









## JAK-STAT Pathway Inhibition and their Implications in COVID-19 Therapy

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### ABSTRACT

As the incidence of COVID-19 increases with time, more and more efforts are made to pave a way out for the therapeutic strategies to deal with the disease progression. Inflammation being a significant influencer in COVID-19 patients, it drives our focus onto the signaling cascades of the JAK/STAT pathway. JAK phosphorylation mediated by cytokine receptor activation leads to phosphorylation of STATs that translocate into the nucleus to translate for inflammatory mediators. The SARS-CoV-2 structural proteins like spike, nucleocapsid, membrane and envelope proteins along with the non- structural proteins 1-16 including proteases like 3CL<sup>PRO</sup> and PL<sup>PRO</sup> promote its entry and survival in hosts. The SARS-CoV-2 infection triggers inflammation via the JAK/STAT pathway leading to recruitment of pneumocytes, endothelial cells, macrophages, monocytes, lymphocytes, natural killer cells and dendritic cells progressing towards cytokine storm. This produces various inflammatory markers in the host that determine the disease severity. The JAK/STAT signaling also mediates immune responses via B cell and T cell differentiation. With an attempt to reduce excessive inflammation, JAK/STAT inhibitors like Ruxolitinib, Baricitinib, Tofacitinib have been employed that mediate its actions via suppressors of cytokine signaling, cytokine inducible SH2 containing protein, Protein inhibitor of activated STAT and protein tyrosine phosphatases. Even though they are implicated with multiple adverse effects, the regulatory authorities have supported its use, and numerous clinical trials are in progress to prove their safety and efficacy. On the contrary, the exact mechanism of JAK/STAT inhibition at molecular levels remains speculative for which further investigations are required.

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## 1. Introduction

The JAK (Janus kinase)/STAT (signal transducers and activators of transcription) are members of a pathway implicated in the SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) induced cytokine storm [1]. Classically, this functional aspect of the JAK/STAT pathway may be related to the cytokine receptor signaling. In unstimulated cells, the STAT proteins with SH2 domains are inactive and confined to the cytoplasm. Following cytokine receptor stimulation, STAT proteins bind to the phospho-tyrosine that contains sequences of the receptor, using SH2 domains [2]. Further, the JAK induced phosphorylation of STAT proteins at the cytokine type receptors causes subsequent dimerization of STAT proteins. The dimerization of STATs enables them to translocate inside the nucleus and stimulate apoptosis, immune regulation, cell cycle differentiation, and gene transcription as shown in Figure 1 [3].

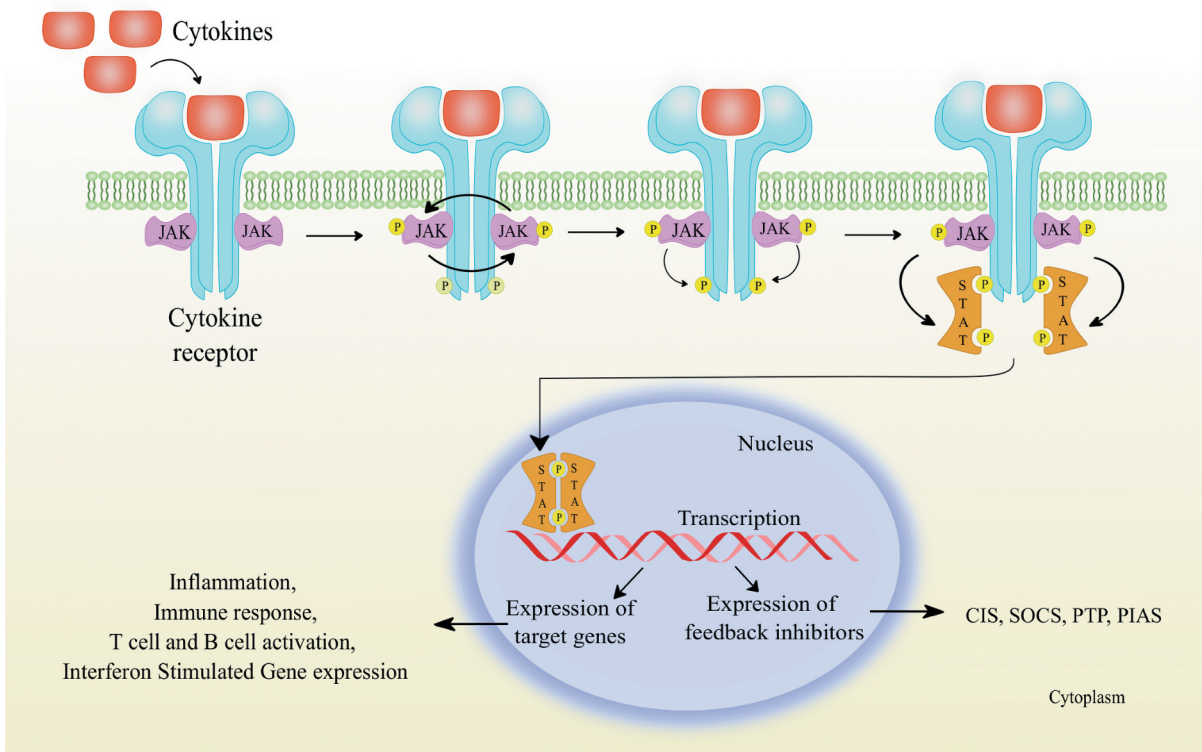
Coronavirus disease 2019 (COVID-19), triggered by SARS-CoV-2 is a viral infection marked by respiratory illness, multiple organ failure, pneumonia, acute respiratory distress syndrome (ARDS), and ultimately death. Besides these symptoms, several systemic symptoms including lymphopenia, abnormalities of prothrombin time, ferritin levels, platelet count, acute partial thromboplastin time, and blood clotting anomalies have been

seen in this disease [2]. While the upsurge in cytokine levels accounts for most of the inflammatory responses, it interrupts the apoptosis of endothelial cells that in turn affect vascular permeability, even though the signaling mechanisms accountable for the cytokine storm in COVID-19 are still unclear.

While the role of JAK/STAT signaling in COVID-19 has been suggested, nevertheless, the intricacy of these networks precludes a flawless understanding of the involvement of cytokine signaling in the COVID-19 pathophysiology. The objective of this review is to recapitulate current reports on the role of JAK/STAT signaling in COVID-19, focusing mainly on therapeutic strategies.

## 2. Stakeholders of SARS-CoV-2 mediated inflammation and its markers in COVID-19

Cytokines are molecular messengers released by the cells having specific actions on the interaction and communication between other cells [4]. They are formed from the critical role players of immunity and inflammation like natural killer (NK) cells, lymphocytes like T and B cells, dendritic cells, and the innate macrophages [5]. Cytokines consist of chemokines, interferons, interleukins, lymphokines, and Tumor Necrosis Factor (TNF) that mediate an essential role in regulating the



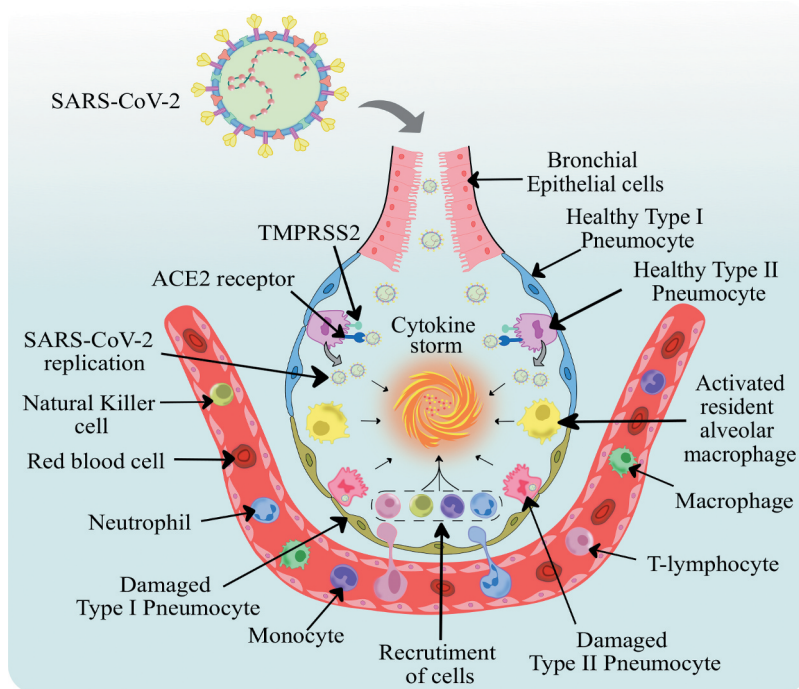
**Figure 1.** The cascade of the JAK/STAT pathway initiated upon the binding of cytokines produced by the SARS-CoV-2 infection onto their corresponding cytokine receptors.

cell inflammation and corresponding immune responses. These constitute the essential messengers that control the signals that enhance or reduce the inflammatory actions to pathogens and any kind of stress or injury [6]. The pro-inflammatory cytokines like Interleukin-1 (IL-1), Tumor Necrosis Factor (TNF), Interleukin-6 (IL-6), and Interleukin-12 (IL-12) promote leukocyte activation. In contrast, anti-inflammatory cytokines like Interleukin 10 (IL-10), Interleukin-14 (IL-14), Tissue Growth Factor (TGF- $\beta$ ) suppress Phospholipase C (PLCs), and the chemokines like Interleukin-8 (IL-8) to recruit cells involved in inflammation [7]. Under normal conditions, cytokine response to infection is important [8]. However, with SARS-CoV-2 infection in the pneumocytes of alveoli, the resident alveolar macrophages, and the epithelial cells of the alveoli and damaged pneumocytes trigger an inflammatory response that releases cytokines and chemokines. This response further attracts more cells like natural killer cells, T-lymphocytes, bloodborne macrophages, and neutrophils which infiltrate from the blood into the alveoli and aggravate the inflammatory response as shown in Figure 2. Interestingly, STAT3 also is known to contribute to the cytokine storm via co-activation of IL-6 amplifier that enhances cytokine production [9]. The COVID-19 patients have been reported to present with high levels of pro-inflammatory cytokines like IL-6 and these levels have been attributed to the severity of the disease [10]. Additionally, GM-CSF, Interferon- $\gamma$  (IFN- $\gamma$ ) mediated protein 10, TNF- $\alpha$ , monocyte chemoattractant protein 1, IL-2 R have also been identified in these patients [11]. Therefore, such

aggravated conditions of hyper inflammation lead to a phenomenon called a cytokine storm that ultimately progresses towards ARDS and even death [12].

