CoDEX) randomized clinical trial showed that intravenous dexamethasone administration plus standard care, in moderate to severe COVID-ARDS patients, had significant effects increasing the number of ventilator-free days[182].

Methylprednisolone has been tested in clinical trials. A multivariate analysis from 175 patients with severe COVID-19 patients showed that it had a protective effect in disease progression from severe to critical illness in those patients younger than 65 years[183]. An individual randomized controlled trial metanalysis showed that, low dose administration of methylprednisolone had no mortality benefits compared to treated patients with control. However, the only trial that administered high doses of methylprednisolone indicated a statistically significant mortality benefit[184]. The use of methylprednisolone is supported by the panel. Other glucocorticoids example such as prednisone, methylprednisolone, hydrocortisone can be used when dexamethasone is not available, despite having less effect evidence than dexamethasone.

Despite having adverse effects like hyperglycemia, neuropsychiatric symptoms, secondary infections,[137][180] [138]corticoids are safe drugs in short-term use. There is not enough evidence to recommend corticosteroids on mild or not hospitalized patients, but more studies are needed for treating mild COVID-19 with corticosteroids.

Chloroquine and hydroxychloroquine

Chloroquine and hydroxychloroquine are antivirals drugs that increase the endosomal pH inhibiting the fusion between the virus and the cells. It was first developed as antimalarial agent, but recently it has been tested as a SARS-CoV-2 preventing and treating drug[185]. However, WHO and the panel strongly do not recommend the use of chloroquine, hydroxychloroquine, or azithromycin, the RECOVERY clinical trial showed that among patients without invasive mechanical ventilation, those treated with the chloroquine and hydroxychloroquine had more chance to require intubation or to die during hospitalization compared to patients with standard care[186]. In addition, cardiac side effects such as QTc prolongation, ventricular arrythmia, and cardiac deaths were reported[187].

Colchicine

Colchicine is an anti-inflammatory agent that irreversibly intercalates into free α/β tubulin dimers blocking their extension[188]. It has been previously used in gout, recurrent pericarditis, and familial Mediterranean fever[188,189]. The RECOVERY reported that hospitalized adults with COVID-19, colchicine did not reduce the 28-day mortality, duration of hospital stay, or risk of progressing to invasive mechanical ventilation or death[190]. In addition, colchicine in non-hospitalized adults is not recommended, a clinical trial named as PRINCIPLE showed no benefit of colchicine, and a larger trial called COLCORONA was not able to reach primary endpoint[191,192].

Discussion: Should we stimulate or inhibit immune response?

This bibliographic research makes an overview of inflammation mechanisms during SARS-CoV-2 infection hypothesizing if the stimulation of immunomodulators could benefit the immune response in early infection stages before a cytokine storm syndrome. Both stimulating and suppressing immunomodulators have been studied.

The SARS-CoV-2 pandemic has showed a wide heterogeneity from asymptomatic patients until severe COVID-19 patients [193]. Hopefully, the vaccination has drastically reduced the severe disease patients, for the two-dose regimens of mRNA vaccines BNT162b2 vaccine effectiveness against COVID-19 was 94.5% (95% confidence interval) 2 months after the first dose and decreased to 66.6% at 7 months[194]. It is hard to predict the severity of the COVID-19 for applying the reviewed therapies, however, some studies were aimed to predict and classify risk factors associated to a serious prognosis. A study reported that smoking male patients over 65 might face a greater risk of developing critical or mortal disease and some comorbidities such as hypertension, diabetes, cardiovascular disease, and respiratory diseases, which can affect the COVID-19 severity[195]. Various genetic factors may also affect the prognosis of COVID-19; for example, the phenotypes of HLA-B *46:01 and HLA-B*15:03[196]. So, vaccinated people in age of risk, immunosuppressed, or have high probabilities of suffering comorbidities should receive targeted interventions like additional primary and booster vaccine doses as well as approved immunomodulators.

Suppressing immunomodulators are being used in severe COVID-19 cases but, could an immune stimulation prevent or treat the disease?

The main concern is that the administration of anti-inflammatory agents in an early stage of infection can lead to a deficient viral clearance and the blocking of inflammatory pathways like the IFN-pathway can lead to a CS [197]. However, the stimulation of the immune system in risk but not immunosuppressed people during first stages of infection is not clearly tested [197].

IFN have a key role in the antiviral immunity[27,30] due to their ability to block virus replication at many levels [123]. In fact, the inefficient or retarded IFN response is associated with hyperinflammation driven by NF-κB and lower viral clearance[124]. Reviewed articles demonstrated the impaired IFN activity and its relationship with COVID-19 severity[6].

The vaccine against COVID-19 is the best way to prevent severe disease[194], however, IFN γ has been tested as a preventive drug in an observational prospective study. The study concluded that nasal IFN γ has a preventive effect on Acute Respiratory Viral Infection like COVID-19 in healthy volunteers[177]. However, the study used healthy volunteers, so more research is required for the possible application of preventive nasal IFN-γ in risk population. Hu, Zhong-Jie et al. showed that lower circulating IFN-γ was a risk factor in COVID-19 patients suggesting that early intervention of anti-viral infection using IFN-γ could benefit patients[67].

Baricitinib can inhibit the mechanism for clathrin-dependent viral endocytosis[7]. For the moment, the unique alternative as a preventive option for people who are in risk of inadequate immune response to vaccination is the administration of both Tixagevimab

and cilgavimab anti-SARS-CoV-2 mAbs. However, the mAbs can only be administrated before the infection[198].

The previous results suggest IFN-γ plus baricitinb as an alternative to prevent SARS-CoV-2 cell entry and replication. Nevertheless, JAK1 and JAK2 are inhibited by baricitinib meaning that IFN-γ antiviral response would be annulled. The mAbs have been used in immunosuppressed patients, so a possible synergy between Tixagevimab/cilgavimab and IFN-γ needs more investigation. More trials are needed to test IFN γ alone or plus Tixagevimab/cilgavimab not only in healthy people but also in population at risk.

In terms of severe disease treatment, clinical trials have also tested different cytokines. On the one hand, a clinical trial showed that adding recombinant IFN-γ to the complex therapy in moderate COVID-19 pneumonia patients resulted in stabilization of vital signs, and reduced length of fever [175]. Furthermore, as mentioned Nguyen, Lee S et al. observed that patients with COVID-19-related pneumonia had decreased mHLA-DR expression levels. This expression decrease is associated with severe respiratory failure. IFN-γ has been shown to induce HLA-DR expression on human malignant melanoma cells lines suggesting that IFN-γ could not only be beneficial in early stages of the disease but also in severe cases. IFN seem to have positive results, however, more investigation is needed due to study reports saying that IFN therapies had little or no effect on hospitalized patients [199]. Additionally, it seems that the inhibition of the TNF-α and IFN-γ synergy had a protective effect in SARS-CoV-2 related dead or cytokine shock[60].

