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The Role of IL-6 and other mediators in the cytokine storm associated with SARS-CoV-2 Infection

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Abstract

Background—The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presents with a spectrum of clinical manifestations from asymptomatic or mild, self-limited constitutional symptoms to a hyperinflammatory state ("cytokine storm") followed by acute respiratory distress syndrome (ARDS) and death.

Objective—To provide an evidence-based review of the associated pathways and potential treatment of the hyperinflammatory state associated with SARS-CoV-2 infection.

Recent findings—Dysregulated immune responses have been reported to occur in a smaller subset of those infected with SARS-CoV-2 leading to clinical deterioration 7 to 10 days following initial presentation. A hyperinflammatory state referred to as "cytokine storm" in its severest form has been marked by elevation of IL-6, IL-10, TNF-alpha and other cytokines and severe CD4+ and

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CD8+ T-cell lymphopenia and coagulopathy. Recognition of at-risk patients could permit early institution of aggressive intensive care, antiviral and immune treatment to reduce the complications related to this pro-inflammatory state. Several reports and ongoing clinical trials provide hope that available immunomodulatory therapies could have therapeutic potential in these severe cases.

Conclusion—This review highlights our current state of knowledge of immune mechanisms and targeted immunomodulatory treatment options for the current COVID-19 pandemic.

Keywords

interleukin −6; sepsis; cytokine storm; cytokines; COVID-19; SARS-CoV-2; tumor necrosis factor-alpha; JAK; STING; proinflammatory; hyperinflammatory; hemophagocytic lymphohistiocytosis

INTRODUCTION

The COVID-19 pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) presents an enormous challenge for public health and clinicians globally. Increased understanding of the immunopathogenesis of SARS-CoV-2 infection as well as ongoing clinical trials of host target drugs such as hydroxychloroquine, direct antivirals, convalescent plasma and other immunomodulatory agents hold promise for future evidence-based and targeted therapies to reduce the morbidity and mortality of the most vulnerable populations.

While infection is often asymptomatic or associated with mild to moderate self-limiting symptoms such as fever, dry cough, myalgia and fatigue (1–6), a subset of patients with severe SARS-CoV-2 infection develop a clinically severe hyperinflammatory state or cytokine storm (CS) for which pulmonary involvement such as acute respiratory distress syndrome (ARDS) is a cardinal feature (1, 7). Further, a subgroup of previously healthy children has been diagnosed with a multi-system inflammatory syndrome associated with acute SARS-CoV-2 infection that appears distinct from the adult CS (8).

While the individual components of CS are varied, interleukin-6 (IL-6) has emerged of particular interest in the context of SARS-CoV-2 infection after being identified as the most significant predictor of mortality in recent retrospective studies of patient survival in COVID-19 (1). Herein, we review the current understanding of the origin and mechanisms of cytokine storm associated with SARS-CoV-2 infection with focus on the identification and implication of IL-6 and other proinflammatory cytokines and pathways in CS-driven ARDS and discuss the potential utility of anti-IL-6 and other cytokine targeting immunomodulatory biologics for the treatment of this critically ill population.

Search strategy and selection criteria

We searched *PubMed* for peer-reviewed articles published between Jan 1st 2000 and April 18th 2020 (date of last search) with the terms (“IL-6” OR “interleukin-6” OR “cytokine”) AND (“sepsis” OR “SIRS” OR “systemic inflammatory response syndrome” OR “non-infectious systemic inflammatory response syndrome” OR “ARDS” OR “acute respiratory

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distress syndrome" OR "cytokine storm" OR "inflammatory response" OR "septic shock" OR "critically ill" OR "organ dysfunction" OR "infection") AND ("ICU" OR "intensive care unit" OR "ED" OR "emergency department"). A second search was oriented on treatment with the terms ("IL-6" OR "interleukin-6" OR "IL-1" OR "interleukin-1" OR "TNF" OR "tumor necrosis factor" OR "interferon gamma" OR "STING" OR "interferon pathway") AND ("sepsis" OR "SIRS" OR "systemic inflammatory response syndrome" OR "ARDS" OR "acute respiratory distress syndrome" OR "cytokine storm" OR "inflammatory response" OR "septic shock" OR "critically ill" OR "organ dysfunction" OR "infection") AND ("IL-6 inhibitor" OR "interleukin-6 inhibitor" OR "JAK-STAT" OR "tocilizumab" OR "humanized IL-6R antibody" OR "anakinra" OR "IL-1 inhibitor"). Please refer to Supplement Figure 1 for details concerning the number of articles entered in *PubMed* with the "cytokine storm" keywords.

All recent articles on COVID-19/SARS-CoV-2 were reviewed including pre-prints from *bioRxiv* and *medRxiv* as a more real-time resources, but realizing the lack of peer review limitation. In order to carefully include the proposed trials for COVID-19, we researched the ClinicalTrials.gov/trials website.

Articles published in English were selected and reviewed. There was a focus on clinical trials, meta-analysis, randomized controlled trials and systematic reviews as well as novel and significant studies. Finally, we also identified several new references from those listed in the reviewed articles. Please note that although there is increasing information about the SARS-CoV-2 virus and its immune consequences, the majority of the literature available on COVID-19 disease and SARS-CoV-2 infection originates from the onset of the pandemic, in China, with various publications from disease phenotype to immunopathogenesis and follow-up.

OVERVIEW OF SEPSIS AND IMMUNE DYSREGULATION

The release of large quantities of proinflammatory cytokines is termed cytokine storm (CS) and is associated with a variety of infective precipitants and other hyperinflammatory states (9, 10). Virally associated causes of particular relevance are the 2003 severe acute respiratory syndrome (SARS) coronavirus Infection (SARS-CoV) that infected over 8000 globally, primarily in Asia and Canada (Toronto) with an 11% mortality rate (11–14), and the 2012 Middle East respiratory syndrome coronavirus (MERS- CoV) with a reported case fatality rate of 35% (15–17). While SARS-CoV-2 belongs to the same *Betacoronavirus* genus as SARS-CoV and MERS, the case fatality associated with both SARS-CoV and MERS significantly exceeds that of SARS-CoV-2 and the numbers of cases worldwide associated with SARS-CoV and MERS are much lower (18). Genomic evidence suggests that SARS-CoV and SARS-CoV-2 share the same human cell receptor for host entry, the angiotensin-converting enzyme 2 (ACE2) (19). SARS-CoV-2 binds with increased affinity to the ACE2 receptor compared to SARS-CoV, a possible explanation for the widely community transmission from asymptomatic hosts (18).

The clinical correlates of increased inflammation or CS are persistent hypotension, hypo or hyperthermia and end-organ dysfunction while the laboratory features include

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haematological anomalies (leukocytosis or leukopenia, thrombocytopenia and disseminated intravascular coagulation with high fibrinogen, triglycerides and ferritin), elevated IL-2R (CD25), elevated IL-6 and general markers of end-organ dysfunction (such as elevated creatinine, deranged liver function tests, etc.) (10). The CS clinical phenotype is variable and regroups systemic signs of inflammation, multi-organ failure and mortality (20).

The hyperinflammatory state associated with severe viral infection shares significant features with cytokine storm and genetic factors associated with hemophagocytic lymphohistiocytosis (HLH) have been identified suggesting these conditions may in fact be part of the same spectrum (21). HLH is caused by an abnormal regulation of activated macrophages and lymphocytes leading to a hyperinflammatory state (21). Interferon-gamma (IFN-γ) is an important cytokine responsible for the organ damage leading to mortality in HLH (20). Other cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), IL-1 and IL-18 are also increased during HLH (22). Several genes that play a role in the pathway of natural killer (NK) cells and CD8+ T-cells have been described as causal for the development of the familial form of HLH. Some examples include the LYST mutation described in the Chediak-Higashi syndrome, the RAB27A mutation in the Griscelli syndrome as well as other mutations in perforin (PRF1). The main clinical and laboratory manifestations of HLH are fever, cytopenia, coagulopathy, liver dysfunction, hyperferritinemia, decreased or absent NK cells function, increased soluble CD25 (IL-2R) and presence of hemophagocytosis on bone marrow biopsy or other organs (21). The secondary form of HLH has been described in influenza during the 2009 H1N1 pandemic, the largest cohort describing 9 cases with a mortality rate of 89% (8/9 cases) (23). The macrophage activation syndrome (MAS) has similar clinical features to HLH and is considered a related disorder that occurs primarily in patients with rheumatologic diseases (24). The excessive inflammatory response described in MAS is characterized by an increased IL-1β with successful reports on the use of anakinra, an IL-1 receptor antagonist (25, 26). In a cohort of 19 adult patients with secondary HLH/MAS, 63.1 % (12/19) had systemic infections as precipitating causes (27). This group used anakinra in 12/19 patients and reported 4 deaths among those patients treated with anakinra (27).

The concept of shared immunological features between HLH, MAS and CS has been entertained with extensive discussion that these diseases could be part of an overlapping immunopathology with shared but also distinctive triggers and genetic features (20). To assess the possible genetic predisposition of individuals with no past medical history to develop HLH secondary to severe H1N1 influenza, Schulert and al. performed whole-exome sequencing (WES) in 14 patients, 13 of whom had evidence of hemophagocytosis at autopsy, and detected 5 heterozygous variants in LYST (two of whom also had a heterozygous PRF1 mutation) (27).