The SARS-CoV-2 uses the ACE2 receptor for accessing the host cells, especially the ones present on the Type II Pneumocytes. The spike protein consists of an S1 subunit that promotes attachment on the ACE2 receptor and an S2 subunit whose proteolytic cleavage is mediated by type II transmembrane serine protease (TMPRSS2), facilitates entry by membrane fusion. Upon entry of the virions in the host cells, they replicate, spread, and create inflammation in the lungs [13].

These inflammatory responses can lead to the release of macrophages, monocytes, and other cytokines and chemokines, leading to cytokine storms, thus deteriorating the conditions in COVID-19 patients [14]. Therefore, inflammatory markers play a crucial role in describing disease severity in COVID-19 patients. Recently, Zheng et.al described the role of markers of inflammation in COVID-19. They concluded that C-reactive protein (CRP), Erythrocyte Sedimentation Rate (ESR), Procalcitonin (PCT), Serum Amyloid A (SAA), IL-6, and serum ferritin were elevated in severe COVID-19 patients compared to non-severe COVID-19 patients [15]. Similarly, along with the ability to release IL-6, SARS-CoV-2 infection is also capable of emancipating granulocyte-macrophage colony-stimulating factor (GM-CSF) from the T cells. The GM-CSF, in turn, can stimulate the inflammatory CD14+ and CD16+ monocytes, elevating IL-6 levels, and several other inflammatory mediators [16]. Apart from these, the COVID-19 patients are often reported to present with lymphopenia and neutrophilia, and



**Figure 2.** Various stakeholders involved in inflammation induced by cytokine storm upon SARS-CoV-2 infection in alveolar cells.

therefore, the Neutrophil-Lymphocyte Ratio (NLR) serves as the potential marker in these patients [17–19]. Feng et al demonstrated that severe conditions of COVID-19 present higher levels of NLR than COVID-19 cases of lesser severity. Thus, this significant increase in neutrophils and a decrease in lymphocyte could be an indicator of severity in COVID-19 progression [20].

### 2.1. Pneumocytes

The alveolar epithelium cells in the lungs express Type 1 and Type 2 pneumocyte cells [21]. SARS-CoV-2 infects type 2 pneumocytes in the alveolar epithelium, abundant in ACE2 receptors, thus promoting effective binding onto the lung tissue [22,23]. Recently a study performed in cynomolgus macaques showed the distribution of SARS-CoV-2 in the type I and type II pneumocytes, the epithelial cells of nasal, bronchial, and bronchiolar mucosa lined with cilia exhibiting marked diffuse alveolar damage (DAD) [24]. The DAD is a peculiar feature observed in cases of ARDS where the patients suffer from the development of inflammation, hyaline membranes, injuries to the epithelial cells of the alveoli, bronchial edema, and even hemorrhage [25]. Recent studies have reported DAD in COVID-19 patients [26,27]. Apart from the known ACE2 receptors, Scott et al demonstrated the ability of a novel receptor CD209L also termed as L-SIGN, to be a potential receptor for SARS-CoV (severe acute respiratory syndrome coronavirus) entry into the host, and synergistically, these receptors were expressed in human alveolar cells type II and endothelial cells of the lungs [28].

Interestingly, another study showed that novel progenitor CD 34<sup>+</sup> Oct 4<sup>+</sup> cells were the primary targets of SARS-CoV infection. The viral infection and degradation of CD 34<sup>+</sup>

Oct 4<sup>+</sup> cells through L-SIGN facilitated entry of the virus into cytokeratin<sup>+</sup> respiratory epithelium could result in cytopathy and inflammation [29]. These cells also possess high levels of ACE2 receptors and therefore share a possibility of getting infected by SARS-CoV. Other stem cells present in the respiratory tract include club cells, bronchioalveolar stem cells, basal cells, along with type II pneumocytes that also contain ACE2 receptors [30].

An interesting recent report describes the correlation between ACE2 receptor distribution and Interferon stimulated gene (ISG)/IFN signaling in the basal cells of nasal epithelium via the JAK/STAT pathway. Upon the release of IFN- $\alpha$ 2 and IFN- $\gamma$ , the levels of STAT1 are enhanced leading to upregulation of the ACE2 receptors promoting binding of SARS-CoV-2 in the host [31].

### 2.2. Endothelial cells

During the early course of infection, endothelial cells are infected [23]. It has high ACE2 expression through which SARS-CoV-2 gain access to enter the host cell. They also constitute CD147 (Extracellular matrix metalloproteinase inducer), TMPRSS2, and sialic acid receptor [32]. They undergo viral replication and apoptosis via Fas/FasL or TRAIL-PR-5 dependent mechanisms. SARS-CoV resulted in endothelial cell dysfunction leading to microcirculation disorder [23].

Infected bronchial epithelium in COVID-19 patient was studied to manifest a strong relation of SARS-CoV with SARS-CoV-2 in immunological pathophysiology and pro-inflammatory cytokines which lead to cytokine storm in affected COVID-19 patients [33]. Endothelial activation in Cytokine Storm Syndrome (CSS) may lead to unstable hemodynamics, capillary leakage, and coagulopathies and

increased production of pro-inflammatory cytokines could release von Willebrand factor and angiopoietin-2 that further stimulate the endothelium activation [34]. The release of cytokines following a SARS-CoV-2 infection was seen to activate the JAK/STAT pathway in the cells of pulmonary endothelia [35].

### 2.3. Macrophages and monocytes

Macrophages are the first-line defense against viral infections and a potent source of inflammatory cytokines. Monocyte and macrophage are rich in ACE2 receptors that cause activation and transcription of pro-inflammatory genes [36]. Several laboratory mice strains are unable to support ACE2 binding studies and therefore effort is made to incorporate human ACE2 into mice out of which one such model is the K18-hACE2-transgenic mice [37]. Interestingly, reports have demonstrated the significant pathogenic potential of SARS-CoV-2 when evaluated in hACE2 transgenic mice [38,39] Upon SARS-CoV-2 infection in K18 type of hACE2 (K18-hACE2)-transgenic mice, high amounts of viral RNA was detected in the lungs accompanied by increased levels of pro-inflammatory cytokines, chemokines, monocytes, neutrophils along with T cells leading to a pneumonia-causing collapse in alveolar cavities [40].

Evidence from COVID-19 patients has demonstrated that CD169+ macrophages rich in ACE2 are present in spleen and lymph nodes [40]. These macrophages were shown to upregulate IL-6 and possess the SARS-CoV-2 nucleoprotein antigen. Other virus-infected tissues also showed an increase in levels of CD95 (Fas), thus suggesting that CD169+ macrophages could promote viral infectivity, inflammation, and lymphocytic necrosis [41].

The low molecular weight fraction of human serum albumin (LMWF5A) can prevent hyper inflammation, development of cytokine storm, and promote vascular permeability [42]. They have shown an immunomodulatory action causing a shift in M1 inflammatory macrophage to M2 anti-inflammatory macrophage as evident in an in-vitro study [43].

COVID-19 patients produce IL-6, IL-10, and TNF along with CD11b, CD14, CD16, CD68, CD163, and CD206 [7]. A higher expression of cytokines was associated with them with HLS. FCN1<sup>+</sup> (High inflammatory derived ficolin-1) macrophages as found in the autopsy reports of bronchoalveolar lavage fluid (BAIF) in ARDS diagnosed COVID-19 patients [44]. STAT 1, STAT 2, and several regulatory factors relating to IFN were activated by Monocyte-derived macrophages [45]. Thus, ill-regulated inflammatory response and T cell apoptosis can expedite activation and accumulation of inflammatory monocyte/macrophage [7].