Most of upregulated cytokines in COVID-19 disease promote strong inflammatory levels related with patient deterioration. The IL-10 is an anti-inflammatory cytokine which protects from exuberant immune responses. The signaling involves induction of a SHIP1-STAT3 complex, which translocate to the nucleus resulting in the inhibition of macrophage activation[200]. However, IL-10 administration does not seem to be a therapeutic option because its high expression can predict poor outcomes in COVID-19 patients. The IL-10 “resistance” in COVID-19 suggest that small SHIP1 agonist molecules could be a therapeutic option, but more investigation is required.

Cytokine such as IL-6 or IL-17 are upregulated during the severe disease and play a key role in the hyperinflammation. This suggest that therapies aimed to promote the immune system with IL-6 or IL-17 are recommended against because they may accelerate patient deterioration.

Current Patient Management Based on Severity

The best way of avoiding the severe disease is the vaccination. However, the risk of developing severe disease is not null. The most efficient way of combating severe disease is the anti-inflammatory treatment initiated at the right moment. It should be tailored in individual patient to achieve the most favorable effects[201]. (Figure 11).

Non-Hospitalized patients

Adults with COVID-19 in an ambulatory care setting SARS-CoV-2 targeting therapies like ritonavir-boosted nirmatrelvir and remdesivir should be prioritized[202]. Risk patients may require additional prophylactic therapies. The management should consider patient’s

vital signs, physical exam findings and risk factors. For the moment, in patients with no signs of venous thromboembolism, a prophylactic heparin dose is strongly suggested.

Hospitalized patients

Patients not requiring supplemental oxygen (PnRSO): According to the RECOVERY trial dexamethasone is not recommended for PnRSO because it had no benefits in patients without respiratory support[181] and there is insufficient evidence of recommending for or against remdesivir in PnRSO[203]. The proposed IFN-γ treatments could be determinant of patient recovery or deterioration suggesting that more investigation is needed to avoid PnRSO deterioration.

PRSO without high flow device: For patients with a low need of oxygen, remdesivir or remdesivir plus dexamethasone have positive results. The CoDEX randomized clinical trial showed that dexamethasone plus standard care had significant effects increasing the number of ventilator-free days[182]. For those with an increasing oxygen need dexamethasone can be administrated with baricitinib (JAKi) or tocilizumab[163]. In case dexamethasone is not available glucocorticoids like methylprednisolone can be used but there is weaker evidence [184].

Patients Who Require Oxygen Through a High-Flow Device or Noninvasive Ventilation: For patients with high flow device dexamethasone plus remdesivir is highly recommended, there are reports demonstrating that remdesivir/dexamethasone group compared to dexamethasone alone had a reduction in hospitalization days and fast improvements in respiratory function and inflammatory markers[204]. For those with an increasing oxygen need dexamethasone can also be administrated with baricitinib (JAKi) or TCZ.

Patients Who Require Mechanical Ventilation or Extracorporeal Membrane Oxygenation: In critical patients, dexamethasone in addition to TCZ is the strongest way of fighting the disease. TCZ can be replaced by sarilumab, REMAP-CAP trial reported that in critical patients, TCZ and sarilumab are improving similarly the survival.

The figure 10 summarizes the therapeutic management of adults hospitalized for COVID-19 based on disease severity.

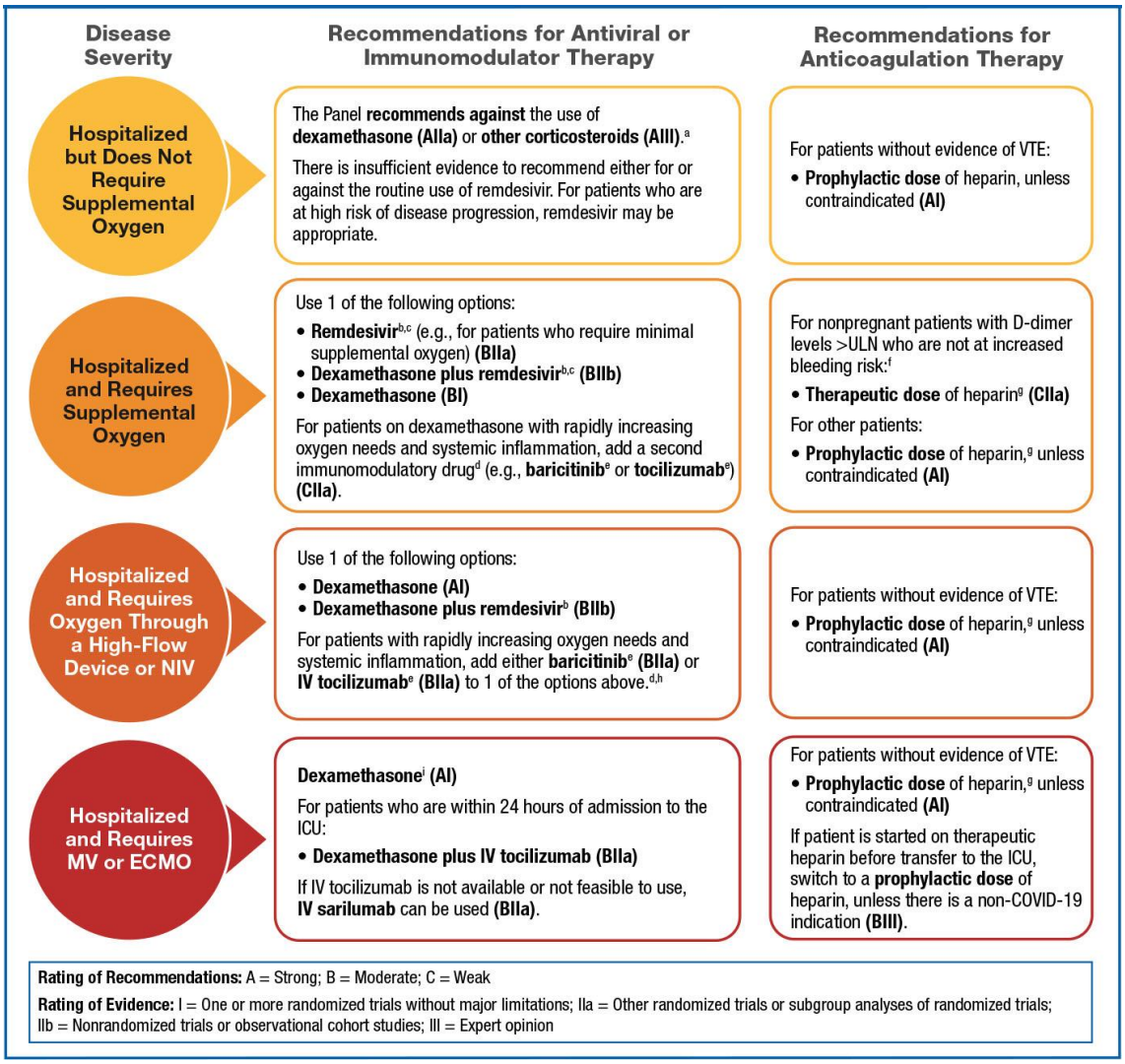

Figure 11 Hospitalized Adults: Therapeutic Management | COVID-19 Treatment Guidelines. https://www.covid19treatmentguidelines.nih.gov/management/clinical-management/hospitalized-adults--therapeutic-management/hospitalized-adults-figure/

Conclusion

The results suggest that vaccination is the most appropriate treatment to prevent severe COVID-19. Nasal IFN-γ administration needs more study as a COVID-19 infection preventive drug since it has only been tested in healthy volunteers and the aim of this therapy should be focus on risk population. IFNs are the first cytokines in the antiviral response, however they can be inhibited by the SARS-CoV-2 proteins or by a decrease in pDC. The objective of this bibliographical research was to demonstrate how the immune system stimulation in the first stages of the disease plays a key role in the elimination of SARS-CoV-2 virus before patient deterioration. The literature suggests that IFN-γ administration could prevent the disease, however, clinical trials are necessary to achieve some evidence.