Pediatric multisystem inflammatory syndrome

Children appear protected from SARS-CoV-2 infection and are more likely to be asymptomatic or have less severe infection compared to their adult counterparts (28, 29). However, more recently, a pediatric multi-system inflammatory syndrome has been described that appears distinct from the adult CS associated with acute SARS-CoV-2

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infection and may follow mild or asymptomatic SARS-CoV-2 infection in previously healthy children. This illness has been reported in children aged from early childhood to adolescence, has Kawasaki-like features, and has been reported in areas with a high SARS-CoV-2 prevalence in children exposed to positive COVID-19 family members. In multiple cases, the child had positive SARS-CoV-2 serology but negative SARS-CoV-2 PCR. Deaths of at least three children associated with this syndrome have been reported in the United States and United Kingdom (8). Children with this syndrome have presented with similar features to Kawasaki disease (29, 30) with high fever, rash, conjunctivitis, diarrhea and shown evidence of myocardial involvement with elevated troponins, arrhythmia, shock, abnormal echocardiographic vascular findings and coronary artery aneurysms. They have shown evidence of a hyperinflammatory state with elevated ferritin, triglycerides and D-dimers. Treatments have included high dose intravenous immunoglobulin and aspirin as suggested for Kawasaki's disease (30). Currently, the specific immunopathogenic link to SARS-CoV-2 infection and the role of adjunctive immunomodulatory and anti-cytokine therapy in this syndrome is unknown.

The immunopathogenesis of cytokine storm in the setting of a viral respiratory tract infection

The airway epithelium is part of the first line of defense in presence of an airborne viral pathogen recognized as a pathogen-associated molecular pattern (PAMPs) and/or damage-associated molecular pattern (DAMPs) that bind to pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) on the surface of macrophages (9). The resident activated alveolar macrophages, after several intracellular signaling cascades, generate tumor necrosis factor (TNF), interleukin-1β (IL-β) and IL-6 and trigger a systemic inflammatory response (Figure 1) (31). This simultaneously prompts a well-coordinated local innate response composed of specific enzymes (defensins, mucins, lysozymes), nitric oxide (NO), reactive oxygen species (ROS), platelet activating factor and other cytokines (32). Other key components of the innate immunity against viral infection are the type I interferons (IFNs). In contrast with findings on the influenza virus, severe COVID-19 patients have minimal peripheral quantities of type 1 IFNs but increased IFNs and IFNs genes in the bronchoalveolar environment, a discovery associated with cytokine storm development in a mouse model of SARS-CoV infection (33). Further, *in vitro,* SARS-CoV-2 failed to produce interferon expression in infected cells (34) indicating a dampened early innate immune response (35).

Monocytes and macrophages play a central role and a disruption in the mononuclear phagocyte compartment is considered to increase the COVID-19 related hyperinflammation (36, 37). Also, an increase in the CD14+CD16+ monocytes producing IL-6 has been noted in the peripheral blood of COVID-19 critically-ill patients (38, 39). Bruton's tyrosine kinase (BTK), an intracellular kinase, also appears to have a role in monocytes and macrophage activation and specifically in infection clearance by macrophages (40).

IL-1β is produced after the inflammasome (especially NLRP3), activated by AIM2 sensing foreign DNA, induces the formation of caspase-1 that cleaves pro-IL-1β into IL-1β (Figure 2). In a study using single-cell RNA sequencing in the peripheral blood mononuclear cells

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(PBMCs) of 10 COVID-19 patients compared with 5 healthy controls, the authors reported an increased quantity of CD14++ monocytes with inflammatory gene expression and CD14+ + IL-1β+ monocytes in the early recovery stages of SARS-CoV-2 (41). IL-1β is also implicated in the activity of nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-KB) inducing the synthesis of various inflammatory genes of mediators such as IL-6 (9). Thus, a reduction in IL-1β activity would reduce IL-6 production (42).

Following initial escape of the innate response, recognition of virus promotes the migration of pulmonary dendritic cells to the lymph nodes for presentation of antigen to passing T-cells for the development of more robust antigen- specific T and B-cell adaptive response. During this response, soluble mediators play a role in cellular function and signal transduction by binding to specific receptors on the surface of target cells. For example, CD8+ T-cells produce excessive amounts of TNF-α and IFN-γ causing direct tissue damage, while activated CD4+ T-cells, in the presence of transforming growth factor β (TGF-β) and IL-6, will differentiate into the T helper 17 (Th 17) cell subset, important for extracellular pathogen elimination and auto-immunity (Figure 1) (43). The defining cytokines secreted by Th17 cells are IL-17A and IL-17F that primarily target macrophages, dendritic cells, endothelial cell and fibroblasts to increase the production of IL-1, IL-6 and TNF-α (43). In this particular setting, IL-6 will also inhibit the TGF-β dependent development of CD4+ regulatory T-cells (Treg), a critical mediator of immune tolerance with a major role in regulating the effector T-cell response (44).

In the setting of CS syndromes, over-activation of effector CD4+ and CD8+ T-cells and production of cytokines and chemokines generate an uncontrolled hyperinflammatory injury at the tissue level resulting in local and distant injury (13). Increased inflammation is associated with peripheral blood lymphopenia, a significant drop in the lymphocyte to neutrophil ratio and CD4+ T-cell dysfunction in observational studies but the mechanisms for these changes are unclear (38, 45, 46). In one of the first studies describing the post-mortem pathological findings in one COVID-19 patient, peripheral flow cytometry indicated a reduced CD4+ and CD8+ cell count but an increased proportion of activation markers such as HLA-DR and CD38 as well as an increased concentration of Th17 cells (47).

THE PLEIOTROPIC ROLE OF INTERLEUKIN-6 (IL-6) IN INFLAMMATORY AND VIRAL RESPONSES

IL-6 is secreted by a plethora of immune and stromal cells including monocytes, macrophages, endothelial cells, B and T-cells, hepatocytes, keratinocytes, adipocytes, dendritic cells and fibroblasts. IL-6 exerts effects on a similarly broad array of cellular targets expressing the functional IL-6 receptor (IL-6R) such as T-cells, B-cells, vascular endothelial cells, monocytes, and hepatocytes (44, 48). As may be expected, such diversity of targets translates into functional pleiotropy including the synthesis of acute phase proteins in the liver, such as C-reactive protein (CRP) which is a surrogate for IL-6; the decreased production of proteins such as albumin; the differentiation of B-cells into plasma cells; hematopoiesis and other metabolic and neurologic processes (44, 48). CRP is an acute phase

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reactant that binds the phospholipid component of microorganisms and damaged cells that is frequently used as a screening marker of infection and/or inflammation (31).

IL-6 affects cellular immunity, with both pro- and anti-inflammatory functions. IL-6 genetic knockout mice present with varied impairments of inflammatory response including a well-documented increased susceptibility to microbial infection, while humans expressing defective IL-6 receptors experience a hyperimmunoglobulin E syndrome (HIES)-like disorder which clinically manifests as dermatitis and recurrent (staphylococcal and mycotic) infections highlighting the important role that IL-6 likely plays in the diverse pathways of IgE mediated allergy and microbial defence (49).

Contrasting inflammatory functions of IL-6 are mediated through its modality of receptor binding. Classical binding of IL-6 to the membrane-bound IL-6 receptor (IL-6R) leads to glycoprotein 130 (gp130) dimerization, Janus kinase (JAK) 1 signalling and activation, among others, of the classical RAS/RAF/MAPK pathways leading to anti-inflammatory responses (Figure 3) (50). While all human cells display preformed, inactive gp130 receptors on their cell surface, this receptor remains inactive without the presence of IL-6R which is only expressed on certain cell types (51). However, pro-inflammatory functions have been found to be mediated through binding of soluble IL-6R, termed trans-signalling, with important ramifications for potential therapeutic targeting (52). It has been shown that an important source of soluble IL-6R is shredded from cells undergoing ADAM17-mediated apoptosis which controls mononuclear phagocyte recruitment, leading to amplified inflammatory response (53). The pro-inflammatory responses of IL-6 are mediated by trans-signaling while the anti-inflammatory functions are probably realized by classic signaling (Figure 3) (50). Selective blockage of this trans-signaling pathway is likely to have the beneficial effect of blocking inflammation without the undesirable off-target effects of broad immune suppression.

The role of IL-6 and other mediators in the response to SARS-CoV-2 infection

A multitude of markers for COVID-19 disease severity have been proposed such as CD4+ and CD8+ T-cell lymphopenia (3, 5, 54) as well as global lymphopenia. Homing of lymphocytes to the lungs is significantly increased in non-survivors compared to survivors (1). A number of publications now highlight that an increase of IL-6 correlates with severe response to SARS-CoV-2; defined as the development of sepsis, ARDS, requirement for mechanical ventilation and death (1, 3, 5, 7, 46, 54–56). The clinical and immunological parameters associated with in-hospital mortality in COVID-19 patients are reported in Table 1. The structural N protein of coronaviruses performs an essential role during host cell entry as well as virus particle assembly and release (11). The N protein from the SARS-CoV activates the transcription factor NF-κB and, subsequently, the expression of IL-6 in human airway epithelial cells, providing a biologically plausible explanation for the role of IL-6 in SARS-CoV-2 infection immune pathogenesis (11).

In a cohort of 60 hospitalised SARS-CoV-2 patients, IL-6 concentrations were 163 ± 153 pg/mL for the group with mild symptoms and 517 ± 796 pg/mL for the patients with severe presentation (ICU admitted) (57). A summary of the observations from studies of IL-6 in hospitalised COVID-19 patients is presented in Table 2. IL-6 may be both a biomarker and a

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potential therapeutic target for hospitalised patients with COVID-19 which is an attractive concept in the absence of alternative direct acting antiviral strategies.