### 2.4. Lymphocytes

T lymphocyte, CD4<sup>+</sup>T, and CD8<sup>+</sup>T play a prominent role in developing autoimmunity and inflammation [45]. Critically ill patients in COVID-19 had a marked decrease in T and B cells [7]. Nevertheless, CD4<sup>+</sup>T and CD8<sup>+</sup>T play a vital part in the elimination of coronaviruses from the infected cell by inducing

immune lesions [45,46]. Helper T cells (Th1) through the NF-κB signaling pathway produce pro-inflammatory conditions that result in further activation of cytokine and chemokine cascades [46]. About 85% of COVID-19 patients revealed peripheral blood lymphopenia and unexpected infiltration in the airways due to the dysfunction of innate T cells against SARS-CoV-2 [45]. IL-6 can suppress T cell activation, causing lymphopenia [23]. Levels in COVID-19 patients confirmed a downstream indicator of cell pyroptosis which is suggested as a cause of lymphopenia [23]. Regulatory T cells (Treg) were upregulated in severe cases; reduced/unchanged in some cases playing a central role in balancing antiviral immunity and cytokine storm [7]. Laboratory reports of severe COVID-19 patients exhibited decreased B cell levels as compared to mild infection [47].

The antiviral role of CD4<sup>+</sup> and CD8<sup>+</sup> T cells is significant in combating pathogens. T-dependent B cell activation by CD4<sup>+</sup> T cells promotes virus-specific antibodies. Virus-infected cells can be destroyed by cytotoxic CD8<sup>+</sup> T cells [48]. In patients infected with SARS-CoV, the pulmonary interstitial cells accounted for, by CD8<sup>+</sup> T cells, have 80% of total inflammatory cells which are infiltrated which is vital for the clearance of coronaviruses from infected cells, thereby resulting in an injured immune system. While the viral replication is not affected or delayed as a result of CD8<sup>+</sup> depletion during the SARS-CoV infection, the depletion of CD4<sup>+</sup> T cell is responsible for the reduction in the neutralizing antibody, pulmonary recruitment of lymphocytes, and cytokine production causing delayed SARS-CoV clearance from lungs and immune-mediated interstitial pneumonitis, etc [48]. While the inhibition of serum containing proteins leads to control of the complement system, it also damages the host tissue. As the viral proteins manage to evade complement system detection, it highlights the significance of the complement system in antiviral activity [49]. The differentiation, growth, and survival of T cells especially the CD8<sup>+</sup> and the CD4<sup>+</sup> T cells is mediated via STAT 3. STAT 3 facilitated stimulation of CD8<sup>+</sup> T cells during a viral attack provides a strong immune response against the virus [50].

### 2.5 Natural Killer (NK) Cells and Dendritic Cells

In peripheral tissues and circulation, NK cells are present. NK cells do not reside permanently in tissue and dynamically move between blood and tissues. Thus, during a viral invasion like SARS-CoV-2, sensing the chemokine gradient, NK cells are recruited to the area of infection [44]. In COVID-19 patients, in peripheral blood, there was an increase in the myeloid cells, but the NK cells were found to be decreased [51]. However, it is presumed that the virally infected cells express specific putative ligands to activate the NK cell receptors to trigger NK cell killing [52]. Through their corresponding ligands, the natural receptors of cytotoxicity such as NKp30, NKp44, NKp46, C-type lectin-like receptors NKG2D and NKp80 (KLRF1) and co-activation receptors as DNAM-1 may get activated in NK cells [52].

In severe cases of COVID-19, the peripheral bloodstream had lesser NK cells. NK cell destroys virus incorporated cells by degranulation, cell apoptosis mediated by receptors, and antibody-dependent cell-facilitated cytotoxicity. There were no

significant differences in mild v/s severe cases, in the number of CD16<sup>+</sup>, and CD56<sup>+</sup>. Anyhow, further studies would be needed to determine the contribution of these cells in cytokine release [7].

Dendritic cells help to release pro-inflammatory mediators like TNF $\alpha$  and aid inactivation of T lymphocytes [46,53]. The exact mechanism of dendritic cells in SARS-CoV-2 is not known. IL-6 from dendritic cells binds to IL-6-sIL-6 R-gp-130 (soluble IL-6 Receptor glycoprotein 130) and activates the JAK-STAT pathway, which can lead to vascular permeability, monocyte recruitment, neutrophil recruitment, and signal amplification [32]. The SARS-CoV-2 infection was also shown to turn down the IFN cascade in the monocyte-derived dendritic cells by preventing the phosphorylation of STAT1 [54]. In the NK cells, the IL-15 mediated by JAK1 regulates their development, growth, and functioning. Similarly, IL-2 signals from JAK1,3 and STAT1,3,5 enhance the cytotoxicity of NK cells [55].

Therefore, a summary of all the above-mentioned stakeholders of inflammation involved in the development and maintenance of the cytokine storm in COVID-19 is given in Table 1.

### 3. Role of JAK/STAT signaling pathway in the immune system

The JAK/STAT signaling pathway can influence the immune system functioning to a significant extent. The JAK/STAT signaling components like JAK1-3 and STAT1-6 have been associated with various roles in immune responses like IL-2, IL-4, IL-6, IL-12 signaling, Th (T-helper)1, Th2, Treg cell and Th9, Th 17 cell differentiation, the proliferation of viral selective CD8<sup>+</sup> T cells and B-cell lymphoma 2 (Bcl-2) [2,56]. It is evident that cytokines are necessary for combating viral infections but the hyperinflammatory conditions created by SARS-CoV-2

infection due to cytokine storm could lead to conditions like ARDS and even death [9]. To prevent this, the inhibition of the JAK/STAT pathway may look to be a promising strategy but having said that, prolonged inhibition of this pathway could lead to compromised immune responses in the body that could promote the proliferation of the SARS-CoV-2. The viral infection could also further promote inhibition of inflammatory signaling pathways causing immunosuppression [57]. Recently, Bouwman and colleagues reported that JAK/STAT signaling system could be a valuable indicator of a strong immune response to SARS-CoV-2 infections [58]. Interestingly, another report stated that the inhibition of the JAK/STAT pathway could reduce the hyperinflammatory conditions but not the viral clearance [59]. This could suggest that COVID-19 patients who are immunocompromised could be susceptible to superinfection via inhibition of the JAK/STAT pathway that would call for cautionary use of JAK/STAT inhibitors.

### 4. Deciphering the link between SARS-CoV-2 proteins and JAK/STAT Signaling

SARS-CoV-2 is a member of  $\beta$ -coronavirus genera in the *Coronaviridae* family that shares about 96.7% sequence similarities with the bat strain RaTG13 and the pangolin isolates [13,60]. It contains a single strand of a positive-sense RNA genome with 29,903 base pairs [61]. The viral RNA is enclosed in an enveloped structure and when viewed under an electron microscope, appears as a crown-like structure due to the incorporation of structural spike proteins on its outer surface that facilitates its entry, proliferation, and infectivity in host cells via ACE2 receptors [62,63]. The ACE2 receptors are expressed in epithelial cells of the kidney, heart lungs, and

**Table 1.** Summary of stakeholders in the development of cytokine storm in COVID-19.

Type of cells	Description
Pneumocytes	<ul style="list-style-type: none"> <li>SARS-CoV-2 bind Type 2 pneumocytes rich in ACE2 receptors</li> <li>SARS-CoV-2 infected pneumocytes show diffused alveolar damage, a peculiar feature in COVID-19 patients diagnosed with ARDS.</li> <li>Alveolar cells express L-SIGN (CD209L), a novel receptor for SARS-CoV binding.</li> <li>Presence of ACE2 receptors on novel progenitor CD 34<sup>+</sup> Oct 4<sup>+</sup> cells could also enhance viral binding.</li> <li>IFN mediated stimulation of STAT1 could upregulate ACE2 receptor levels in nasal epithelium.</li> </ul>
Endothelial Cells	<ul style="list-style-type: none"> <li>The presence of ACE2 receptors along with CD147, TMPRSS2, and sialic acid receptors facilitate SARS-CoV-2 binding and penetration.</li> <li>Viral infectivity occurs via Fas/FasL or TRAIL-PR-5 mediated mechanisms.</li> <li>Cytokine storm induced by the uncontrolled release of pro-inflammatory cytokines can release von Willebrand factor and angiotensin-2 that further stimulate the endothelium activation.</li> <li>Activation of JAK/STAT signaling follows upon cytokine release.</li> </ul>
Macrophages and Monocytes	<ul style="list-style-type: none"> <li>Both rich in ACE2 receptors</li> <li>Transfection of SARS-CoV-2 onto ACE2 receptors of K18-hACE2-transgenic mice macrophages showed significant infectivity.</li> <li>CD169<sup>+</sup> macrophages rich in ACE2 receptors possess SARS-CoV-2 nucleoprotein antigen and stimulate IL-6 levels.</li> <li>High levels of HLS.FCN1<sup>+</sup> was found in bronchoalveolar lavage fluid of COVID-19 diagnosed with ARDS.</li> <li>Monocyte-derived macrophages activate STAT1, STAT2, and IFN regulatory factors.</li> </ul>
Lymphocytes	<ul style="list-style-type: none"> <li>T lymphocyte, CD4<sup>+</sup>T, and CD8<sup>+</sup>T develop autoimmunity and inflammation.</li> <li>T cell and B cell levels in COVID-19 patients are decreased.</li> <li>CD4 + T cells and CD8<sup>+</sup> cells eliminate viral particles via immune lesions.</li> <li>IL-6 and IL1-<math>\beta</math> lead to lymphopenia in COVID-19 patients.</li> <li>STAT3 mediated CD8 + T cell stimulation provides a greater immune response against SARS-CoV-2.</li> </ul>
NK cells and Dendritic cells	<ul style="list-style-type: none"> <li>NK cell reduction is evident in SARS-CoV-2 infection.</li> <li>Viral cells mediate NK cell destruction.</li> <li>NK cells neutralize SARS-COV-2 via degranulation, apoptosis, and cytotoxicity.</li> <li>Dendritic cells release IL-6 that activates the JAK/STAT pathway to combat SARS-CoV-2.</li> <li>The prevention of STAT1 phosphorylation by SARS-CoV-2 in dendritic cells reduces IFN signaling.</li> <li>JAK/STAT signaling plays an essential role in the development and functioning of NK cells.</li> </ul>