There are some studies that reported a reduction in circulating IFN-γ is associated with lung fibrosis. Moreover, it has been reported that a decreased in mHLA-DR expression is associated with severe respiratory failure. Adding recombinant IFN-γ to the complex therapy in moderate COVID-19 pneumonia patients resulted in stabilization of vital signs and was able to promote a fast increase in HLA-DR monocyte expression[67,175,176]. The research suggests a possible IFN-γ preventive treatment for inhibiting SARS-CoV-2 replication. A synergy between Tixagevimab/cilgavimab and IFN-γ could prevent COVID-19 disease. More trials are needed to test the synergy of IFN γ not only in healthy people but also in risk population.

Other IFN like IFN-λ can inhibit the recruitment of neutrophils to the site of infection and promote antiviral gene expression without over-stimulating the immune system [179] but they are not just approved for clinical trial.

However, IFNs, besides the antiviral activity, also play an important inflammatory role, meaning that an early treatment in risk patients could lead to a faster hyperinflammation. In fact, there are studies which have concluded that the inhibition of TNF-α and IFN-γ synergy had a protective effect in SARS-CoV-2 related dead[60]. However, more clinical trials using IFN as a therapy need to be carried out.

The Therapeutic Management of Hospitalized Adults With COVID-19 panel and WHO recommend anti-inflammatory treatments with antiviral, JAKi, IL-6 blockers and corticoids. Newest WHO COVID-19 therapies include the use of antiviral and antiretrovirals like nirmatrelvir-ritonavir in patients without severe illness. It is vital to avoid drugs that have been discarded like chloroquine and hydroxychloroquine and to study each COVID-19 case individually. All the risk factors and comorbidities need to be considered in each patient with the objective of avoiding deterioration and improving the recovery by individualizing the treatment.

The most efficient way of combating severe disease is the use of suppressing immunomodulators, antiviral-antiretroviral drugs and neutralizing monoclonal antibody treatment initiated at the right moment. They should be tailored individually to each patient to achieve the most favorable effects [201,] Nevertheless, new clinical trials testing new drugs are still needed to develop new therapeutic strategies and new knowledge of COVID-19 disease.

Abbreviations

Abbreviations	Meaning
ADAM	A Desintegrin And Metalloproteinase Domain Containing Protein
ARDS	Acute Respiratory Distress Syndrome
AT1R	Angiotensin II type-1 receptor
ACE2	Angiotensin-converting enzyme 2
AAK1	AP2 Associated Protein Kinase
CRP	C Reactive Protein
COVID-19	Coronavirus Disease 2019
CS	Cytokine Storm
DAMP	Damage Associated Molecular Patterns
EC	Endothelial Activation
E	Envelope
ESR	Erythrocyte Sedimentation Rate
GAK	G-Protein Associated Kinase
G-CSF	Granulocyte Colony Stimulating Factor
HLH	Hemophagocytic Lymphohistiocytosis
iNOS	Inducible Nitric Oxide Synthase
ILC	Innate Lymphoid cell
IFN	Interferon
IL	Interleukin
JAK	Janus Kinase
MAS	Macrophage Activating Syndrome
MDA5	Melanoma Differentiation Associated-Protein
M	Membrane
mIL-6R	Membrane Bound IL-6 Receptor
MCP-1	Monocyte Chemoattractant Protein 1
MAPK	Mitogen Activated Protein Kinase
NK	Natural Killer
NSP	Non-Structural Protein
NFκB	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
N	Nucleocapsid
ORF	Open Reading Frame
PRR	Pattern Recognizing Receptor
PKC	Protein Kinase C
RIG	Retinoic Acid Inducible Gen
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
STAT	Signal Transducer and Activator of Transcription
sIL-6R	Soluble IL-6 Receptor
S	Spike Protein
SOCS	Suppressor Of Cytokine Signaling
TCZ	Tocilizumab
TLR	Toll-Like Receptor
TMPRSS2	Transmembrane Serin Protease

Bibliography

1. Hu, B., Guo, H., Zhou, P. & Shi, Z.-L. Characteristics of SARS-CoV-2 and COVID-19. doi:10.1038/s41579-020-00459-7.

2. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733. doi:10.1056/NEJMoa2001017.

3. Fehr, A. R. & Perlman, S. Coronaviruses: An Overview of Their Replication and Pathogenesis. *Coronaviruses* **1282**, 1 (2015).

4. Li, W. *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450 (2003).

5. Kim, J. S. *et al.* Immunopathogenesis and treatment of cytokine storm in COVID-19. *Theranostics* **11**, 316 (2021).

6. Hadjadj, J. *et al.* Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science (New York, N.y.)* **369**, 718 (2020).

7. The interplay between inflammatory pathways and COVID-19: A critical review on pathogenesis and therapeutic options - PMC. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7709793/.

8. Liu, J. *et al.* Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine* **55**, (2020).

9. Wong, C. K. *et al.* Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clinical and Experimental Immunology* **136**, 95 (2004).

10. Ye, Q., Wang, B. & Mao, J. The pathogenesis and treatment of the `Cytokine Storm' in COVID-19. *The Journal of Infection* **80**, 607 (2020).

11. Lopez Bernal, J. *et al.* Effectiveness of the Pfizer-BioNTech and Oxford-AstraZeneca vaccines on covid-19 related symptoms, hospital admissions, and mortality in older adults in England: test negative case-control study. (2021) doi:10.1136/bmj.n1088.

12. Su, S. *et al.* Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends in Microbiology* **24**, 490 (2016).

13. Hu, B., Guo, H., Zhou, P. & Shi, Z. L. Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews. Microbiology* **19**, 1 (2021).

14. Walls, A. C. *et al.* Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein. *bioRxiv* 2020.02.19.956581 (2020) doi:10.1101/2020.02.19.956581.

15. Andersen, K. G., Rambaut, A., Lipkin, W. I., Holmes, E. C. & Garry, R. F. The proximal origin of SARS-CoV-2. *Nature Medicine* **26**, 1 (2020).

16. Kadam, S. B., Sukhramani, G. S., Bishnoi, P., Pable, A. A. & Barvkar, V. T. SARS-CoV-2, the pandemic coronavirus: Molecular and structural insights. *Journal of Basic Microbiology* **61**, 180 (2021).

17. Scialo, F. *et al.* ACE2: The Major Cell Entry Receptor for SARS-CoV-2. *Lung* **198**, 867 (2020).

18. Samavati, L. & Uhal, B. D. ACE2, Much More Than Just a Receptor for SARS-COV-2. *Frontiers in Cellular and Infection Microbiology* **10**, 317 (2020).