Importantly, proposed clinical cut-off values for IL-6 in this setting have started to emerge with > 80 pg/ml determined to predict respiratory failure in a study with 40 COVID-19 patients in which 13 required medical ventilation (4) and > 100 pg/ml in patients with detectable serum SARS-CoV-2 nucleic acid was found to correlate with mortality (N=17/48) (55). However, other authors have reported specificity at much lower concentrations with a retrospective study of 43 COVID-19 patients from China indicating that severe cases could be predicted using an IL-6 value greater than 24.3 pg/mL (sensitivity of 73.3% and a specificity of 89.3%) (56). While studies with larger sample sizes are urgently required to determine the true IL-6 cut-off associated with severe disease, ICU admission and mortality, these initial results are promising. The presented data is limited by its retrospective nature and uncertainty if IL-6 levels can be ascertained by clinical laboratories in an expedited fashion to guide therapy in real-time.

Lessons from IL-6 and other proinflammatory states including sepsis

IL-6 levels are considered to be undetectable or below 10 pg/mL (with some inter-test variability) in heathy controls (12, 58, 59). Conversely, mean IL-6 levels at presentation appear highest in severe sepsis (51.4 pg/mL) compared to patients that do not develop severe sepsis (36.5 pg/mL; $p < 0.03$) in a study of community-acquired pneumonia (60). This response is highly specific for severe disease and some studies indicate a role in disease progression, demonstrating up to a four-fold decrease in IL-6 three days after initial diagnosis. Moreover, IL-6 values appear to drop abruptly in survivors while remaining higher in non-survivor groups (59–61). Nonetheless, IL-6 currently represents one of the best characterised markers of disease severity and an early rise in IL-6 is associated with sepsis, organ failure and death (62–64).

Assigning discrete cut-off values for IL-6 to enable its use as a clinical diagnostic tool has remained ill-defined due to variations in the literature. Song *et al.* demonstrated in 142 patients that an IL-6 cut-off value of 52.60 pg/mL and 348.9 pg/mL was associated with a diagnostic and prognostic value, respectively, in patients with SIRS (65). In contrast, in another SIRS cohort (N=177), a cut-off of 75 pg/mL for sepsis and 145 pg/mL for septic shock was defined (61). Thus, even if further clarification is required, the literature demonstrates that elevated IL-6 values are associated with sepsis or septic shock development (59, 66–68). This is also supported by a 2016 meta-analysis of 2680 critically-ill patients from 22 studies - the use of IL-6 was of moderate diagnostic capacity and relatively high specificity in defining sepsis from other SIRS (69) and, thus, IL-6 may be of utility to confirm infectious causation in patients with complex presentation while considering the limitation in terms of availability of IL-6 levels. The specific IL-6 values from critically ill patients are represented in Table 3.

Lessons from IL-6 response in CAR T-cell associated cytokine release syndrome

There are similarities between the immunopathology of sepsis-associated cytokine storm and the cytokine release syndrome (CRS), a well described complication of chimeric antigen

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receptor T-cell (CAR T-cell) therapy or hematopoietic cell transplantation (HCT). While these terms should not be used interchangeably, CRS was described as part of the cytokine storm syndromes [14]. The CRS is a cytokine-mediated systemic inflammatory disease that groups signs and symptoms of multiple organ damage ranging from mild constitutional symptoms (grade 1) to end-organ damage (grade 4) [23, 24]. Multiple grading systems for CRS have been provided in the literature and a commonly used one is presented in Table 4. In the case of CRS, the cytokines are released directly by the infused CAR T-cells or by other immune cells such as macrophages in response to the cytokines produced by the CAR T-cells [25]. In some series of CRS, serum IL-6 levels correlated with the activation of potent T lymphocytes and CAR T-cell expansion, predicting subsequent therapeutic response and tumour control (70, 71). Humanized IL-6R inhibitors such as tocilizumab have been integrated into CAR T-cell treatment protocols to pre-emptively manage CRS.

ROLE OF BIOLOGICAL IMMUNOTHERAPIES IN SARS-CoV-2

Targeting IL-6

The use of biomarkers such as IL-6 and downstream CRP to recognise early the hyperinflammatory state of SARS-CoV-2 infection has been proposed as a trigger point for employing immunological therapies. Importantly, many such immunotherapies are already available for different treatment indications including those that target the IL-6 and IL-6R. Tocilizumab is a humanized anti-IL-6R antibody engineered by grafting the complementarily determining regions (CDR) of a mouse anti-human IL-6R antibody into a human IgGk to create a human antibody with a human IL-6R binding site. Critically for the opposing pro- and anti-inflammatory functions previously discussed, tocilizumab binds to both membrane-bound and soluble IL-6R for total inhibition of IL-6 signal transduction (Figure 3). The main side effects of completely blocking IL-6 signaling are neutropenia, thrombocytopenia and liver enzyme abnormalities (72). Serious infections have been reported in patients treated long term with tocilizumab so caution should be used (73). Nonetheless, tocilizumab is FDA approved for not only rheumatoid arthritis for which it was originally developed and provides beneficial relief of this largely Th17-driven disease, but, more recently, for severe or life-threatening (grade 3 or 4) CRS associated with CAR T-cell therapy (Table 4) with a dramatic reversal of the clinical manifestations (20). For CRS, initial studies dosed patients at 8 mg/kg and 12 mg/kg infused intravenously over 60 minutes with up to three additional doses if needed (minimum 8 hours between consecutive doses) (74). Responders were defined as patients with symptom resolution within 14 days.

Because of the proposed benefits of using tocilizumab in patients with CAR T-cell induced CRS and the described similarities between CRS and CS following infection, randomized trials are recruiting in COVID-19. In certain centres, tocilizumab has been employed in a compassionate access fashion in critically severe COVID-19 patients. A retrospective study from China (N=21) which used tocilizumab 400 mg intravenous drip (single dose) with or without lopinavir/ritonavir and methylprednisolone demonstrated improvement in fever, hypoxemia, CRP levels and pulmonary CT imaging, without adverse events (75). The mean CRP levels before the drug were 75.06 ± 66.80 mg/L and decreased to 38.13 ± 54.21 mg/L at day one, 10.61 ± 13.79 mg/L at day three and 2.72 ± 3.60 mg/L at day five (75). The

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mean IL-6 level prior to the first dose of tocilizumab was 132.38 ± 278.54 pg/ml (75). Although follow-up IL-6 levels were not subsequently ascertained in this study, the pre-treatment IL-6 concentration aligns with severe disease cut-offs in those studies mentioned earlier. Of immense importance for monitoring, increased serum IL-6 may be expected after initial treatment with tocilizumab (76). Indeed, it is considered that the usual IL-6R mediated consumption of IL-6 is altered by the bound between tocilizumab and IL-6R and that the IL-6 level during tocilizumab treatment probably reflects disease activity (76). Furthermore, in this study, IL-6 was also significantly increased in 20 healthy volunteers 7 days after a single dose of tocilizumab (3.0 +/− 0.6 pg/mL at baseline and 9.3 +/−1.0 pg/mL at day 7) (76). Therefore, it is proposed that post tocilizumab use, monitoring of CRP may be a more appropriate assay for monitoring inflammation (44, 76). A French center has also shared their experience with tocilizumab 8 mg/kg (up to two doses) in 30 severe SARS-CoV-2 patients defined as requiring more than 6 L/min oxygen therapy with rapid changes in oxygen needs (increase of more than 3 L/min in 12 hours) and having a more than 5 days disease diagnosis (77). The authors found that, when compared with a matched control group, the drug decreased the need for mechanical ventilation and ICU admission (23/30) (77). Finally, in an observational study from the United States, 153 patients with severe COVID-19 (defined as patients requiring supplemental oxygen and critical disease) were treated with an 8 mg/kg intravenous tocilizumab dose (maximum 800 mg). When compared to the non-severe group, survival rates were similar (p=0.11) (78).

In light of these promising results, the FDA has approved a randomized, double-blinded, placebo-controlled phase III clinical trial (COVACTA) with 55 locations in North America and Europe. This trial aims to test the efficacy and safety of intravenous tocilizumab in patients with severe SARS-CoV-2 infection (NCT04320615). Similarly, a multicenter, randomized controlled trial was started in China to test the efficacy and safety of tocilizumab in the treatment of patients with COVID-19 pneumonia and elevated IL-6 levels (ChiCTR2000029765). The Italian Regulatory Drug Agency (AIFA) has approved a multicenter, single-arm, open-label, phase 2 study (TOCIVID-19) where all the patients will be treated with tocilizumab 8 mg/kg intravenously (up to a maximum of 800 mg per dose), the primary goal being to assess the mortality rate after the first month (EudraCT: 2020–001110-38). There are currently more than 20 registered COVID-19 associated tocilizumab trials (Table 5). A study registered in Greece proposes to individualise immunomodulatory treatment including tocilizumab or anakinra in COVID-19 depending on their cytokine profile (NCT04339712).

Sarilumab, a monoclonal antibody to IL-6 receptor, is also being investigated in COVID-19 trials. A French multicenter randomized controlled trial (CORIMUNO-SARI) aiming to assess the efficacity and safety of sarilumab versus standard of care is ongoing (NCT04324073). Two additional industry driven clinical trials (NCT04315298, NCT04327388) aiming to assess the efficacy and safety of sarilumab in COVID-19 hospitalized patients are recruiting. While the clinical outcome data for sarilumab is lacking, the comparative response with tocilizumab will be of interest given the longer half-life of sarilumab and greater affinity for the IL-6R (72).