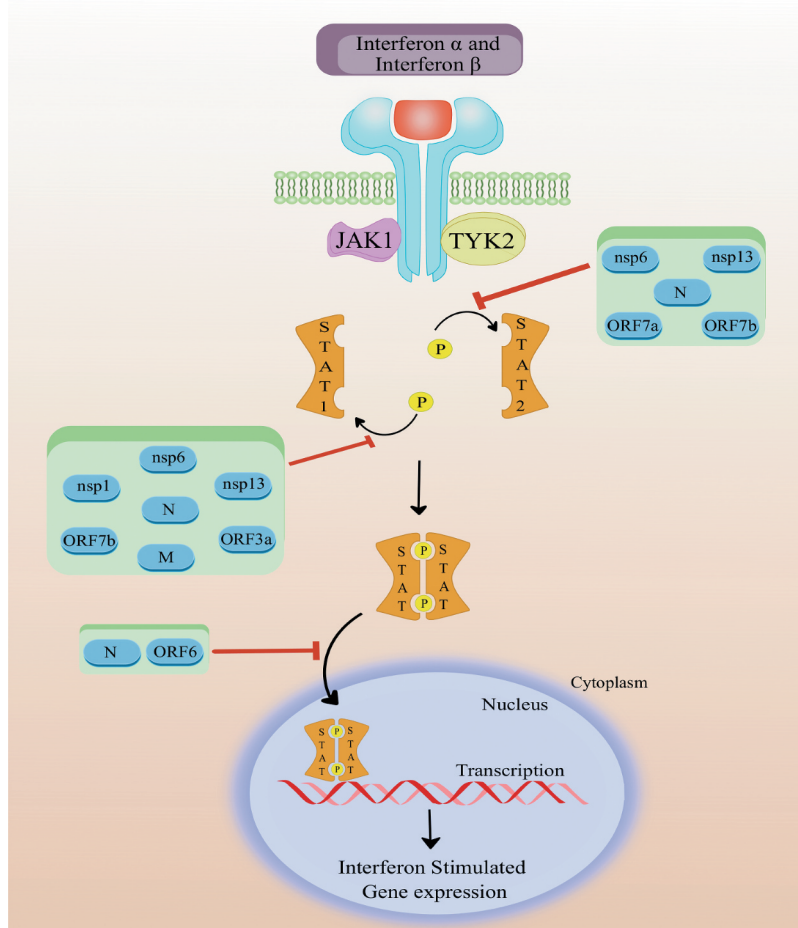
blood vessel endothelial cells. They are also expressed on monocyte and macrophages [36]. Differentiated enterocytes, lymph nodes, and spleen macrophages like CD68+ and CD169 + also express ACE2 receptors [7]. In the CNS, ACE2 is expressed in the glial cells and cell bodies of neurons [64,65]. Another manifestation of SARS-CoV-2 infection is the development of diarrhea in infected patients that suggest the expression of these receptors in the gastrointestinal (GI) system [66].

The genome comprises of 10 Open Reading Frames (ORFs), out of which ORF1a/b encodes for polyprotein 1a (pp1a), polyprotein 1b (pp1b), and 16 nonstructural proteins (nsp). The residual ORFs encode for structural proteins like Spike, Nucleocapsid, Envelope, and Membrane protein abbreviated as S, N, E, and M [67,68]. The pp1a and pp1b are transformed into two proteases namely 3CL<sup>PRO</sup> (3 chymotrypsin-like proteases) and PL<sup>PRO</sup> (papain-like protease). The PL<sup>PRO</sup> facilitates cleaving of nsp1, nsp2, and nsp3 while 3CL<sup>PRO</sup> is responsible for the processing of nsp4- nsp16 [69]. The nsps especially play a crucial role in the development of the replicase complex that is required for genome transcription and virus replication [70].

Among the several nsps' of SARS-CoV-2, the nsp1, nsp3, nsp 6, nsp13, inhibits STAT1 dependent expression of ISGs by suppressing the STAT1 phosphorylation and reducing

inflammatory conditions. Amongst the ORF, ORF3a and ORF 7b inhibit STAT1. The nsp6 and nsp13 also inhibit STAT2 phosphorylation along with ORF 7a and ORF 7b [71–73]. It is also reported that ORF 6 could inhibit nuclear transportation of STAT1 via binding to karyopherin - $\alpha$ 2 [73]. The SARS-CoV-2 3CL<sup>PRO</sup> enzymatically causes a reduction in STAT1 protein levels and STAT1 induced IFN phosphorylation and hampers STAT1 nuclear translocation. It is also suggested that 3CL<sup>PRO</sup> could promote autophagic degradation of STAT1. In conditions where 3CL<sup>PRO</sup> was seen to be expressed in high amounts, the viral replication was enhanced due to the reduction in IFN and ISG signaling pathways [74]. The PL<sup>PRO</sup> also houses de-ubiquitination and de- ISGylation properties via the JAK-STAT pathway. Specifically, it is known to cleave STAT2 and affect the interferon signaling [31,75]. Among the structural proteins of SARS-CoV-2, the N protein has also been shown to inhibit the type 1 IFN pathway mediated inflammatory cascade by preventing the phosphorylation and nuclear passage of STAT 1 and STAT 2 as shown in Figure 3 [76].

Besides hyper inflammation, coagulopathy also contributes to the severity of the condition. Thus, modulation of monocyte and endothelial cell activation via Oxidized Phospholipids (OxPLs) produced on oxidative stress were studied to avert thrombotic complications in those with



**Figure 3.** Interaction of SARS-CoV-2 proteins with JAK/STAT signaling.

cardiovascular and other metabolic comorbidities [77]. From the studies conducted, various strategies that block ACE2 receptor; TMPRSS2 inhibitors like Camostat mesylate; anti-IFN- $\gamma$  drugs like Emapalumab that target STAT1; JAK1 and JAK2 inhibitors like Baricitinib, Ruxolitinib that inhibit ACE2 mediated endocytosis; monoclonal antibody targeting S protein; PARPs intervening antiviral ADP-ribosylation can aid in the treatment of this disease [47,60,78].

## 5. Modulating JAK/STAT signaling pathway in COVID-19

Various regulatory agencies now uphold JAK inhibitors (JAKinibs) for the treatment of immune-mediated diseases [79]. Aberrant activation of STAT3 is a characteristic feature of abnormal cellular differentiation and proliferation [80]. The FERM and SH<sub>2</sub> like domains of JAKs regulate the kinase activity, whereas gene transcription is modulated by STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6 [81]. The inhibitory mechanism of the SOCS (Suppressors of Cytokine Signalling) protein family or autoinhibition of JH1-JH2 interactions are few driving techniques in controlling the anomalous activation of the pathway [81,82]. SOCS proteins induced downregulation of the pathway is either by the direct disruption of JAK phosphorylation or by its proteasomal degradation [83]. Even so, a negative feedback loop is formed where transcription of SOCS1 to SOCS7 and Cytokine Inducible SH2 containing protein (CIS) is upregulated by the STATs [82]. Moreover, STAT1 stimulation causes hyperactivation of pro-inflammatory molecules like COX2 promoting immune suppression, cancer persistence, and drug resistance to doxorubicin, cisplatin, docetaxel [84]. Studies suggest that phosphorylation of Activation loop (AL) of JH1 can intramolecularly regulate JAK Tyrosine Kinase activity [82]. Another mechanism of regulation includes the dephosphorylation of activated JAKs and STATs by multiple Protein Tyrosine Phosphatase (PTP) including SHP1; SHP2 (both activator and suppressor) and TC-PTP for STAT1, STAT3, STAT5; PTPRT for STAT3; CD45 for JAKs [82,83]. Intermolecular regulation is mostly involved in the termination of signaling after cytokine stimulation [82]. Also, termination of the pathway is initiated by various inhibitory residues causing dissociation of JH2 from a receptor, strengthening JH1-JH2 inhibitory interactions, production of inhibitor proteins, and removal of signaling complexes through internalization. Upon the evidence, inhibition of activated STAT dimers by Protein Inhibitors of Activated STATs (PIAS) namely PIAS1, PIAS2 (PIASx), PIAS3, PIAS4 (PIASy) also are crucial in modulating JAK/STAT signaling [82,83].