19. Jackson, C. B., Farzan, M., Chen, B. & Choe, H. Mechanisms of SARS-CoV-2 entry into cells. *Nature Reviews. Molecular Cell Biology* **23**, 3 (2022).

20. Glowacka, I. *et al.* Evidence that TMPRSS2 Activates the Severe Acute Respiratory Syndrome Coronavirus Spike Protein for Membrane Fusion and Reduces Viral Control by the Humoral Immune Response. *Journal of Virology* **85**, 4122 (2011).

21. Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271 (2020).

22. Shang, J. *et al.* Cell entry mechanisms of SARS-CoV-2. *Proc Natl Acad Sci U S A* **117**, 11727 (2020).

23. V'kovski, P., Kratzel, A., Steiner, S., Stalder, H. & Thiel, V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews. Microbiology* **19**, 155 (2021).

24. Stertz, S. *et al.* The intracellular sites of early replication and budding of SARS-coronavirus. *Virology* **361**, 304 (2007).

25. Ghosh, S. *et al.* β-Coronaviruses use lysosomal organelles for cellular egress. *bioRxiv* 2020.07.25.192310 (2020) doi:10.1101/2020.07.25.192310.

26. Yang, L. *et al.* The signal pathways and treatment of cytokine storm in COVID-19. *Signal Transduction and Targeted Therapy* **6**, (2021).

27. Ramasamy, S. & Subbian, S. Critical Determinants of Cytokine Storm and Type I Interferon Response in COVID-19 Pathogenesis. *Clinical Microbiology Reviews* **34**, (2021).

28. Moore, J. B. & June, C. H. Cytokine release syndrome in severe COVID-19. *Science (1979)* **368**, 473–474 (2020).

29. del Valle-Mendoza, J. *et al.* Comparison of cytokines levels among COVID-19 patients living at sea level and high altitude. *BMC Infectious Diseases* **22**, 1–6 (2022).

30. Honda, K., Takaoka, A. & Taniguchi, T. Type I Inteferon Gene Induction by the Interferon Regulatory Factor Family of Transcription Factors. *Immunity* **25**, 349–360 (2006).

31. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen Recognition and Innate Immunity. *Cell* **124**, 783–801 (2006).

32. Kawasaki, T. & Kawai, T. Toll-Like Receptor Signaling Pathways. *Frontiers in Immunology* **5**, (2014).

33. Khanmohammadi, S. & Rezaei, N. Role of Toll-like receptors in the pathogenesis of COVID-19. *Journal of Medical Virology* **93**, 2735–2739 (2021).

34. Yang, L. *et al.* The signal pathways and treatment of cytokine storm in COVID-19. *Signal Transduction and Targeted Therapy* **6**, (2021).

35. Kopecky-Bromberg, S. A., Martínez-Sobrido, L., Frieman, M., Baric, R. A. & Palese, P. Severe Acute Respiratory Syndrome Coronavirus Open Reading Frame (ORF) 3b, ORF 6, and Nucleocapsid Proteins Function as Interferon Antagonists. *Journal of Virology* **81**, 548 (2007).

36. Hu, Y. *et al.* The Severe Acute Respiratory Syndrome Coronavirus Nucleocapsid Inhibits Type I Interferon Production by Interfering with TRIM25-Mediated RIG-I Ubiquitination. *Journal of Virology* **91**, (2017).

37. Minakshi, R. *et al.* The SARS Coronavirus 3a Protein Causes Endoplasmic Reticulum Stress and Induces Ligand-Independent Downregulation of the Type 1 Interferon Receptor. *PLoS ONE* **4**, 8342 (2009).

38. Li, J. Y. *et al.* The ORF6, ORF8 and nucleocapsid proteins of SARS-CoV-2 inhibit type I interferon signaling pathway. *Virus Research* **286**, 198074 (2020).

39. Konno, Y. *et al.* SARS-CoV-2 ORF3b Is a Potent Interferon Antagonist Whose Activity Is Increased by a Naturally Occurring Elongation Variant. *Cell Reports* **32**, 108185 (2020).

40. Park, A. & Iwasaki, A. Type I and Type III Interferons – Induction, Signaling, Evasion, and Application to Combat COVID-19. *Cell Host & Microbe* **27**, 870 (2020).

41. Wilk, A. J. *et al.* A single-cell atlas of the peripheral immune response in patients with severe COVID-19. *Nat Med* **26**, 1070 (2020).

42. Chen, L. *et al.* Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* **9**, 7204 (2018).

43. O'Shea, J. J. *et al.* The JAK-STAT Pathway: Impact on Human Disease and Therapeutic Intervention. *Annu Rev Med* **66**, 311 (2015).

44. O'Shea, J. J. & Plenge, R. JAKs and STATs in Immunoregulation and Immune-Mediated Disease. *Immunity* **36**, 542 (2012).

45. Bronte, V. *et al.* Baricitinib restrains the immune dysregulation in patients with severe COVID-19. *The Journal of Clinical Investigation* **130**, 6409 (2020).

46. Kang, S., Tanaka, T., Narazaki, M. & Kishimoto, T. Targeting Interleukin-6 Signaling in Clinic. *Immunity* **50**, 1007–1023 (2019).

47. Ulich, T. R., del Castillo, J. & Guo, K. In Vivo Hematologic Effects of Recombinant Interleukin-6 on Hematopoiesis and Circulating Numbers of RBCs and WBCs. *Blood* **73**, 108–110 (1989).

48. Tanaka, T., Narazaki, M. & Kishimoto, T. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harbor Perspectives in Biology* **6**, 16295–16296 (2014).

49. Bettelli, E. *et al.* Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature 2006 441:7090* **441**, 235–238 (2006).

50. Tanaka, T., Narazaki, M. & Kishimoto, T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. *Immunotherapy* **8**, 959–970 (2016).

51. Kuba, K. *et al.* A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. *Nature Medicine* **11**, 875 (2005).

52. Iwasaki, M. *et al.* Inflammation Triggered by SARS-CoV-2 and ACE2 Augment Drives Multiple Organ Failure of Severe COVID-19: Molecular Mechanisms and Implications. *Inflammation* **44**, 13 (2021).

53. Horwitz, D. A., Zheng, S. G., Wang, J. & Gray, J. D. Critical role of IL-2 and TGF-β in generation, function and stabilization of Foxp3+ CD4+ Treg. *European Journal of Immunology* **38**, 912–915 (2008).

54. Abbas, A. K., Trotta, E., Simeonov, D. R., Marson, A. & Bluestone, J. A. Revisiting IL-2: Biology and therapeutic prospects. *Science Immunology* **3**, (2018).

55. Huang, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497 (2020).

56. Shi, H. *et al.* The inhibition of IL-2/IL-2R gives rise to CD8+ T cell and lymphocyte decrease through JAK1-STAT5 in critical patients with COVID-19 pneumonia. *Cell Death & Disease* **11**, (2020).

57. Schroder, K., Hertzog, P. J., Ravasi, T. & Hume, D. A. Interferon-γ: an overview of signals, mechanisms and functions. *Journal of Leukocyte Biology* **75**, 163–189 (2004).