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Siltuximab, a chimeric monoclonal antibody targeting IL-6 directly and preventing binding to both soluble and membrane bound IL-6 receptors, is FDA approved for the multicentric Castleman's disease (79). There are currently 3 European trials recruiting COVID-19 diagnosed patients (NCT04322188, NCT04329650, NCT04330638).

Targeting IL-1β

IL-1β leads to an increase in body temperature, lung inflammation and fibrosis (36). Increased levels of IL-1β were noted in patients diagnosed with SARS-CoV (80) and similar to IL-6, were associated with increased mortality in sepsis (9). Anakinra is a non-glycosylated human decoy interleukin-1 receptor antagonist (IL-1Ra) that binds to the type 1 IL-1 receptor and inhibits IL-1α and IL-1β signal transduction (81). This drug is FDA approved for rheumatoid arthritis and neonatal-onset multisystem inflammatory disease (NOMID) (82) and suggested in the treatment algorithm for secondary HLH/MAS (83).

A recent study found that the serum I L-1β levels were undetectable in 100% (N=17) of the patients with severe or moderate SARS-CoV-2 infection (5), an expected result considering the mechanism of action of this exocrine cytokine. Anakinra was used in a cohort from Italy to treat 29 adult patients diagnosed with COVID-19 related moderate-to-severe ARDS and hyperinflammation (defined as serum CRP ≥100 mg/L, ferritin ≥900 ng/mL, or both) (84). Survival was 90% compared to 56% in a standard treatment group (N=16) (p=0.009) (84). Other improvements included a reduction in CRP and a decrease in mechanical ventilation use. Post-treatment inflammatory relapse was not reported and the treatment was well tolerated (84).

Further, the post-hoc analysis of a phase III randomized control trial studying the use of anakinra in severe sepsis indicated a significant improvement in survival of septic patients with features of macrophage activation syndrome (MAS) in the absence of any severe adverse reactions (26). CORIMUNO-ANA is a trial that aims to determine the efficacy of anakinra in SARS-CoV-2 infected patients (NCT04341584). Anakinra will be administered twice daily as decreasing doses of intravenous infusions (400 mg on day one, two and three; 200 mg on day four and 100 mg on day five). Canakinumab is a human anti-IL-1β monoclonal antibody that blocks IL-1β interaction with the IL-1 receptor for which there is currently one registered observational study (NCT04348448).

Targeting TNF-α and Interferon-γ

Similar to IL-1β, TNF-α has a direct role in acute systemic inflammation and is increased in patients with severe SARS-CoV-2 infection (2, 5, 85). However, this finding is not consistent among the different studies (54). Besides the observational reports that indicate an increase in the levels of this cytokine, a direct pathogenic mechanism of cellular viral entry involving the shedding of the coronavirus' functional receptor, the angiotensin-converting enzyme 2 (ACE2), was studied. This process of binding and shedding of the ACE2 is coupled with TNF-α production and the production of a TNF-α-converting enzyme (TACE) (86). Thus, it has been suggested that an anti-TNF drug could not only inhibit TNF-α directly but also down-regulate the expression and shedding of the ACE2. Also, some studies showed a decrease in sepsis related mortality with anti-TNF treatment (9).

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There are multiple commercialised anti-TNF biologics. Adalimumab is a recombinant human IgG1 monoclonal antibody that specifically binds to human tumor necrosis factor alpha (TNF-α) and blocks its interaction with the p55 and p75 cell surface TNF receptors. This drug could be potentially useful in managing severe COVID-19 manifestations. To analyze the benefits of an anti-TNF-α treatment in COVID-19, a randomized-controlled trial of adalimumab injection in severe COVID-19 patients has been registered (ChiCTR2000030089).

Similar to TNF, the major pro-inflammatory cytokine IFN-γ is also increased in the cytokine storm associated with COVID-19. IFN-γ was particularly well described in SARS-CoV patients (12) and may be targeted by emapalumab for which a comparative multicentre randomized clinical trial is also underway in combination with anakinra (NCT04324021).

Targeting IL-17

Another cytokine that could have a role in the cytokine storm caused by COVID-19 is IL-17. This was not a cytokine of interest in the recent SARS-CoV-2 studies and the only study that characterized IL-17 in COVID-19 found normal levels using a flow cytometry method (54).

As described in this review, IL-17 stimulates the production of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α. Secukinumab is a human IgG1K monoclonal antibody that binds to IL-17A (inhibits the interaction with the IL-17 receptor) and is currently used for plaque psoriasis and several rheumatological conditions (87). The further rational for inhibiting IL-17 is that it is a proximal target to IL-1 and IL-6 and, hence, could reduce neutrophil recruitment to the lungs and prevent organ dysfunction in ARDS. To our knowledge, there are no ongoing trials involving this drug.

Targeting JAK

Targeting the Th17 pathway, research on murine models showed promising results with the use of fedratinib, a JAK2 inhibitor. In this study, the drug decreased the expression of IL-17. As IL-6 and IL-23 are signals for Th17 cell initial differentiation and effector function through the JAK2-STAT3 pathway, the use of this inhibitor could decrease the pro-inflammatory function of Th17 (88). This drug is currently FDA approved for myelofibrosis (89). To our knowledge, there are no current registered trials involving this drug.

As mentioned, the cell-surface ACE2 receptor is needed for coronavirus endocytosis and one of the regulators of this process is the AP2-associated protein kinase 1 (AAK1), part of the numb-associated kinase (NAK) family (90). AAK1 inhibitors have been shown to prevent virus infections by disrupting viral cell invasion. Baricitinib is an oral JAK inhibitor (JAK1/JAK2, JAK1/JAK3, JAK1/tyrosine kinase 2 (TYK2) and JAK2/TYK2) but also an AAK1 inhibitor, having direct antiviral activity, that is currently FDA approved for rheumatoid arthritis resistant to anti-TNF drugs (91). Several trials are ongoing to confirm its safety and efficacy and it is also being investigated in combination therapy with remdesivir (NCT04340232, NCT04321993, NCT04320277). Remdesivir, an adenosine analogue with demonstrated antiviral activity against a broad range of RNA virus families, has been used in a randomized, placebo-controlled trial showing a decrease in time to recovery (15 versus 11

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days) and a trend towards decrease in mortality (92). This drug gained an FDA approval for use in children and adults with severe COVID-19.

Ruxolitinib is a JAK1 and JAK2 inhibitor that mediates the signaling of numerous cytokines such as IL-6, IFN-γ and growth factors with essential roles in immune function and hematopoiesis. This drug is FDA approved for myelofibrosis, hydroxyurea resistant polycythemia vera and steroid-refractory acute graft-versus-host disease (93). A multicenter, single-blinded, randomized trial (1:1) of 44 patients with COVID-19 showed a tendency (not statistically significant) towards improvement in clinical outcomes in the ruxolitinib group (94). Several larger clinical trials from North American and Europe are ongoing (Table 5).

Another attractive drug is tofacitinib that has been shown to inhibit the *in vitro* activity of JAK1/JAK2, JAK1/JAK3 and JAK2/JAK2 and thus decrease the related cytokines. According to the FDA, it can be used for rheumatoid arthritis, psoriatic arthritis and ulcerative colitis (73). There is one planned Italian trial that aims to assess the advantage of early administration of tofacitinib in SARS-COv2 related interstitial pneumonia (NCT04332042).

Serious bacterial, mycobacterial, fungal and viral infections have been reported with the use of JAK inhibitors. This potential off-target effect of these drugs combined with the decreased interferon innate response can lead to severe complications and caution should be used in the SARS-CoV-2 context with theoretical benefit for the anti-JAK molecules that have more specific targets.

Other targeted immunomodulatory therapies and combination therapies

As described, the production of cytokines and chemokines by macrophages is regulated by the BTK. Thus, inhibition of this protein could be a promising strategy for reducing COVID-19 related complications with therapeutic inhibition of BTK in patients with lymphoid malignancies resulting in decreased proinflammatory cytokines.

Company sponsored trials with acalabrutinib, a small-molecule inhibitor of BTK enzymatic activity, that aim to study its efficacy and safety compared to best supportive care in hospitalized patients with COVID-19 are currently listed and will begin recruitment shorty in the United States and Europe (Table 5).

By sensing self or pathogenic cytosolic double-stranded DNA (dsDNA), the cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthetase (cGAS) stimulator of interferon (IFN) genes (STING) plays an important role in innate immunity and tumor development (95). STING is expressed in T-cells, monocytes, NK cells, and dermal fibroblasts and cGAS-STING signaling promotes the production of IL-6 and the downstream activation of STAT3 (95). The STING-IFN-β pathway is triggered by the binding of cGAS to STING that leads to IFN regulatory factor 3 (IRF-3) phosphorylation and subsequent transcription of the gene encoding interferon-β (IFNB) (96). The JAK receptors and their specific pathways are activated by the IFN-β binding to its receptor. The regulation of STING and other proinflammatory cytokine genes is also achieved with the

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synthesis and release of interferons. Thus, this pro-inflammatory loop can be obstructed by JAK inhibition (96).