## 6. JAK/STAT inhibitors as therapeutic agents against COVID-19

The role of cytokines in COVID-19 has been a contributor to the worsening of the disease and inhibiting these cytokines to reduce inflammation has been a strategy to keep the disease under check. The cytokines via the cytokine receptors are known to activate the JAK/STAT cascade [85]. Evidence states that activation of the JAK/STAT pathway mainly via cytokines like IL-6 is correlated with subsequent

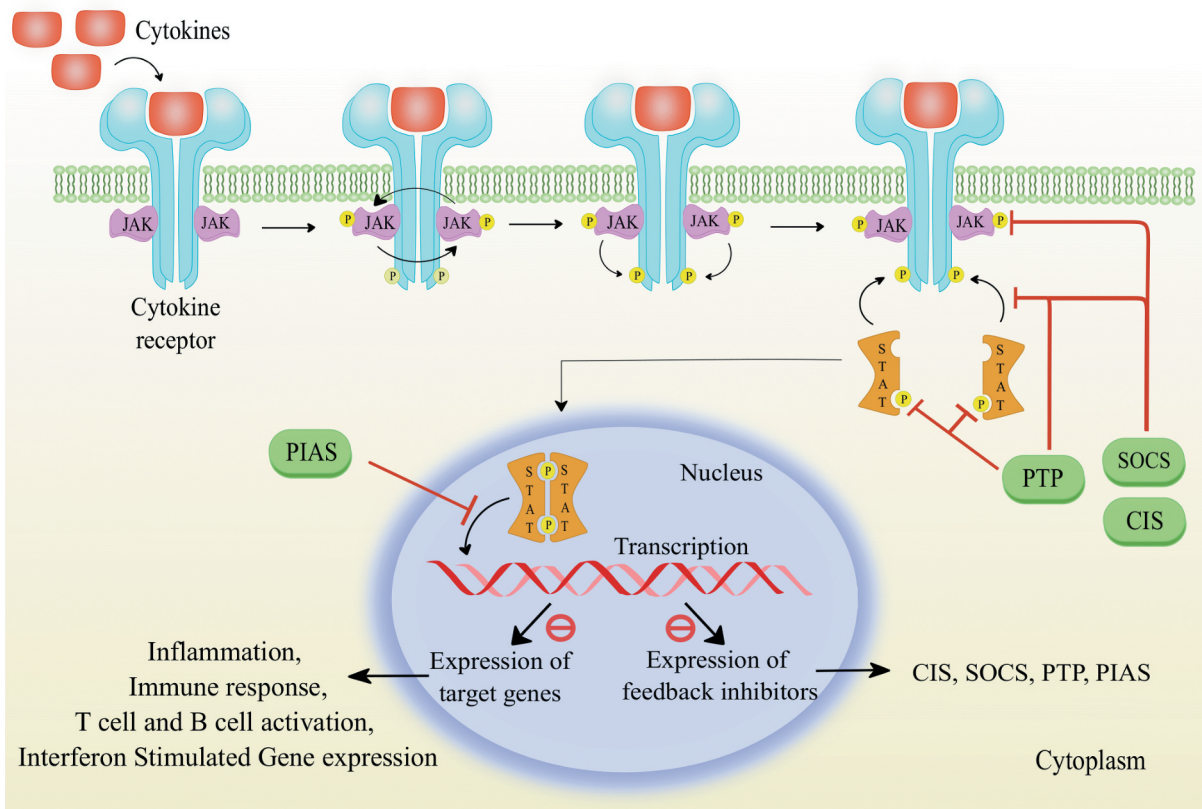
aggravation of COVID-19 [86]. Therefore, a significant focus has shifted towards the development of JAK inhibitors as a therapeutic aid in COVID-19. Interestingly, TH 17 signaling has shown to lead towards cytokine storm in COVID-19 patients causing damage to tissues and pulmonary edema [87]. IL-6 and IL-23 signaling facilitated by STAT-3 is essential in maintaining cell differentiation of Th17 cells and its effector functions [88]. In turn, the IL-6 and IL-23 activate STAT-3 via JAK-2 and thus JAK2 inhibitors could prevent the inflammatory cascade [89]. Similarly, SOCS include members namely SOCS1- SOCS7 and CIS. They inhibit JAK/STAT signals either by JAK kinase activity inhibition or the prevention of STAT binding to JAK [90]. The ability of SOCS to inhibit JAK kinase is supported by the kinase inhibitory region (KIR) in SOCS [83]. Within the SOCS family, SOCS1 is regarded as the most important and highly potent inhibitor of JAK1 and JAK2. This could be possibly due to the use of both KIR and SH2 binding domains by SOCS1 in inhibiting JAK kinase [91]. Along with SOCS, another set of specific proteins namely PIAS also negatively regulates the JAK/STAT pathway. The PIAS family mainly contains PIAS<sub>1</sub>, PIAS<sub>x</sub>, PIAS<sub>β</sub>, PIAS<sub>3</sub>, PIAS<sub>y</sub> [92]. They could prevent the transcription by hindering the transcription factor's DNA-binding process, enhancing co-regulators like histone deacetylases to repress transcription, and promoting the transcription factor's SUMOylation process where SUMO refers to Small Ubiquitin-like Modifier [93]. The PTPs are also involved in inhibiting the JAK/STAT pathways. Their extensive family includes protein encoded by genes namely Protein Tyrosine Phosphatase Non-Receptor (PTPN) Type 1, PTPN Type 2 (PTPN2), PTPN Type 6 (PTPN6), PTPN Type 11 (PTPN11), Dual Specificity Phosphatase 3 (DUSP3), and Protein Tyrosine Phosphatase Receptor Type C (PTPRC) [93]. They are mainly known to dephosphorylate JAK/STAT proteins. The PTPs like Src homology region 2 domain-containing phosphatase-2 (SHP-2) also known as PTPN11 mainly dephosphorylate nuclear STAT1 and cytoplasmic STAT5. The T cell PTP (TC-PTP) dephosphorylates STAT1 and STAT3 [94]. The TC-PTP is cytosolic phosphatases that also inhibits the JAK/STAT signaling through the dephosphorylation of JAK/STAT proteins [95]. Similarly, the Protein Tyrosine Phosphatase 1B (PTP1B) formed from the PTPN1 gene promotes STAT1 and STAT3 dephosphorylation thus affecting the JAK/STAT pathway [96].

Thus, the role of SOCS, CIS, PIAS, and PTP is crucial in regulating the JAK/STAT pathway as depicted in Figure 4.

Therefore, even though it appears simple that the JAK/STAT signaling is influenced by a wide variety of proteins, the exact mechanism of action occurring at molecular levels is still foggy to draw concrete conclusions of the same. Having said that, employing the evidence of enormous current knowledge about these JAK/STAT inhibitors further detailed investigations are necessary.

### 6.1 Ruxolitinib

Ruxolitinib, an orally bioavailable JAK inhibitor with European Union approval is preferred to treat primary myelofibrosis (PMF) that hampers normal blood cell formation capabilities, post essential thrombocythemia myelofibrosis (PET-MF) or



**Figure 4.** The effect of inhibitory proteins on the functioning of the JAK/STAT pathway.

post polycythemia vera myelofibrosis (PPV-MF) [97]. It inhibits JAK 1 and JAK 2 by terminating the kinase activity, thereby preventing STAT activation and nuclear translocation. Ruxolitinib induced inhibition of the IL-6/JAK/STAT3 pathway could cause a significant reduction of IL-6 levels [98]. It can overcome complications due to immune hyperactivation by the JAK/STAT signal transduction pathway and potentially knockdown the hyper inflammation reducing the lung impairment and restoring the PaO<sub>2</sub>/FiO<sub>2</sub> ratio in COVID-19 patients [99].

Ruxolitinib is used to treat severe COVID-19 disease associated with ARDS by inhibition of cytokine signaling [100]. Numerous clinical trials are underway to evaluate the role of Ruxolitinib in COVID-19 patients.

A multi-centered phase II clinical trial (NCT04348071) employing Ruxolitinib in COVID-19 patients characterized by CSS was proved to be safe and efficacious in the intervention and prevented multi-organ failure. Similarly, Incyte and Novartis have initiated a phase III trial for Ruxolitinib (NCT04362137), evaluating the effectiveness of Ruxolitinib in severe COVID-19 patients having respiratory disorders. Table 1 enlists the registered clinical trials employing Ruxolitinib to treat COVID-19 patients.

## 6.2 Baricitinib

Baricitinib is a kinase inhibitor that competes with ATP (adenosine triphosphate) to effectively and reversibly inhibit both

JAK1 and JAK2 with high selectivity hindering the pro-inflammatory signals of several cytokines like IL-6, IL-12, IL-23, etc., intracellularly [101]. Recently a pilot study done by Cantini et.al showed that Baricitinib possesses good safety and efficacy profiles in COVID-19 patients showing no side effects [102].

Baricitinib is evident to be the safer approach in patients with moderate COVID-19 inhibiting release of cytokines as an anti-inflammatory agent and endocytosis of SARS-CoV-2 virions [103]. It also plays a vital role in blocking proteins of the host cell responsible for the viral reproduction, and being an anti-inflammatory agent, also reduces the inflammation in ARDS patients as well [101].

Recently, phase III clinical trials were initiated by Eli Lilly employing Baricitinib (NCT04421027) to analyze its safety and effectiveness. It is a randomized interventional trial with nearly 600 participants, possessing at least one elevated inflammatory marker but without the requirement of medical ventilation. Lilly expects a reduction of cytokine storms associated with the virus through Baricitinib treatments. A regime including Baricitinib 4 mg daily with background therapy or placebo with background therapy was received by the patients for up to 14 days.

Likewise, several on-going studies are to be progressed with Baricitinib. A report by Bronte et.al stated that it aids to amend the immune abnormalities in COVID-19 hospitalized patients [104]. Another phase III clinical trial evaluates the role of Baricitinib + Remdesivir combination (NCT04401579) for the treatment of COVID-19. It is an interventional randomized study aimed to primarily assess the recovery time of



COVID-19 patients taking a combination of Baricitinib + Remdesivir as compared to patients taking only Remdesivir.