58. Salazar-Mather, T. P., Hamilton, T. A. & Biron, C. A. A chemokine-to-cytokine-to-chemokine cascade critical in antiviral defense. *Journal of Clinical Investigation* **105**, 985 (2000).

59. Li, H., Gade, P., Xiao, W. & Kalvakolanu, D. v. The Interferon Signaling Network and Transcription Factor C/EBP-β. *Cell Mol Immunol* **4**, 407 (2007).

60. Karki, R. *et al.* Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Shock Syndromes. *Cell* **184**, 149 (2021).

61. Janka, G. E. Hemophagocytic syndromes. *Blood Reviews* **21**, 245–253 (2007).

62. Zandvakili, I., Conboy, C. B., Ayed, A. O., Cathcart-Rake, E. J. & Tefferi, A. Ruxolitinib as first-line treatment in secondary hemophagocytic lymphohistiocytosis: A second experience. *American Journal of Hematology* **93**, E123–E125 (2018).

63. Henderson, L. A. & Cron, R. Q. Macrophage Activation Syndrome and Secondary Hemophagocytic Lymphohistiocytosis in Childhood Inflammatory Disorders: Diagnosis and Management. *Paediatr Drugs* **22**, 29 (2020).

64. Al-Samkari, H. & Berliner, N. Hemophagocytic Lymphohistiocytosis. *Annual Review of Pathology: Mechanisms of Disease* **13**, 27–49 (2018).

65. Han, H. *et al.* Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerging Microbes & Infections* **9**, 1123 (2020).

66. Chen, G. *et al.* Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of Clinical Investigation* **130**, 2620 (2020).

67. Deng, K. *et al.* Lower Circulating Interferon-Gamma Is a Risk Factor for Lung Fibrosis in COVID-19 Patients. *Front. Immunol* **11**, 585647 (2020).

68. Chan, A. H. & Schroder, K. Cytokines Focus: Inflammasome signaling and regulation of interleukin-1 family cytokines. *The Journal of Experimental Medicine* **217**, (2020).

69. Shakoory, B. *et al.* Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of the macrophage activation syndrome: Re-analysis of a prior Phase III trial. *Crit Care Med* **44**, 275 (2016).

70. Aouba, A. *et al.* Targeting the inflammatory cascade with anakinra in moderate to severe COVID-19 pneumonia: case series. *Ann Rheum Dis* **79**, 1381–1382 (2020).

71. Catanzaro, M. *et al.* Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. *Signal Transduction and Targeted Therapy* **5**, (2020).

72. Hayden, M. S. & Ghosh, S. Regulation of NF-κB by TNF Family Cytokines. *Semin Immunol* **26**, 253 (2014).

73. Kunnumakkara, A. B. *et al.* COVID-19, cytokines, inflammation, and spices: How are they related? *Life Sciences* **284**, (2021).

74. Yadav, V. R., Prasad, S., Sung, B., Kannappan, R. & Aggarwal, B. B. Targeting Inflammatory Pathways by Triterpenoids for Prevention and Treatment of Cancer. *Toxins (Basel)* **2**, 2428 (2010).

75. Hayden, M. S. & Ghosh, S. Shared Principles in NF-κB Signaling. *Cell* **132**, 344–362 (2008).

76. Lawrence, T. The Nuclear Factor NF-κB Pathway in Inflammation. *Cold Spring Harbor Perspectives in Biology* **1**, (2009).

77. Halim, C., Mirza, A. F. & Sari, M. I. The Association between TNF-α, IL-6, and Vitamin D Levels and COVID-19 Severity and Mortality: A Systematic Review and Meta-Analysis. *Pathogens* **11**, (2022).

78. Merza, M. Y. *et al.* Analysis of cytokines in SARS-CoV-2 or COVID-19 patients in Erbil city, Kurdistan Region of Iraq. *PLoS ONE* **16**, (2021).

79. Mulchandani, R., Lyngdoh, T. & Kakkar, A. K. Deciphering the COVID-19 cytokine storm: Systematic review and meta-analysis. *European Journal of Clinical Investigation* **51**, (2021).

80. Qin, C. *et al.* Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **71**, 762–768 (2020).

81. Huang, W. *et al.* The Inflammatory Factors Associated with Disease Severity to Predict COVID-19 Progression. *The Journal of Immunology* **206**, 1597–1608 (2021).

82. Saraiva, M. & O'Garra, A. The regulation of IL-10 production by immune cells. *Nature Reviews Immunology* **10**, 170–181 (2010).

83. Moore, K. W., de Waal Malefyt, R., Coffman, R. L. & O'Garra, A. Interleukin-10 and the Interleukin-10 Receptor. *https://doi.org/10.1146/annurev.immunol.19.1.683* **19**, 683–765 (2003).

84. Saraiva, M., Vieira, P. & O'Garra, A. Cytokines Focus: Biology and therapeutic potential of interleukin-10. *The Journal of Experimental Medicine* **217**, (2020).

85. Han, H. *et al.* Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. *Emerging Microbes & Infections* **9**, 1123 (2020).

86. Ma, X. & Trinchieri, G. Regulation of interleukin-12 production in antigen-presenting cells. *Adv Immunol* **79**, 55–92 (2001).

87. Tait Wojno, E. D., Hunter, C. A. & Stumhofer, J. S. The immunobiology of the Interleukin-12 family: Room for discovery. *Immunity* **50**, 851 (2019).

88. Moll-Bernardes, R. *et al.* IL-10 and IL-12 (P70) Levels Predict the Risk of Covid-19 Progression in Hypertensive Patients: Insights From the BRACE-CORONA Trial. *Frontiers in Cardiovascular Medicine* **8**, 702507 (2021).

89. Espíndola, O. M. *et al.* Inflammatory Cytokine Patterns Associated with Neurological Diseases in Coronavirus Disease 2019. *Annals of Neurology* **89**, 1041–1045 (2021).

90. Östling, J. *et al.* IL-17–high asthma with features of a psoriasis immunophenotype. *Journal of Allergy and Clinical Immunology* **144**, 1198–1213 (2019).

91. Majumder, S. & McGeachy, M. J. IL-17 in the Pathogenesis of Disease: Good intentions gone awry. *Annu Rev Immunol* **39**, 537 (2021).

92. Muir, R. *et al.* Innate Lymphoid Cells Are the Predominant Source of IL-17A during the Early Pathogenesis of Acute Respiratory Distress Syndrome. *Am J Respir Crit Care Med* **193**, 407–416 (2016).

93. Martonik, D., Parfieniuk-Kowerda, A., Rogalska, M. & Flisiak, R. The Role of Th17 Response in COVID-19. *Cells* **10**, (2021).

94. Yu, Z. xin *et al.* The ratio of Th17/Treg cells as a risk indicator in early acute respiratory distress syndrome. *Critical Care* **19**, (2015).

95. Lin, S., Wu, H., Wang, C., Xiao, Z. & Xu, F. Regulatory T Cells and Acute Lung Injury: Cytokines, Uncontrolled Inflammation, and Therapeutic Implications. *Frontiers in Immunology* **9**, 1 (2018).