Combination therapy with lopinavir-ritonavir, ribavirin and IFN-p-1b compared with lopinavir-ritonavir monotherapy was evaluated in an intention to treat multicenter, randomized phase 2 clinical trial from China. The primary endpoint was the time before a negative nasopharyngeal swab (RT-PCR) in SARS-CoV-2 patients with the median time reported for the combination group (N=86) being 7 days and the time in control group (N=41) was 12 days (p=0.0010) (97). Anti-Coronavirus Therapies (ACT) COVID-19 is a clinical trial that aims to evaluate the combination of chloroquine and azithromycin with subcutaneous injection of IFN-β1b for SARS-CoV-2 prevention by assessing admission to intensive care, mechanical ventilation and/or death (NCT04324463).

The complement system is also a potential therapeutic target in SARS-CoV-2 infection. Complement is key to the innate immune response to all viruses and complement inhibition is a potential treatment for severe SARS-CoV-2 infection by reducing the severity and end-organ consequences of the innate immune response (98, 99). A recent mouse model suggested that complement activation through C3 exacerbates SARS-CoV associated ARDS and that C3-deficient mice infected with SARS-CoV showed less respiratory decline (100). Lung biopsy samples from patients with SARS-CoV-2 associated ARDS showed evidence of complement activation with C3 fragment deposition and associated increased serum 5a levels (98). However, there is little clinical data on the potential role of complement activation and its role in ARDS associated with SARS-CoV-2. There are now several proposed and ongoing studies examining the role of C5 inhibitors such as eculizumab and ravulizumab (Table 5).

Convalescent plasma

Given the lack of evidence-based treatment and the novelty of this disease, convalescent plasma (CP) has reemerged as an emergency intervention passive immunization strategy aiming to decrease morbidity and mortality in critically ill COVID-19 patients (101, 102). This treatment has been shown favourable during the SARS-CoV infection with a decrease in hospital stays and mortality compared to controls (103, 104). Also, a recent systematic review, while acknowledging the limited data, indicated that CP is safe, clinically effective and can play a role in reducing mortality (105). The described mechanisms of action are direct neutralization of the virus aimed at the spike (S) viral protein (106, 107) as well as other immunomodulatory and anti-inflammatory functions such as neutralization of cytokines, complement and autoantibodies (101). A clinical report on the use of CP in critically ill SARS-CoV-2 patients showed a hypothetical benefit with decrease of body temperature, increase in respiratory function and ARDS resolution in 4 of the 5 patients included (107). In an open-label, multicenter, randomized clinical trial from China, adding convalescent plasma to the treatment plan did not result in increased clinical recovery (108). Several questions remain unanswered regarding CP and there is a need for larger randomized controlled trials to answer these questions but emerging successful reports related with its use in severe COVID-19 highlights the intense inflammatory response that accompanies this infection.

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CONCLUSION

Severe SARS-CoV-2 infection is associated with cytokine storm producing a hyperinflammatory state and a clinical and laboratory picture similar to hemophagocytic lymphohistiocytosis that typically occurs 7–10 days following the onset of acute illness. In this setting, IL-6 levels correlate with respiratory failure, poor outcomes and mortality. Blocking this and other appealing cytokines and signaling pathways at an early stage shows promise to target specific and undesirable immune responses in the setting of acute SARS-CoV-2 infection. Currently, studies examining the combination of direct antiviral agents with immunomodulatory therapy are ongoing and will be important in the quest to prevent acute respiratory deterioration, ventilation use, morbidity and mortality from SARS-CoV-2 infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AAK1	AP2-associated protein kinase 1
ACE2	angiotensin-converting enzyme 2
AIFA	Italian Regulatory Drug Agency
AIM2	Absent in melanoma 2
AMP	adenosine monophosphate
ARDS	Acute respiratory distress syndrome
BRD4	Bromodomain-containing protein 4
BTK	Bruton’s tyrosine kinase
CAR	Chimeric antigen receptor
CP	convalescent plasma
COVID-19	Coronavirus disease 2019
CDR	Complementarily determining regions
cGAS	cyclic guanosine monophosphate-adenosine monophosphate synthetase

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CRP	C-reactive protein
CRS	Cytokine release syndrome
CS	Cytokine storm
CTCAE	Common Terminology Criteria for Adverse Events
DAMP	Damage-associated molecular pattern
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
ED	Emergency department
ELISA	Enzyme-linked immunosorbent assay
gp130	glycoprotein 130
FDA	Food and Drug Administration
FLT3	fms-like tyrosine kinase 3
GMP	guanosine monophosphate
HCT	Hematopoietic cell transplantation
HIES	Hyper Immunoglobulin E syndrome
HLH	hemophagocytic lymphohistiocytosis
ICU	Intensive care unit
IL	Interleukin
IFN	Interferon
IQR	Interquartile range
IRF-3	interferon regulatory factor 3
JAK	Janus Associated Kinase
LRR	leucine-rich repeats
MAPK	Mitogen-activated protein kinase
MAS	macrophage activation syndrome
MERS	Middle East respiratory syndrome
NAK	numb-associated kinase
NF-kB	nuclear factor kappa-light-chain-enhancer of activated B-cells

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NK	natural killer
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
NOD	Nucleotide-binding and oligomerization domain
NO	Nitric oxide
NOMID	neonatal-onset multisystem inflammatory
PAMP	Pathogen-associated molecular pattern
PBMC	peripheral blood mononuclear cells
pg/ml	picogram/milliliter
PRRs	pattern recognition receptors
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SARS	Severe acute respiratory syndrome
SARS-CoV-1	SARS-associated coronavirus 1
SIRS	Systemic inflammatory response syndrome
STAT	signal transducer and activator of transcription
STING	stimulator of interferon genes
TACE	TNF-α-converting enzyme
TGF-β	transforming growth factor β
Th 17	T helper 17
TLRs	Toll-like receptors
TNF	Tumour Necrosis Factor
Treg	regulatory T-cells
TYK2	tyrosine kinase 2
WES	whole-exome sequencing

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Inflammatory cytokines and chemokines, including IL-6, IL-1β and TNF-α, are significantly elevated in patients with severe SARS-CoV-2 infection suggesting that cytokine storm may play a role in the SARS-CoV-2 severity, morbidity and mortality.

IL-6 has major effects on cellular immunity, with both pro- and anti-inflammatory functions.

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Several recent publications have shown that an increase in the pro-inflammatory cytokine IL-6 correlates with disease severity, defined as sepsis, ARDS or mechanical ventilation and mortality in SARS-CoV-2.

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As with the development of any novel diagnosis and as increased levels of IL-6 are associated with sepsis and septic shock, clinical cut-offs must be defined.

Clinical trials are urgently warranted to evaluate a therapeutic strategy targeting upstream and downstream pathways in SARS-CoV-2. The effective dose and the ideal administration timing of the immunomodulatory drugs remain under investigation.

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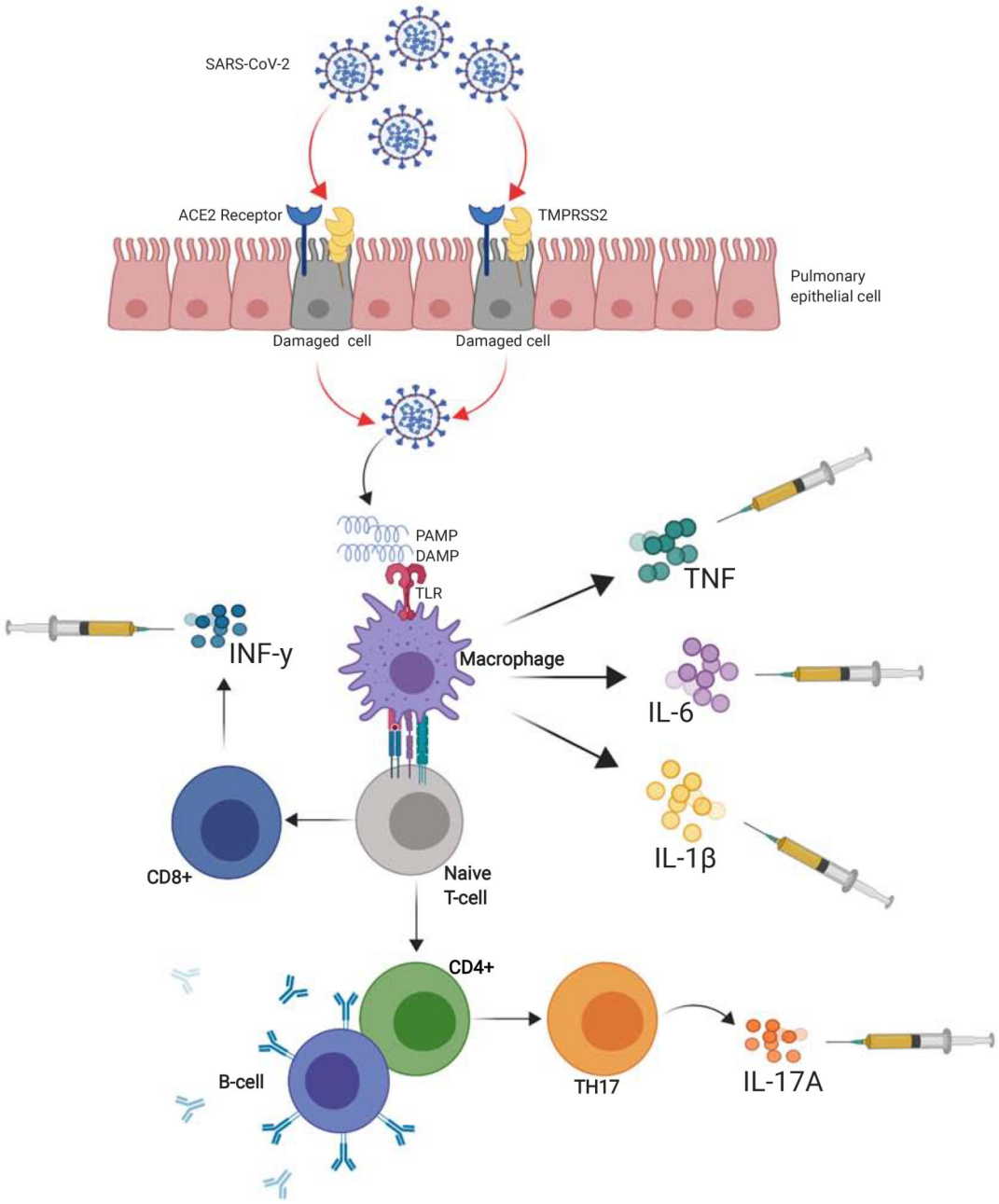