### 6.3 Tofacitinib

Tofacitinib is frequently indicated in rheumatoid arthritis (RA), colitis, and spondylitis owing to its anti-rheumatic effects [105]. It inhibits mainly JAK1 and JAK 3 and to a lesser range the JAK2 and TYK 2. The inhibition of JAK, especially JAK1 and JAK3 blocks the signaling of multiple interleukins as shown in Figure 4, thus reducing inflammatory cascade [106].

With such an effective role as a JAK inhibitor, clinical trial investigations on Tofacitinib are now targeted for COVID-19. Although clinical trials are undergoing in different parts of the world, the studies are bounded in contrast to other JAK inhibitors. Nevertheless, it is currently undergoing phase II trials through Investigator-Initiated Studies (IIS) in which everyone enrolled in the trial has received experimental therapy. Pfizer mainly intended on how safe and efficacious Tofacitinib was, in COVID-19 patients (NCT04469114), that were hospitalized on account of pneumonia and received standard of care therapy. It is designed as a multi-centric study, that is randomized and double-blinded, having a placebo-control employing 260 participants.

A study developed by Yale University focuses on the safety and even the efficacy potential of Tofacitinib for moderate COVID-19 patients (NCT04415151). It is also a randomized study, with double-blinding and placebo-control at the phase II stage with 60 participants. It is carried out in COVID-19 patients having pneumonia, requiring supplemental

oxygen and serological markers of inflammation but not mechanical ventilation. This study primarily establishes whether tofacitinib improves the clinical outcomes in patients with moderate SARS-CoV-2 infection.

Therefore, a summary of these inhibitors used in COVID-19 having actions on their respective cytokine receptors is shown in Figure 5.

### 6.4 Other drugs modulating the JAK-STAT pathway in COVID-19

Other multiple ways to modulate the functioning of the JAK-STAT signaling also have paved the way out. One such method is using oligodeoxynucleotide (ODN) decoys that inhibit the DNA binding domains and inhibit gene expression [107]. Anti-sense oligonucleotides have also been used to interact with STAT3 signaling in cancer cells [108]. Like the above mentioned JAK inhibitors, Fedratinib, an FDA approved JAK2 inhibitor also has shown potential in providing relief to COVID-19 patients. Through the IL-6 and IL-23 signaling, STAT 3 facilitates the differentiation of T Helper 17 (TH17) cells. Both these interleukins promote STAT3 activation via JAK2 and therefore the JAK2 inhibitors could prevent the inflammatory actions of TH17 cells thus reducing inflammation in COVID-19 patients [87]. While the JAK inhibition may hamper the activity of the inflammatory signaling in the host, combination therapies of JAK inhibitors along with antivirals like remdesivir/lopinavir/ritonavir could be helpful in controlling inflammation in COVID-19 cases. This could prevent unwanted inflammatory consequences in the host

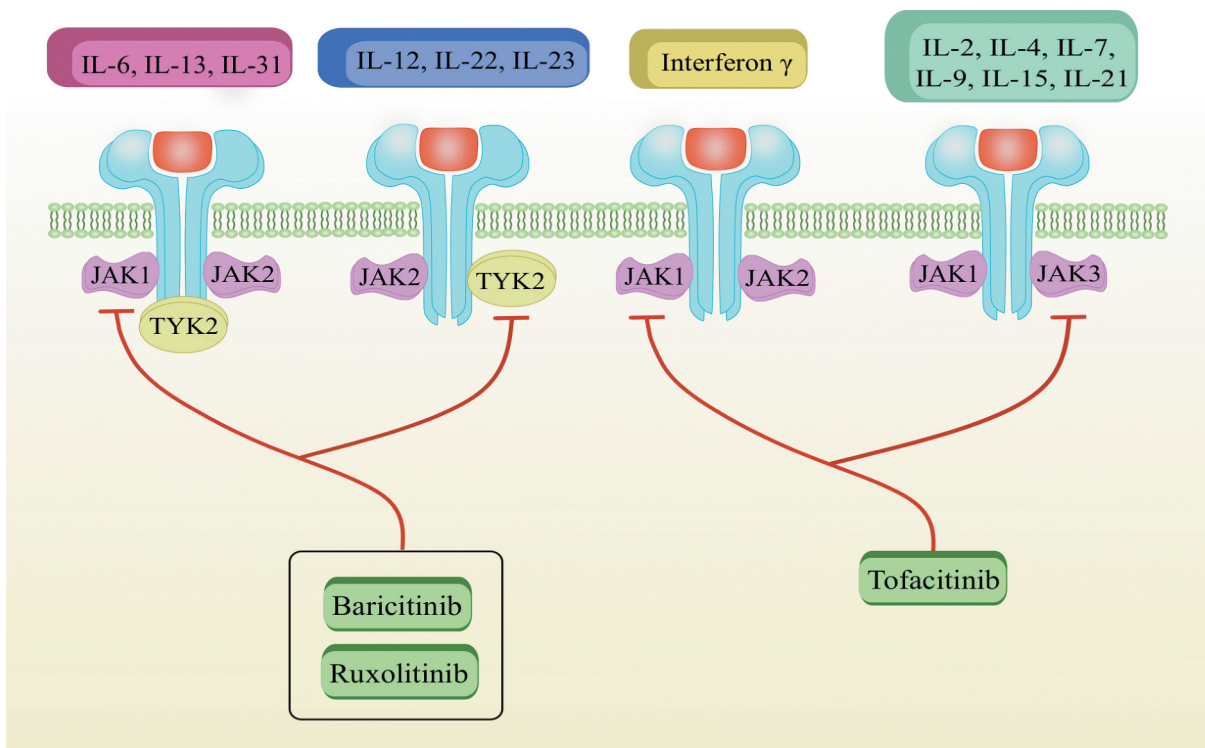


Figure 5. Some of the JAK/STAT inhibitors used in COVID-19.

and provide an effective way to reduce viral infectivity and spread [1]. Interestingly the JAK-STAT pathway is also activated by the IL-6 [3]. The IL-6 is produced by the binding of Angiotensin II to the type 1 Angiotensin II receptor (AT1 receptor) through JAK/STAT initiation [109]. The SARS-CoV-2 S protein serves as a down regulator of ACE2 ultimately causing overexpression of angiotensin II thus in-turn increasing IL-6 production.

Therefore it could be concluded that drugs like tocilizumab and Sarilumab that inhibit IL-6 could prove effective in the management of inflammation in COVID-19 patients.

## 7. Possible side effects of drugs altering the JAK-STAT signaling in COVID-19

The use of Ruxolitinib has shown side-effects like purpuric lesions on the skin of limbs and erythrodermic rashes in 2 SARS-CoV-2 patients. Anemia was also diagnosed in many studies and trials, increase the risk for opportunistic infections [110]. Whereas common side effects include myelosuppression, GI toxicity, immunosuppression, bladder pain, dizziness, headache, sore throat, skin rashes, weight gain, flatulence, etc [111].

The side effects like headache, upper respiratory tract infection (UTI), nasopharyngitis are commonly seen in patients with Baricitinib therapy. The long term use of Baricitinib may increase the risks of developing serious infections and thromboembolic events in patients [112]. Consumption of Immunosuppressants concomitantly with Baricitinib was more likely prone to these infections. Other side effects include fungal infections (candidiasis), pneumocystis, bacterial, viral infections, pneumonia, Herpes zoster, UTI, acute histoplasmosis, cryptococcosis, etc [101].

Elevation in Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Deep Vein Thrombosis (DVT), Pulmonary Embolism (PE), Anemia (Hb<8 g/dl), neutropenia, lipid level elevation (increased TC, LDL, HDL), GI perforation, malignancy, and lymphoproliferative disorders were reported [113].

Tofacitinib also exhibited side effects like abdominal pain, acne vulgaris, anemia, angioedema, diarrhea, dehydration, dyspepsia, headache, hepatotoxicity, hyperlipidemia, hepatitis, lymphoma, lymphopenia, nausea, neutropenia, pulmonary embolism, rashes, vomiting, blood clots, GI perforations [113].

## 8. Regulatory authorities' verdict on the usage of JAK Inhibitors against COVID-19

WHO declared the outbreak of the COVID-19 as pandemic on February 11<sup>th</sup>, 2020, which to date has exceeded 34 million confirmed cases with greater than 10 lakhs deaths across the world [114,115]. The International Solidarity trial was launched by WHO to compare agents that could prove effective against COVID-19 and discover effective drugs to slow disease progression or improve survival in COVID-19 patients [116]. US FDA and European Medicine Association have approved JAK inhibitors like Ruxolitinib, Baricitinib, Tofacitinib, Fedratinib, Oclacitinib, Upadacitinib [86]. Four effective strategies established by

FDA in repurposing already approved agents include priority screening of agents, selection of therapies that have attained breakthroughs, accelerating the approval, and applying fast track to selective processes. The Indian Council of Medical Research (ICMR) collaborated with the Australian authorities for expediting attainment and approvals [117]. National Institute of Health (NIH), has conducted various studies on these agents to frame treatment guidelines. Baricitinib, selective JAK1, and JAK2 inhibitor is postulated to inhibit AP2 associated protein kinase 1 (AAK 1), which regulates endocytosis in alveolar type 2 (AT2) epithelial cells in the lungs and has the greatest theoretical antiviral efficacy [118]. National Institute of Allergy and Infectious Diseases (NIAID) has developed a clinical trial undergoing phase III to test the efficacious nature of Baricitinib (NCT04401579) with antiviral Remdesivir in COVID-19 patients in the US with nearly 1034 participants. It is a randomized study, that is double-blinded having placebo-control which evaluates the putative benefit of Baricitinib. An independent Data and Safety Monitoring Board (DSMD) concluded that the study showed positive results with improved recovery time over Remdesivir alone. Besides, another trial justified that Ruxolitinib in addition to standard of care (SOC) treatment mitigated exuberant cytokine storm associated with COVID-19 [100]. To date, no specific drug or vaccine is recommended by WHO to prevent or cure COVID-19, but the efforts in the development of the therapeutic strategies are still ongoing.