96. Chen, B. D. M., Clark, C. R. & Chou -h., T. Granulocyte/Macrophage Colony-Stimulating Factor Stimulates Monocyte and Tissue Macrophage Proliferation and Enhances Their Responsiveness to Macrophage Colony-Stimulating Factor. *Blood* **71**, 997–1002 (1988).

97. Bhattacharya, P. *et al.* Dual Role of GM-CSF as a Pro-Inflammatory and a Regulatory Cytokine: Implications for Immune Therapy. *Journal of Interferon & Cytokine Research* **35**, 585 (2015).

98. Hamilton, J. A. Cytokines Focus: GM-CSF in inflammation. *The Journal of Experimental Medicine* **217**, (2020).

99. Hu, B., Huang, S. & Yin, L. The cytokine storm and COVID-19. *Journal of Medical Virology* **93**, 250–256 (2021).

100. Crayne, C. B., Albeituni, S., Nichols, K. E. & Cron, R. Q. The Immunology of Macrophage Activation Syndrome. *Frontiers in Immunology* **10**, 119 (2019).

101. Hermans, I. F., Ritchie, D. S., Yang, J., Roberts, J. M. & Ronchese, F. CD8+ T Cell-Dependent Elimination of Dendritic Cells In Vivo Limits the Induction of Antitumor Immunity. *The Journal of Immunology* **164**, 3095–3101 (2000).

102. Retamozo, S. *et al.* Haemophagocytic syndrome and COVID-19. *Clinical Rheumatology* **40**, 1233 (2021).

103. Bandaru, S. S., Capace, A., Busa, V. & Williams, A. Secondary Hemophagocytic Lymphohistiocytosis in a Post-COVID-19 Patient. *Cureus* **14**, (2022).

104. Obstfeld, A. E. *et al.* Cytokine release syndrome associated with chimeric-antigen receptor T-cell therapy: clinicopathological insights. *Blood* **130**, 2569–2572 (2017).

105. Gao, Y. M., Xu, G., Wang, B. & Liu, B. C. Cytokine storm syndrome in coronavirus disease 2019: A narrative review. *J Intern Med* **289**, 147–161 (2021).

106. Gust, J. *et al.* Endothelial activation and blood-brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. *Cancer Discov* **7**, 1404 (2017).

107. Roy, R. K. *et al.* Macrophage Activation Syndrome and COVID 19: Impact of MAPK Driven Immune-Epigenetic Programming by SARS-Cov-2. *Frontiers in Immunology* **12**, 4162 (2021).

108. Merad, M. & Martin, J. C. Author Correction: Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages (Nature

Reviews Immunology, (2020), 20, 6, (355-362), 10.1038/s41577-020-0331-4). *Nature Reviews Immunology* **20**, 448 (2020).

109. Jiang, Y. *et al.* Cytokine storm in COVID-19: from viral infection to immune responses, diagnosis and therapy. *International Journal of Biological Sciences* **18**, 459 (2022).

110. Sanz, J. M., Gómez Lahoz, A. M. & Martín, R. O. Papel del sistema inmune en la infección por el SARS-CoV-2: inmunopatología de la COVID-19. *Medicine* **13**, 1917 (2021).

111. Ziegler, C. G. K. *et al.* SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell* **181**, 1016 (2020).

112. Ziegler, C. G. K. *et al.* SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell* **181**, 1016 (2020).

113. Imai, Y. *et al.* Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* **436**, 112 (2005).

114. Zou, Z. *et al.* Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nature Communications* **5**, (2014).

115. Chen, R. *et al.* Cytokine Storm: The Primary Determinant for the Pathophysiological Evolution of COVID-19 Deterioration. *Frontiers in Immunology* **12**, 1409 (2021).

116. Marrero, M. B. *et al.* Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor. *Nature* **375**, 247–250 (1995).

117. Ziegler, C. G. K. *et al.* SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. *Cell* **181**, 1016 (2020).

118. Issa, H. *et al.* Combination of Angiotensin (1-7) Agonists and Convalescent Plasma as a New Strategy to Overcome Angiotensin Converting Enzyme 2 (ACE2) Inhibition for the Treatment of COVID-19. *Frontiers in Medicine* **8**, 620990 (2021).

119. Naqvi, I. *et al.* DAMPs/PAMPs induce monocytic TLR activation and tolerance in COVID-19 patients; nucleic acid binding scavengers can counteract such TLR agonists. *Biomaterials* **283**, 121393 (2022).

120. Kawai, T. & Akira, S. Signaling to NF-κB by Toll-like receptors. *Trends in Molecular Medicine* **13**, 460–469 (2007).

121. Sokol, C. L. & Luster, A. D. The Chemokine System in Innate Immunity. *Cold Spring Harbor Perspectives in Biology* **7**, 1–20 (2015).

122. Kimura, A. & Kishimoto, T. IL-6: Regulator of Treg/Th17 balance. *European Journal of Immunology* **40**, 1830–1835 (2010).

123. Katze, M. G., He, Y. & Gale, M. Viruses and interferon: a fight for supremacy. *Nature Reviews Immunology 2002 2:9* **2**, 675–687 (2002).

124. Wang, Z., Pan, H. & Jiang, B. Type I IFN deficiency: an immunological characteristic of severe COVID-19 patients. *Signal Transduction and Targeted Therapy 2020 5:1* **5**, 1–2 (2020).

125. Naqvi, I. *et al.* DAMPs/PAMPs induce monocytic TLR activation and tolerance in COVID-19 patients; nucleic acid binding scavengers can counteract such TLR agonists. *Biomaterials* **283**, 121393 (2022).

126. Cao, X. COVID-19: immunopathology and its implications for therapy. *Nature Reviews. Immunology* **20**, 269 (2020).

127. Huang, C. *et al.* Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* **395**, 497 (2020).

128. Frater, J. L., Zini, G., d'Onofrio, G. & Rogers, H. J. COVID-19 and the clinical hematology laboratory. *International Journal of Laboratory Hematology* **42**, 11–18 (2020).

129. Luo, X. H., Zhu, Y., Mao, J. & Du, R. C. T-cell immunobiology and cytokine storm of COVID-19. *Scandinavian Journal of Immunology* **93**, (2021).

130. Zhang, W. *et al.* The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clinical Immunology (Orlando, Fla.)* **214**, 108393 (2020).

131. Levy, G., Guglielmelli, P., Langmuir, P. & Constantinescu, S. JAK inhibitors and COVID-19. *Journal for Immunotherapy of Cancer* **10**, 2838 (2022).

132. Richardson, P. *et al.* Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. *Lancet* **395**, e30 (2020).

133. Honda, S. & Harigai, M. The safety of baricitinib in patients with rheumatoid arthritis. *https://doi.org/10.1080/14740338.2020.1743263* **19**, 545–551 (2020).

134. Bronte, V. *et al.* Baricitinib restrains the immune dysregulation in patients with severe COVID-19. *The Journal of Clinical Investigation* **130**, 6409 (2020).

135. Cantini, F. *et al.* Beneficial impact of Baricitinib in COVID-19 moderate pneumonia; multicentre study. *Journal of Infection* **81**, 647–679 (2020).