Figure 1: COVID-19 Immunological mechanisms for cytokine storm and possible role of biologics

When SARS-CoV-2 pathogen-associated molecular pattern (PAMPs) and/or Damage-associated molecular patterns (DAMPs) bind to toll-like receptors (TLRs) on the surface of resident alveolar macrophages, they become activated and secrete tumor necrosis factor (TNF), interleukin-1β (IL-1β) and IL-6. In increased levels, these cytokines will be the hallmark of the cytokine storm responsible for the acute respiratory distress syndrome (ARDS) and cytokine storm (CS) in COVID-19. The different targets of biologics are illustrated in the figure. Specifically, the downstream effect IL-6 can be blocked with tocilizumab, sarilumab or siltuximab and the effects of IL-1β with anakinra or canakinumab.

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CD8+ T-cells produce IFN-γ causing direct tissue damage, while activated CD4+ T-cells, in the presence of transforming growth factor β (TGF-β) and IL-6, will differentiate into the T helper 17 (Th 17) cell subset, responsible for secreting IL-17A and IL-17F who, among numerous roles, target macrophages, dendritic cells, endothelial cell and fibroblasts to increase the production of IL-1, IL-6 and TNF.

ACE2, Angiotensin converting enzyme 2; ARDS, DAMP, damage-associated molecular patterns; PAMP, pathogen-associated molecular pattern; *SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF, tumour necrosis factor; TMPRSS2, transmembrane protease, serine 2.*

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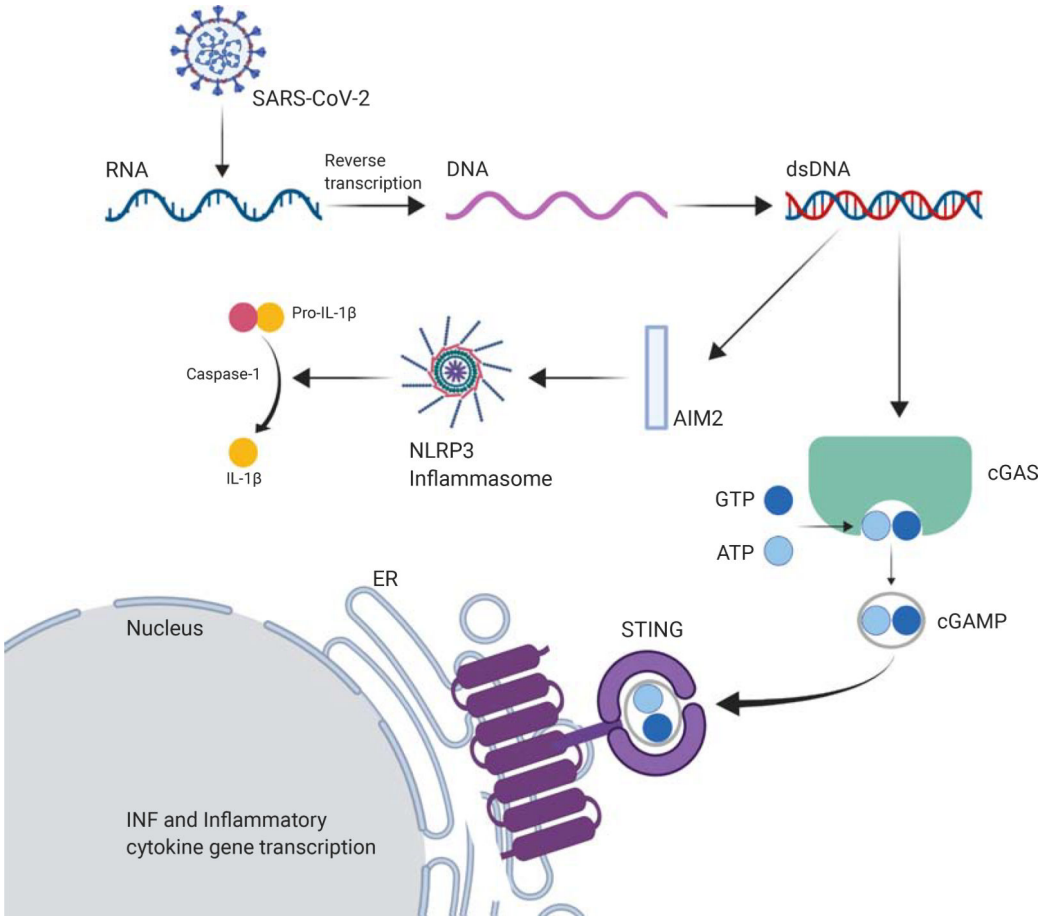

Figure 2: The implications of the STING pathway in coronavirus

The cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthetase (cGAS) stimulator of interferon (IFN) genes (STING) pathway is activated by sensing foreign cytosolic DNA (obtained after reverse transcription from the SARS-CoV-2). cGAS catalyzes the generation of cyclic GMP-AMP (cGAMP) that binds and activates STING in the ER (endoplasmic reticulum) leading to the expression of IFNs and other cytokines. IL-1β is produced after the NLRP3 inflammasome, activated by AIM2 sensing foreign DNA, induces the formation of caspase-1 that will cleave pro-IL-1β into IL-1β.
AMP, adenosine monophosphate; ATP, Adenosine triphosphate; cGAMP, cyclic GMP-AMP; DNA, deoxyribonucleic acid; GMP, guanosine monophosphate; GTP, Guanosine triphosphate; cGAS, cyclic GMP-AMP synthetase; ER, endoplasmic reticulum; IFN, interferon; RNA, ribonucleic acid; STING, stimulator of interferon.

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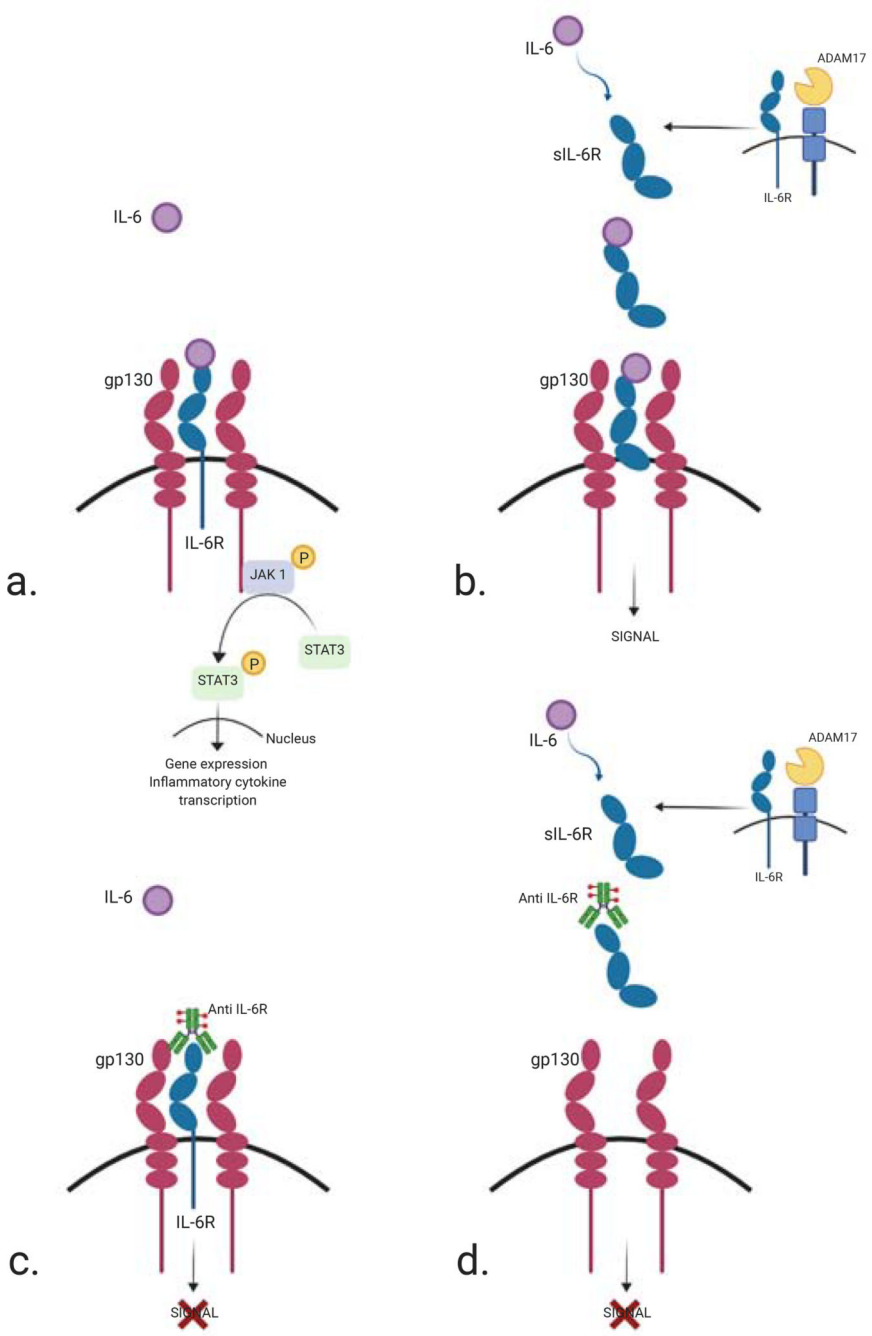