## 9. Current status of clinical trials

Currently, a total of 40 studies have been enlisted under clinicaltrials.gov that involve the use of JAK inhibitors like Ruxolitinib, Baricitinib, Tofacitinib, and Pacritinib for COVID-19 either as a single or a combined regimen with other drugs. Out of these 40 studies, 20 trials are related to Ruxolitinib, 15 trials to Baricitinib, 4 trials to Tofacitinib, and 1 trial to Pacritinib. In general, 14 studies have not started recruiting subjects while 21 studies are already recruiting subjects, the subjects for 2 studies are available. Amongst these studies 1 is completed and 2 are withdrawn.

Among 20 studies of Ruxolitinib, 1 study was withdrawn (NCT04354714) as the changes suggested by the FDA could not be made. Out of the 19 studies, two were expanded access type (NCT04337359) and (NCT04355793). The remaining 17 studies included mainly interventional studies and few observational studies with Ruxolitinib as a single regimen and in combination with other drugs as shown in Table 2.

Out of 14 Baricitinib studies, 1 was completed (NCT04358614) and 13 were active. Out of the 13, 10 were of interventional design and 3 were observational. Most of the studies were having Baricitinib as the sole regimen while a few studies employed combinations of Baricitinib with other drugs as mentioned in Table 3.

Out of 5 studies of Tofacitinib, 1 study (NCT04412252) is withdrawn due to other SARS-CoV-2-related research including alternative trials with tofacitinib. Mostly all the studies

Table 2. Summary of active clinical trials employing Ruxolitinib in COVID-19 patients.

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04348071	<ul style="list-style-type: none"> <li>• Single-arm</li> <li>• Open-label</li> <li>• Single site</li> <li>• Interventional</li> <li>• n = 80</li> </ul>	<ul style="list-style-type: none"> <li>• Ruxolitinib</li> <li>Ruxolitinib 10 mg- orally twice daily</li> </ul>	<ul style="list-style-type: none"> <li>• Cumulative events affecting daily living, clinical status, and life-threatening events.</li> </ul>
NCT04377620	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Double-blinded</li> <li>• Placebo-controlled</li> <li>• Multicentered</li> <li>• Interventional</li> <li>• n = 500</li> </ul>	<ul style="list-style-type: none"> <li>• Ruxolitinib</li> <li>Ruxolitinib 5 mg + SOC – BID 12 hours apart orally or enterically</li> <li>Ruxolitinib 15 mg + SOC – BID 12 hours apart orally or enterically</li> </ul>	<ul style="list-style-type: none"> <li>• Participants dead due to non-specific reasons</li> </ul>
NCT04414098	<ul style="list-style-type: none"> <li>• Experimental</li> <li>• Open-label</li> <li>• Prospective</li> <li>• Single centered</li> <li>• Add-on</li> <li>• Interventional</li> <li>• n = 100</li> </ul>	<ul style="list-style-type: none"> <li>• Placebo</li> <li>• Placebo + SOC – BID 12 hours apart orally or enterically.</li> <li>Ruxolitinib- 5 mg/12 hours up to 14 days and taken orally.</li> </ul>	<ul style="list-style-type: none"> <li>• Evaluation of Ruxolitinib efficiency in COVID-19 treatment</li> </ul>
NCT04366232	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Open label</li> <li>• Parallel assignment</li> <li>• Interventional</li> <li>• n = 54</li> </ul>	<ul style="list-style-type: none"> <li>• Anakinra</li> <li>Anakinra- 300 mg, 1/day Intravenous (I.V) route lasting for 5 days and subsequently tapering of dose.</li> <li>• Anakinra + Ruxolitinib</li> <li>Anakinra-300 mg od I.V for 14 days</li> <li>Ruxolitinib-5 mg B.I.D per up to 28 days.</li> </ul>	<ul style="list-style-type: none"> <li>• Measurement of:                             <ul style="list-style-type: none"> <li>• C-Reactive Protein (CRP)</li> <li>• Ferritinemia,</li> <li>• Serum creatinine,</li> <li>• AST/ALT,</li> <li>• Eosinophils</li> </ul> </li> </ul>
NCT04334044	<ul style="list-style-type: none"> <li>• Single group assignment</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 20</li> </ul>	<ul style="list-style-type: none"> <li>• Standard of Care</li> <li>• Ruxolitinib- 5 mg twice a day orally</li> </ul>	<ul style="list-style-type: none"> <li>• Recovery from pneumonia</li> </ul>
NCT04362137	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Double-blind</li> <li>• Placebo-controlled</li> <li>• Multicentered</li> <li>• Interventional</li> <li>• n = 402</li> </ul>	<ul style="list-style-type: none"> <li>• Ruxolitinib</li> <li>Ruxolitinib- twice daily 5 mg (B.I.D) for 14–28 days.</li> <li>• Placebo</li> <li>• Placebo for 14–28 days.</li> <li>• Ruxolitinib</li> <li>Ruxolitinib-10 mg, 2x/day, for 14 days. Following this, 5 mg, 2x/day for 2 days, and then 5 mg, x/day for a day.</li> </ul>	<ul style="list-style-type: none"> <li>• Patients with respiratory failure, require intensive care unit (ICU)</li> <li>• The proportion of dead patients</li> </ul>
NCT04331665	<ul style="list-style-type: none"> <li>• Single arm</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 64</li> </ul>	<ul style="list-style-type: none"> <li>• Placebo for 14–28 days.</li> <li>• Ruxolitinib</li> <li>Ruxolitinib-10 mg, 2x/day, for 14 days. Following this, 5 mg, 2x/day for 2 days, and then 5 mg, x/day for a day.</li> </ul>	<ul style="list-style-type: none"> <li>• The proportion of ill patients with COVID-19 pneumonia</li> <li>• The number of adverse events.</li> </ul>

(Continued)

Table 2. (Continued).

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04361903	<ul style="list-style-type: none"> <li>Cohort</li> <li>Retrospective</li> <li>Monocentric</li> <li>Non-profit</li> <li>Observational</li> <li>n = 13</li> </ul>	<ul style="list-style-type: none"> <li>Ruxolitinib</li> <li>Ruxolitinib- 20 mg 2 times a day in the first 48 hours.</li> </ul>	<ul style="list-style-type: none"> <li>Patients avoiding mechanically assisted ventilation in ARDS occurring in COVID-19 patients.</li> </ul>
NCT04338958	<ul style="list-style-type: none"> <li>Single arm</li> <li>Non-randomized</li> <li>Interventional</li> <li>n = 200</li> </ul>	<ul style="list-style-type: none"> <li>Ruxolitinib</li> <li>Ruxolitinib- 10 mg 2x/day increased to 20 mg 2x/day for 7 days.</li> </ul>	<ul style="list-style-type: none"> <li>Patients obtaining a 25% reduction in hyper-inflammation score on day 7.</li> </ul>
NCT04424056	<ul style="list-style-type: none"> <li>Open-label</li> <li>Randomized</li> <li>Parallel assignment</li> <li>Interventional</li> <li>n = 216</li> </ul>	<ul style="list-style-type: none"> <li>Anakinra and Ruxolitinib</li> <li>Tocilizumab and Ruxolitinib</li> <li>Standard of Care</li> </ul>	<ul style="list-style-type: none"> <li>The number of days without mechanical ventilation at day 28.</li> </ul>
NCT04331665	<ul style="list-style-type: none"> <li>Single-arm</li> <li>Open-label</li> <li>Interventional</li> <li>n = 64</li> </ul>	<ul style="list-style-type: none"> <li>Ruxolitinib</li> <li>Ruxolitinib-10 mg, 2x/day (Day 0–14), then 5 mg, 2x/day for 2 days again 5 mg, but 1x/day, for a day.</li> </ul>	<ul style="list-style-type: none"> <li>Pneumonia struck critically ill COVID-19 patients</li> <li>No. of adverse events</li> </ul>
NCT04374149	<ul style="list-style-type: none"> <li>Non-randomized</li> <li>Sequential assignment</li> <li>Open-label</li> <li>Interventional</li> <li>n = 20</li> </ul>	<ul style="list-style-type: none"> <li>Therapeutic Plasma Exchange (TPE)</li> <li>5 single plasma volume exchanges over 7 days 2/d then 3/d with albumin or FFP replacement if underlying coagulopathy</li> <li>Therapeutic Plasma Exchange (TPE) + Ruxolitinib</li> <li>TPE intervention as stated above + Ruxolitinib 5 mg p.o B.I. D. beginning day before first TPE and continuing b.i.d for 14 days.</li> </ul>	<ul style="list-style-type: none"> <li>Patients with <math>\geq</math> 33% decrease in cytokine load in one-third or more participants</li> </ul>
NCT04359290	<ul style="list-style-type: none"> <li>Single group assignment</li> <li>Open-label</li> <li>Interventional</li> <li>n = 15</li> </ul>	<ul style="list-style-type: none"> <li>Ruxolitinib</li> <li>Ruxolitinib-10 mg 2/d b.i.d (day 1) and increased up to 15 mg 2/d b.i.d (days 2–8) depending on platelet counts and renal function.</li> </ul>	<ul style="list-style-type: none"> <li>Overall survival of COVID-19 patients.</li> </ul>
NCT04477993	<ul style="list-style-type: none"> <li>Randomized</li> <li>Double-blind</li> <li>Parallel assignment</li> <li>Interventional</li> <li>n = 200</li> </ul>	<ul style="list-style-type: none"> <li>Ruxolitinib</li> <li>Ruxolitinib- oral 5 mg, (B.I.D) at days 0–14 days. The dose is reduced if neutrophils go below 500/mm<sup>3</sup> or platelets below 50,000/mm<sup>3</sup>.</li> <li>Placebo</li> <li>Oral placebo for 14 days. B.I.D.</li> </ul>	<ul style="list-style-type: none"> <li>Death, ICU admission, mechanical ventilation at day 14.</li> </ul>