136. Agarwal, A. *et al.* A living WHO guideline on drugs for covid-19. *BMJ* **370**, (2020).

137. Hospitalized Adults: Therapeutic Management | COVID-19 Treatment Guidelines. https://www.covid19treatmentguidelines.nih.gov/management/clinical-management/hospitalized-adults--therapeutic-management/.

138. Guimarães, P. O. *et al.* Tofacitinib in Patients Hospitalized with Covid-19 Pneumonia. *The New England Journal of Medicine* **385**, 406–415 (2021).

139. Stebbing, J. *et al.* COVID-19: combining antiviral and anti-inflammatory treatments. *The Lancet. Infectious Diseases* **20**, 400 (2020).

140. Innes, A. J. *et al.* Ruxolitinib for tocilizumab-refractory severe COVID-19 infection. *British Journal of Haematology* **190**, e198–e200 (2020).

141. Assessment of Efficacy and Safety of Ruxolitinib in Participants With COVID-19-Associated ARDS Who Require Mechanical Ventilation (RUXCOVID-DEVENT) - Full Text View - ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04377620.

142. Han, M. K. *et al.* Ruxolitinib in addition to standard of care for the treatment of patients admitted to hospital with COVID-19 (RUXCOVID): a randomised, double-blind, placebo-controlled, phase 3 trial. *The Lancet Rheumatology* (2022) doi:10.1016/S2665-9913(22)00044-3.

143. Seif, F. *et al.* JAK Inhibition as a New Treatment Strategy for Patients with COVID-19. *International Archives of Allergy and Immunology* **181**, 467 (2020).

144. Kalil, A. C. *et al.* Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. *The New England Journal of Medicine* **384**, 795–807 (2021).

145. Kinase Inhibitors | COVID-19 Treatment Guidelines. https://www.covid19treatmentguidelines.nih.gov/therapies/immunomodulators/kinase-inhibitors/.

146. Remdesivir | COVID-19 Treatment Guidelines. https://www.covid19treatmentguidelines.nih.gov/therapies/antiviral-therapy/remdesivir/.

147. Therapeutics and COVID-19: living guideline. https://www.who.int/publications/i/item/WHO-2019-nCoV-therapeutics-2022.3.

148. Treon, S. P. *et al.* The BTK inhibitor ibrutinib may protect against pulmonary injury in COVID-19-infected patients. *Blood* **135**, 1912–1915 (2020).

149. Roschewski, M. *et al.* Inhibition of Bruton tyrosine kinase in patients with severe COVID-19. *Sci Immunol* **5**, (2020).

150. van de Veerdonk, F. L. & Netea, M. G. Blocking IL-1 to prevent respiratory failure in COVID-19. *Critical Care* **24**, (2020).

151. Kyriazopoulou, E. *et al.* An open label trial of anakinra to prevent respiratory failure in COVID-19. *Elife* **10**, (2021).

152. Cavalli, G. *et al.* Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. *The Lancet Rheumatology* **2**, e325 (2020).

153. Yang, L. *et al.* The signal pathways and treatment of cytokine storm in COVID-19. *Signal Transduction and Targeted Therapy* **6**, (2021).

154. Wu, D. & Yang, X. O. TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. *Journal of Microbiology, Immunology, and Infection* **53**, 368 (2020).

155. Ly, K. *et al.* Anti IL-17 in psoriasis. *Expert Rev Clin Immunol* **15**, 1185–1194 (2019).

156. Gisondi, P. *et al.* The impact of the COVID-19 pandemic on patients with chronic plaque psoriasis being treated with biological therapy: the Northern Italy experience. *The British Journal of Dermatology* **183**, 373–374 (2020).

157. Weber, J. & Keam, S. J. Ustekinumab. *BioDrugs* **23**, 53–61 (2009).

158. López-Ferrer, A., Laiz, A. & Puig, L. The safety of ustekinumab for the treatment of psoriatic arthritis. *https://doi.org/10.1080/14740338.2017.1323864* **16**, 733–742 (2017).

159. Chen, X. Y., Yan, B. X. & Man, X. Y. TNFα inhibitor may be effective for severe COVID-19: learning from toxic epidermal necrolysis. *Therapeutic Advances in Respiratory Disease* **14**, (2020).

160. Duret, P. M. *et al.* Recovery from COVID-19 in a patient with spondyloarthritis treated with TNF-alpha inhibitor etanercept. *Annals of the Rheumatic Diseases* **79**, 1251 (2020).

161. Fisher, B. A. *et al.* Namilumab or infliximab compared with standard of care in hospitalised patients with COVID-19 (CATALYST): a randomised, multicentre, multi-arm, multistage, open-label, adaptive, phase 2, proof-of-concept trial. *The Lancet. Respiratory Medicine* **10**, 255 (2022).

162. Lin, P. Targeting interleukin-6 for noninfectious uveitis. *Clinical Ophthalmology (Auckland, N.Z.)* **9**, 1697 (2015).

163. Abani, O. *et al.* Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* **397**, 1637 (2021).

164. Investigators, T. R.-C. Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. *The New England Journal of Medicine* **384**, 1491–1502 (2021).

165. Rosas, I. O. *et al.* Tocilizumab in Hospitalized Patients with Severe Covid-19 Pneumonia. *The New England Journal of Medicine* **384**, 1503–1516 (2021).

166. Soin, A. S. *et al.* Tocilizumab plus standard care versus standard care in patients in India with moderate to severe COVID-19-associated cytokine release syndrome (COVINTOC): an open-label, multicentre, randomised, controlled, phase 3 trial. *The Lancet. Respiratory Medicine* **9**, 511 (2021).

167. Salama, C. *et al.* Tocilizumab in Patients Hospitalized with Covid-19 Pneumonia. *The New England Journal of Medicine* **384**, 20–30 (2021).

168. Stone, J. H. *et al.* Efficacy of Tocilizumab in Patients Hospitalized with Covid-19. *The New England Journal of Medicine* **383**, 2333–2344 (2020).

169. Salvarani, C. *et al.* Effect of Tocilizumab vs Standard Care on Clinical Worsening in Patients Hospitalized With COVID-19 Pneumonia: A Randomized Clinical Trial. *JAMA Internal Medicine* **181**, 1 (2021).

170. Veiga, V. C. *et al.* Effect of tocilizumab on clinical outcomes at 15 days in patients with severe or critical coronavirus disease 2019: randomised controlled trial. *The BMJ* **372**, (2021).

171. Hermine, O. *et al.* Effect of Tocilizumab vs Usual Care in Adults Hospitalized With COVID-19 and Moderate or Severe Pneumonia: A Randomized Clinical Trial. *JAMA Internal Medicine* **181**, 1 (2021).

172. Chamlagain, R., Shah, S., Sharma Paudel, B., Dhital, R. & Kandel, B. Efficacy and Safety of Sarilumab in COVID-19: A Systematic Review. *Interdisciplinary Perspectives on Infectious Diseases* **2021**, (2021).

173. Gritti, G. *et al.* IL-6 signalling pathway inactivation with siltuximab in patients with COVID-19 respiratory failure: an observational cohort study. *medRxiv* 2020.04.01.20048561 (2020) doi:10.1101/2020.04.01.20048561.