Figure 3: Classic and trans-signaling IL-6R

a. and **b.** Different signaling pathways stimulated by IL-6. Binding of IL-6 to the membrane-bound or soluble IL-6 receptor (IL-6R) leads to gp130 dimerization and Janus kinase 1 (JAK1) - STAT 3 signalling and activation leading to gene expression of inflammatory cytokines. This pathway is only represented in **a.** and replaced by the word “SIGNAL” in **b.** **a.** Classic signaling, which is restricted to several cell types, is initiated through binding of IL-6 to the membrane IL-6R and forms a complex with gp130.

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b. Trans-signaling is driven by IL- 6 in all gp130-expressing cells. Pro-inflammatory functions have been found to be mediated through binding of soluble IL-6R shredded from cells undergoing ADAM17-mediated apoptosis.
c. and **d**. IL-6 blockade therapy using a humanized anti-IL-6 receptor monoclonal antibody A humanized anti-IL-6R antibody blocks IL-6-mediated signaling pathway by inhibiting IL-6 binding to the membrane (**c.**) and soluble (**d.**) receptors.
gp130, glycoprotein 130; IL, Interleukin; IL-6R, IL-6 receptor; sIL-6R, soluble IL-6R; JAK, Janus Associated Kinase; STAT, signal transducer and activator of transcription.

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Table 1 –

Clinical and Immunological Parameters associated with in-hospital mortality in COVID-19

Studies	**Zhou et al. 2020 (1)**	**Ruan et al. 2020 (7)**	**Wu et al. 2020 (3)**	**Wang et al. 2020 (6)**	**Li et al. 2020 (85)**
Country	China	China	China	China	China
Type of study	Retrospective cohort	Retrospective cohort	Retrospective cohort	Retrospective cohort	Retrospective cohort
Patients	191	150	201	33	548
Comorbidities *	Age > 69 years HTN CAD Diabetes	Age > 68 years HTN CAD	Age > 65 years HTN	n/a	Age > 65 years HTN Male
Clinical *	↑ Sofa score£ > 4.5	Dyspnea Respiratory failure, ARDS AKI Other infection	Dyspnea	n/a	n/a
Laboratory *	Lymphopenia < 0.6 × 10^9 /L Leucopenia < 4 × 10^9 /L ↑ Procalcitonin < 0.1 ng/ml ↑ Creatinine > 133 µmol/L ↑ D-dimer >1 µg/ml ↑ ALT> 40 U/L ↑ LDH > 245 U/L ↑ Troponin I > 28 pg/mL ↑ CK > 185 U/L ↑ Ferritin> 300 µg/L	Lymphopenia < 0.6 × 10^9 /L Leucocytosis > 10.6 × 10^9 /L ↑ CRP > 126.6 mg/L ↑ Creatinine > 91.2 µmol/L ↑ urea> 8.6 µmol/L ↑ Troponin I > 30 pg/mL ↑ Myoglobin > 258.9 ng/mL	Lymphopenia < 0.6 × 10^9 /L ↑ urea > 7.4 µmol/L ↑ D-dimer >3.95 µg/ml ↑ LDH > 484 U/L	Lymphopenia < 1.1 × 10^9 /L Neutrophilia > 6 × 10^9 /L ↑ creatinine> 100 µmol/L ↑ urea > 7.5 µmol/L ↑ D-dimer >500 µg/L	Leucocytosis ↑ LDH > 445 U/L
IL-6	Non-survivors (N=54) 11 (7.5 – 14.4) pg/mL Survivors (N=137) 6.3 (5.0 – 7.9) pg/mL	Non-survivors ¶ (N=68) 11.4 ± 8.5 ng/mL Survivors ¶ 6.8 ± 3.6 ng/mL (N=82)	Non-survivors ¶ (N = 44) 10.1 (7.4 – 14.8) pg/L Survivors ¶ (N = 117) 6.3 (5.4 – 7.8) pg/L	n/a	n/a

Abbreviations: *AKI, Acute kidney injury; ARDS, acute respiratory distress syndrome; CAD, coronary artery disease; HTN, hypertension; n/a, non-available;*

Values expressed as means ± standard deviation or mean [interquartile range (IQR)].

*Only variables statistically significant are presented ($p < 0.05$).

£SOFA score: Sequential Organ Failure Assessment. This score includes multiples parameters such as assessment of respiratory status (partial pressure of oxygen, fraction of inspired oxygen and oxygen saturation), coagulation parameters (platelets), liver function (bilirubin), hypotension, central nervous assessment with Glasgow coma score and renal function (creatinine) (109).

¶The IL-6 units reported in these studies do not compare with the units generally presented. Unfortunately, the method used for measuring IL-6 was not provided.

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Table 2 –

Review of hospital admission IL-6 values in COVID-19 patients

Reference	Setting Country	N ▽	Control * (pg/mL)	Cut-off (pg/mL)	Critically ill patients □ (pg/mL)	Predictor of Complications ♦ (pg/mL)	Predictor of Mortality ♦ (pg/mL)	Method for IL-6 monitoring
(4)	Hospital Germany	40	19.6 [0 – 76.5]	80	n/a	121.0 [19.2 – 430.0]	n/a	n/a
			N = 27			N=13		
(3) ¶	Hospital China	201	6.3 [5.4 – 7.8] pg/L	n/a	6.1 (5.1– 6.7] pg/L	7.4 [5.6 – 10.9] pg/L	10.1 [7.4 – 14.8] pg/L	n/a
			N=117		N=40	N=84	N=44	
(7) ¶	Hospital China	150	6.8 ± 3.6 ng/mL	n/a	n/a	n/a	11.4 ± 8.5 ng/mL	n/a
			N = 82				N = 68	
(1)	Hospital China	191	6.3 [5.0 – 7.9]	n/a	n/a	n/a	11.0 [7.5 – 14.4]	n/a
			N = 137				N = 54	
(55)	Hospital China	48	10.4 [3.8 – 31.0]	100	64 [25.6–111.9]	n/a	n/a	ECLIA (Roche Ltd)
			N =21		N = 17			
(56)	Hospital China	43	10.6 [5.1 – 24.2]	24.3	n/a	36.1 [23.0 – 59.2]	n/a	ECLIA (Rochecobase601)
			N = 28			N = 17		
(110)	Hospital China	43	6.7 [4.4 – 12.4]	n/a	n/a	51.7 [34.3 – 161.7]	n/a	n/a
			N = 36			N = 7		
(54)	Hospital China	53	13.4 ± 1.8	n/a	37.8 ± 7.8	n/a	n/a	FMBA (Qingdao Raisecare Biotechnology Co.)
			N = 45		N =18			

Abbreviations: *n/a, non-available; ARDS, acute respiratory distress syndrome; ECLIA, electrochemiluminescence method; FMBA, flow cytometer microsphere-based assay.*

Values expressed as means ± standard deviation or mean [interquartile range (IQR)]

▽ Number of patients included in each study

* The "Control" IL-6 value represents the COVID-19 diagnosed patients with mild symptoms included in the studies (no healthy controls included).

□ Some studies included IL-6 levels dosed after hospital admission and during disease progression.

♦ This value was recorded upon hospital admission and predicted either sepsis or mortality.

¶ The IL-6 units reported in these studies do not match the units generally presented. Unfortunately, the method used for measuring IL-6 was not provided.

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Table 3 –

Literature review of hospital admission IL-6 values in critically ill patients

Reference	Population IL-6 dosage technique	Setting Country	Study Design	N ∇	Control * (pg/mL)	Cut-off ¥ (pg/mL)	Sepsis ϖ (pg/mL)	Predictor of Sepsis ♦ (pg/mL)	Predictor of Mortality ♦ (pg/mL)
(12)	SARS-CoV patients Detection level n/a (CBA)	Hospital Taiwan	MCRC	88	7.5 ± 30.4	n/a	245.7 ± 770.2	n/a	387.2 ± 911.82
(57)	SARS-CoV patients Detection >10 pg/ml (ELISA)	Hospital China	SCPC	228	61.0 ± 10.1	n/a	n/a	163 ± 153 517 ± 796 (severe)	n/a
(65)	SIRS, sepsis (S) and septic shock (C) patients Detection level n/a (ELISA)	ED Korea	SCPC	142	23.6 [11.2–43.5]	52.60 (S) 348.9 (C)	n/a	89.9 [45.2–272.6] 1378.6 [256.411,062.1] (S)	≥ 348.92 7609.5 [4526.0–12,208.4] (28 days)
(67)	Critically ill patients with organ dysfunction Detection level n/a (RT)	ICU Japan	SCPC	100	104 [46–152]	152	n/a	720 [183–7,656]	n/a
(59)	Severe sepsis patients Detection level n/a (ELISA)	ED Taiwan	SCPC	76	32.9 [0–663.5]	n/a	n/a	223.4 [3.1–979.1] septic shock	196.3 [0.5–979.1]
(111)	Sepsis patients Detection level n/a (ELISA)	ICU Finland	MCPC	61	426 [234–1000]	n/a	n/a	n/a	1000 [269–2000]
(112)	SIRS patients Detection >9.7pg/mL (ELISA)	ICU Malaysia	SCPC	239	183 [61–358]		238 [86–3159]		1127 [218–8643] (30 days)
(61)	SIRS patients Detection level n/a (ECLIA)	ED Korea	SCPC	177	55.3 ± 100.9	75 (sepsis) 145(shock)	n/a	900.1 ± 1643.4	1018.8
(66)	Infection suspicion patients Detection level n/a (ECLIA)	ED Finland	SCPC	539	15.3 [1.5– 653]	n/a	n/a	93.5 [1.5–43 790]	n/a
(68)	Infection and SIRS patients Detection level n/a (ECLIA)	ICU Switzerland	SCPC	78	44.2	200	n/a	n/a	1000
(113)	Major trauma patients (female vs male) (ELISA)	ED Germany	SCPC	343	163.7 ± 25.98	n/a	n/a	363.9 ± 72.58	n/a
(58)	Major trauma patients Detection >7.8pg/ml (ELISA)	ICU France	SCPC	100	55.7 [45.9–83.8]	n/a	n/a	95.1 [71.3–210.3]	n/a
(62)	Major trauma patients Detection level n/a (ELISA)	Trauma unit Switzerland	SCRC	1032	282.1 ± 39.8	n/a	n/a	551.6 ± 124.1	n/a
(60)	CAP patients Detection >5.9pg/mL (ECLIA)	ED US	MCPC	1426	38.7	n/a	98.7	51.4	109.4 (90 days)

Abbreviations: *CBA, Human Th1/Th2 Cytokine or Chemokine Bead Array Kit; CAP, community acquired pneumonia, ED, emergency department; ECLIA, electrochemiluminescence method; ELISA, enzyme-linked immunosorbent assay; ICU, intensive care unit; n/a, non-available; RT, routine testing; SARS-CoV, severe acute respiratory syndrome coronavirus; SIRS, Systemic inflammatory response syndrome; US, United States.*

Types of study design: SCRC: single-center retrospective cohort; SCPC: single-center prospective cohort; MCRC: multi-center retrospective cohort; MCPC: multi-center prospective cohort.

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Values expressed as means ± standard deviation or mean [interquartile range (IQR)].

▽ Number of patients included in each study

* The control group does not include any healthy controls.

ϖ Some studies included IL-6 levels dosed after sepsis diagnostic.

◆ This initial value was recorded upon admission to the hospital (ED) or ICU depending on the study and predicted either sepsis or mortality.

¥ The authors calculated a cut-off value that could predict sepsis.

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Table 4 –

Cytokine Release Storm - Grades and treatment (114)

Cytokine release storm: A multi-organ clinical diagnostic involving constitutional, cardiovascular, hematological, gastrointestinal, cutaneous and neurological manifestations.		
Grades	**Clinical Manifestations**	**Recommended treatment**
1 - Mild	Patients require symptomatic treatment only Fever +/− other constitutional symptoms (no organ dysfunction)	Supportive care (fluids, antipyretics, analgesics as needed)
2 - Moderate	Symptoms respond to moderate intervention Hypoxia (oxygen requirement <40% Fi02) OR Hypotension (responsive to IV fluids or low dose vasopressors) Grade 2 organ toxicity *	Supportive care Cardiac and other organ function monitoring If co-morbidities or older age, consider treatment as per for **Grade 3**
3 - Severe	Symptoms respond to aggressive intervention Hypoxia (oxygen requirement ≥40% Fi02) OR Hypotension requiring high dose or multiple vasopressors Grade 3 organ toxicity ** or Grade 4 transaminitis ***	Tocilizumab ▽ Adults: 4 mg/kg in adults Children: 8 mg/kg in children Repeat the dose if clinical improvement does not occur within 24 to 48 hours. +/− Low dose corticosteroids ¥
4 - Life threatening	Requirement for mechanical ventilation OR Grade 4 organ toxicity *** (excluding transaminitis)	Tocilizumab +/− Low dose corticosteroids ¥

* Common Terminology Criteria for Adverse Events (CTCAE) - Grade 2 - Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL).

** CTCAE - Grade 3 - Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care.

*** CTCAE - Grade 4 - Life-threatening consequences; urgent intervention indicated.

▽ Dose of tocilizumab approved for adults and children with rheumatoid arthritis

¥ Data concerning the use of steroids in COVID-19 is limited. Please refer to the National Institutes of Health (NIH) treatment guidelines (115).

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Table 5 –

Potential therapies for COVID-19 ARDS and CS

Name	Commercial name	Target	Role	FDA indications	Trials Country	Planned Clinical Trials
Tocilizumab	Actemra® RoActemra®	membrane or soluble IL-6R	Inhibits IL-6 signal transduction	Cytokine Release Syndrome Rheumatoid arthritis Giant Cell Arteritis Juvenile Idiopathic Arthritis	COVACTA -United States Italy Spain France Belgium Greece Switzerland Denmark Malaysia China	NCT04320615 NCT04356937 NCT04331795 NCT04346355 NCT04332913 NCT04317092 NCT04315480 NCT04335305 NCT04332094 NCT04331808 NCT04330638 NCT04339712 NCT04335071 NCT04322773 NCT04345445 NCT04310228 NCT04306705
Sarilumab	Kevzara®	membrane or soluble IL-6R	Inhibits IL-6 signal transduction	Rheumatoid arthritis	International United States Canada France Spain Denmark	NCT04327388 NCT04315298 NCT04321993 NCT04324073 NCT04357808 NCT04357860 NCT04322773 NCT04345289
Siltuximab	Sylvant®	IL-6	Inhibits IL-6 signal transduction	Multicentric Castleman's disease	Italy Spain Belgium	NCT04322188 NCT04329650 NCT04330638
Anakinra	Kineret®	type 1 IL-1 receptor	Inhibits IL-1α and IL-1β signal transduction.	Rheumatoid arthritis NOMID	United States Italy Greece France	NCT04362111 NCT04324021 NCT04339712 NCT04357366 NCT04341584
Canakinumab	Ilaris®	IL-1β	blocking IL-1β interaction with IL-1 receptors	Periodic Fever Syndromes Juvenile Idiopathic Arthritis	Italy	NCT04348448
Ruxolitinib	Jakafi® Jakavi®	JAK1, JAK2 inhibitor	Inhibits cytokine-induced STAT phosphorylation	Myelofibrosis Polycythemia Vera Acute Graft Versus Host Disease	United States Canada Mexico Germany Spain	NCT04354714 NCT04348071 NCT04331665 NCT04334044 NCT04359290 NCT04348695

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Name	Commercial name	Target	Role	FDA indications	Trials Country	Planned Clinical Trials
Tofacitinib	Xeljanz®	JAK1, JAK2, JAK3, TYK2 inhibitor		Rheumatoid Arthritis Psoriatic Arthritis Ulcerative Colitis	Italy	NCT04332042
Baricitinib	Olumiant®	JAK2 (JAK 1/3, TYK2), AAK1 inhibitor		Rheumatoid arthritis	United States Canada Italy	NCT04340232 NCT04321993 NCT04320277
Fedratinib	Inrebic®	JAK2, FLT3 and BRD4 inhibitor		Myelofibrosis	None	None
Acalabrutinib	Calquence®	BTK	Inhibits BTK signaling/ B-cell activation	Mantle cell lymphoma Chronic lymphocytic leukemia Small lymphocytic lymphoma	United States Europe	NCT04380688 NCT04346199
Eculizumab	Soliris®	complement protein C5	inhibits C5 cleavage to C5a and C5b (prevents formation of C5b-9)	Paroxysmal nocturnal hemoglobinuria (PNH)	United States France	NCT04288713 NCT04346797 NCT04355494
Ravulizumab	Ultomiris®				United States	NCT04369469 NCT04390464
Emapalumab	Gamifant®	IFN-γ	binds to and neutralizes IFN-γ	Primary HLH	Italy	NCT04324021
Adalimumab	Humira®	TNFα	inhibits TNF-α signal transduction	Rheumatoid Arthritis, Psoriatic Arthritis Ankylosing Spondylitis Crohn's Disease Ulcerative Colitis Plaque Psoriasis Hidradenitis Suppurativa	China	ChiCTR2000030089
Secukinumab	Cosentyx®	IL-17A	Inhibits IL-17A signal transduction (via IL-17 receptor)	Psoriatic Arthritis Ankylosing Spondylitis Plaque Psoriasis	None	None

Abbreviations: *AAK1, AP2-associated protein kinase 1; BRD4, Bromodomain-containing protein 4; BTK, Bruton's tyrosine kinase: FLT3, fms-like tyrosine kinase 3; HLH, hemophagocytic lymphohistiocytosis; IFN, Interferon; IL, Interleukin; JAK, Janus Associated Kinase; NOMID, neonatal-onset multisystem inflammatory; STAT, Signal transducer and activator of transcription; TYK2, tyrosine kinase 2; TNF, Tumour Necrosis Factor.*

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