174. Kalil, A. C. *et al.* Efficacy of interferon beta-1a plus remdesivir compared with remdesivir alone in hospitalised adults with COVID-19: a double-blind, randomised, placebo-controlled, phase 3 trial. *The Lancet. Respiratory Medicine* **9**, 1365 (2021).

175. Myasnikov, A. L. Z., Berns, S. A., Talyzin, P. A. & Ershov, F. I. [Interferon gamma in the treatment of patients with moderate COVID-19]. *Vopr Virusol* **66**, 47–54 (2021).

176. Nguyen, L. S., Ait Hamou, Z., Gastli, N., Chapuis, N. & Pène, F. Potential role for interferon gamma in the treatment of recurrent ventilator-acquired pneumonia in patients with COVID-19: a hypothesis. *Intensive Care Medicine* **47**, 619 (2021).

177. Myasnikov, A. L., Berns, S. A., Zverev, K. v., Lartseva, O. A. & Talyzin, P. A. Efficacy of interferon gamma in the prevention of sars-cov-2 infection (Covid-19): Results of a prospective controlled trial. *International Journal of Biomedicine* **10**, 182–188 (2020).

178. Davidson, S. *et al.* IFNλ is a potent anti-influenza therapeutic without the inflammatory side effects of IFNα treatment. *EMBO Molecular Medicine* **8**, 1099 (2016).

179. Feld, J. J. *et al.* Peginterferon lambda for the treatment of outpatients with COVID-19: a phase 2, placebo-controlled randomised trial. *Lancet Respir Med* **9**, 498–510 (2021).

180. Mattos-Silva, P. *et al.* Pros and cons of corticosteroid therapy for COVID-19 patients. *Respiratory Physiology & Neurobiology* **280**, 103492 (2020).

181. Group, T. R. C. Dexamethasone in Hospitalized Patients with Covid-19 — Preliminary Report. *The New England Journal of Medicine* **384**, 693–704 (2021).

182. Tomazini, B. M. *et al.* Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients With Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19: The CoDEX Randomized Clinical Trial. *JAMA* **324**, 1 (2020).

183. Yang, R., Xiong, Y., Ke, H., Chen, T. & Gao, S. The role of methylprednisolone on preventing disease progression for hospitalized patients with severe COVID-19. *European Journal of Clinical Investigation* **50**, (2020).

184. Hasan, S. S., Kow, C. S., Mustafa, Z. U. & Merchant, H. A. Does methylprednisolone reduce the mortality risk in hospitalized COVID-19 patients? A meta-analysis of randomized control trials. *https://doi.org/10.1080/17476348.2021.1925546* **15**, 1049–1055 (2021).

185. Fantini, J., Chahinian, H. & Yahi, N. Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: What molecular dynamics studies of virus-host interactions reveal. *International Journal of Antimicrobial Agents* **56**, 106020 (2020).

186. P, H. *et al.* Effect of Hydroxychloroquine in Hospitalized Patients with Covid-19. *N Engl J Med* **383**, 2030–2040 (2020).

187. Kamp, T. J., Hamdan, M. H. & January, C. T. Chloroquine or Hydroxychloroquine for COVID-19: Is Cardiotoxicity a Concern? *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease* **9**, 16887 (2020).

188. Andreu, J. M. & Timasheff, S. N. Tubulin bound to colchicine forms polymers different from microtubules. *Proc Natl Acad Sci U S A* **79**, 6753 (1982).

189. van Echteld, I., Wechalekar, M. D., Schlesinger, N., Buchbinder, R. & Aletaha, D. Colchicine for acute gout. *Cochrane Database Syst Rev* **2014**, (2014).

190. Colchicine in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet Respir Med* **9**, 1419–1426 (2021).

191. Tardif, J. C. *et al.* Colchicine for community-treated patients with COVID-19 (COLCORONA): a phase 3, randomised, double-blinded, adaptive, placebo-controlled, multicentre trial. *Lancet Respir Med* **9**, 924–932 (2021).

192. Group, P. T. C. *et al.* Colchicine for COVID-19 in adults in the community (PRINCIPLE): a randomised, controlled, adaptive platform trial. *medRxiv* 2021.09.20.21263828 (2021) doi:10.1101/2021.09.20.21263828.

193. Beck, D. B. & Aksentijevich, I. Susceptibility to severe COVID-19. *Science (1979)* **370**, 404–405 (2020).

194. Lin, D.-Y. *et al.* Effectiveness of Covid-19 Vaccines over a 9-Month Period in North Carolina. *N Engl J Med* **386**, 933–941 (2022).

195. Zheng, Z. *et al.* Risk factors of critical & mortal COVID-19 cases: A systematic literature review and meta-analysis. *The Journal of Infection* **81**, e16 (2020).

196. Goker Bagca, B. & Biray Avci, C. The potential of JAK/STAT pathway inhibition by ruxolitinib in the treatment of COVID-19. *Cytokine & Growth Factor Reviews* **54**, 51 (2020).

197. Prioritization of Therapeutics | COVID-19 Treatment Guidelines. https://www.covid19treatmentguidelines.nih.gov/overview/prioritization-of-therapeutics/.

198. Major Changes----------, ----------recent. FACT SHEET FOR HEALTHCARE PROVIDERS: EMERGENCY USE AUTHORIZATION FOR EVUSHELD™ (tixagevimab co-packaged with cilgavimab) HIGHLIGHTS OF EMERGENCY USE AUTHORIZATION (EUA) These highlights of the EUA do not include all the information needed to use EVUSHELD™ under the EUA. See the FULL FACT SHEET FOR HEALTHCARE PROVIDERS for EVUSHELD. EVUSHELD (tixagevimab) injection; (cilgavimab) injection, co-packaged for intramuscular use Original EUA Authorized Date: 12/2021. (2022).

199. Consortium, W. S. T. Repurposed Antiviral Drugs for Covid-19 — Interim WHO Solidarity Trial Results. *The New England Journal of Medicine* **384**, 497–511 (2021).

200. Islam, H., Chamberlain, T. C., Mui, A. L. & Little, J. P. Elevated Interleukin-10 Levels in COVID-19: Potentiation of Pro-Inflammatory Responses or Impaired Anti-Inflammatory Action? *Frontiers in Immunology* **12**, 2485 (2021).

201. Zhang, W. *et al.* The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clinical Immunology (Orlando, Fla.)* **214**, 108393 (2020).

202. Deng, K. *et al.* Lower Circulating Interferon-Gamma Is a Risk Factor for Lung Fibrosis in COVID-19 Patients. *Front. Immunol* **11**, 585647 (2020).

203. Beigel, J. H. *et al.* Remdesivir for the Treatment of Covid-19 - Final Report. *N Engl J Med* **383**, 1813–1826 (2020).

204. Adinolfi, L. E. *et al.* Remdesivir Plus Dexamethasone Versus Dexamethasone Alone for the Treatment of Coronavirus Disease 2019 (COVID-19) Patients Requiring Supplemental O2 Therapy: A Prospective Controlled Nonrandomized Study. *Clinical Infectious Diseases* (2022) doi:10.1093/CID/CIAC014.