(Continued)

Table 2. (Continued).

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04403243	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Open-label</li> <li>• Parallel assignment</li> <li>• Interventional</li> <li>• n = 70</li> </ul>	<ul style="list-style-type: none"> <li>• Colchicine</li> <li>Colchicine- oral 0.5 mg 2x/day (0–3 days) followed by 0.5 mg 1x/day oral if weight is less than 86 kg or 0.5 mg 2x/day oral if weight is more than 85 kg for up to 7 days.</li> <li>• Ruxolitinib</li> <li>Ruxolitinib- 5 mg 2/d orally for ten days</li> <li>• Secukinumab</li> <li>Secukinumab- 300 mg s.c first dose and 150 mg 2/d s.c for ten days</li> <li>• Standard therapy</li> <li>• Ruxolitinib and Simvastatin</li> <li>Ruxolitinib- Every 12 h, 5 mg orally (0–7 days), later increased to 10 mg/12 h for 14 days.</li> <li>Simvastatin- Orally 40 mg/24 h for 14 days.</li> <li>• Standard of Care</li> <li>• SARS-CoV-2 infected patients</li> <li>Study A: Data on clinical outcomes and features of SARS-CoV-2 infection.</li> <li>Study B: Data on epidemiological surveillance to describe the epidemiology of the SARS-CoV-2.</li> <li>Study C: Data on viral evolution.</li> <li>• Non-SARS-CoV-2 infected patients</li> <li>Study A: Data on clinical outcomes and features of SARS-CoV-2 infection.</li> </ul>	<ul style="list-style-type: none"> <li>• Measurement of respiratory rate, body temperature, ventilation, CRP, D-dimer levels</li> <li>• Cases developing severe respiratory failure</li> <li>• Identification of factors associated with infection, hospitalization, and requirement of ICU treatment.</li> <li>• Duration of hospitalization</li> <li>• Duration of ICU stay</li> <li>• Clearance</li> <li>• Half-life</li> <li>• Volume of distribution</li> <li>• Elimination rate constant</li> <li>• Half-life</li> </ul>
NCT04348695	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Open-label</li> <li>• Parallel assignment</li> <li>• Interventional</li> <li>• n = 94</li> </ul>	<ul style="list-style-type: none"> <li>• Standard of Care</li> <li>• SARS-CoV-2 infected patients</li> <li>Study A: Data on clinical outcomes and features of SARS-CoV-2 infection.</li> <li>Study B: Data on epidemiological surveillance to describe the epidemiology of the SARS-CoV-2.</li> <li>Study C: Data on viral evolution.</li> <li>• Non-SARS-CoV-2 infected patients</li> <li>Study A: Data on clinical outcomes and features of SARS-CoV-2 infection.</li> </ul>	<ul style="list-style-type: none"> <li>• Cases developing severe respiratory failure</li> <li>• Identification of factors associated with infection, hospitalization, and requirement of ICU treatment.</li> <li>• Duration of hospitalization</li> <li>• Duration of ICU stay</li> <li>• Clearance</li> <li>• Half-life</li> <li>• Volume of distribution</li> <li>• Elimination rate constant</li> <li>• Half-life</li> </ul>
NCT04278404	<ul style="list-style-type: none"> <li>• Prospective</li> <li>• Observational</li> <li>• n = 500</li> </ul>	<ul style="list-style-type: none"> <li>• Collection of body fluids of children to compare outcomes with the standard of care</li> </ul>	<ul style="list-style-type: none"> <li>• Clearance</li> <li>• Half-life</li> <li>• Volume of distribution</li> <li>• Elimination rate constant</li> <li>• Half-life</li> </ul>

**Table 3.** Summary of active clinical trials employing Baricitinib in COVID-19 patients.

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04340232	<ul style="list-style-type: none"> <li>Single-arm</li> <li>Open-label</li> <li>Single site</li> <li>Interventional</li> <li>n = 80</li> </ul>	<ul style="list-style-type: none"> <li>Baricitinib</li> <li>Baricitinib 2 mg- once daily orally for 14 days</li> </ul>	<ul style="list-style-type: none"> <li>Cumulative events affecting daily living, clinical status, and life-threatening events.</li> </ul>
NCT04421027	<ul style="list-style-type: none"> <li>Randomized</li> <li>Double-Blind</li> <li>Placebo-Controlled,</li> <li>Parallel assignment</li> <li>Interventional</li> <li>n = 600</li> </ul>	<ul style="list-style-type: none"> <li>Baricitinib</li> <li>Baricitinib 4mg orally</li> <li>Placebo-control</li> </ul>	<ul style="list-style-type: none"> <li>Cases needing: <ul style="list-style-type: none"> <li>Non-invasive ventilation</li> <li>High-Flow Oxygen</li> <li>Invasive Mechanical Ventilation</li> </ul> </li> </ul>
NCT04373044	<ul style="list-style-type: none"> <li>Randomized</li> <li>Parallel Assignment</li> <li>Interventional</li> <li>n = 144</li> </ul>	<ul style="list-style-type: none"> <li>Placebo and Hydroxychloroquine</li> <li>Placebo p.o daily, and standard of care hydroxychloroquine PO TID for 14 days.</li> <li>Baricitinib and Hydroxychloroquine</li> <li>Baricitinib p.o + Hydroxychloroquine p.o for 14 days.</li> </ul>	<ul style="list-style-type: none"> <li>Patients needing invasive mechanical ventilation</li> <li>Dead patients</li> </ul>
NCT04393051	<ul style="list-style-type: none"> <li>Randomized</li> <li>Multicentered</li> <li>Open-label</li> <li>Parallel Assignment</li> <li>Interventional</li> <li>n = 126</li> </ul>	<ul style="list-style-type: none"> <li>Baricitinib</li> <li>Baricitinib- orally 4 mg or 2 mg/day (1–14 days) for 14 days.</li> <li>Standard therapy</li> </ul>	<ul style="list-style-type: none"> <li>The decrease in patients requiring invasive ventilation</li> </ul>
NCT04362943	<ul style="list-style-type: none"> <li>Retrospective</li> <li>Observational</li> <li>n = 576</li> </ul>	<ul style="list-style-type: none"> <li>Baricitinib or Anakinra</li> </ul>	<ul style="list-style-type: none"> <li>Mortality rate.</li> </ul>
NCT04390464	<ul style="list-style-type: none"> <li>Randomized</li> <li>Parallel assignment</li> <li>Open-label</li> <li>Interventional</li> <li>n = 1167</li> </ul>	<ul style="list-style-type: none"> <li>Baricitinib + Standard of care (SOC)</li> <li>Baricitinib- orally 4 mg 1/day for 14 days with SOC.</li> <li>Ravulizumab + SOC</li> <li>Ravulizumab on day 1 as per patient weight with SOC.</li> <li>Standard of care</li> </ul>	<ul style="list-style-type: none"> <li>The number of days taken for death, mechanical ventilation, renal failure.</li> </ul>
NCT04401579	<ul style="list-style-type: none"> <li>Randomized</li> <li>Double-blinded</li> <li>Multicentre</li> <li>Adaptive</li> <li>Interventional</li> <li>n = 1034</li> </ul>	<ul style="list-style-type: none"> <li>Remdesivir + Baricitinib</li> <li>Remdesivir –200 mg I.V (Day 1), following maintenance dose of 100 mg 1/d till 10 days and Baricitinib 2 × 2 mg tablets for 14 days via the oral route.</li> <li>Remdesivir + Baricitinib</li> <li>Remdesivir –200 mg I.V (Day 1), following maintenance dose of 100 mg 1/d till 10 days and Baricitinib placebo 2 × 2 mg tablets for 14 days via the oral route.</li> </ul>	<ul style="list-style-type: none"> <li>Time to recovery</li> </ul>
NCT04346147	<ul style="list-style-type: none"> <li>Randomized</li> <li>Open-label</li> <li>Parallel assignment</li> <li>Interventional</li> <li>n = 165</li> </ul>	<ul style="list-style-type: none"> <li>Hydroxychloroquine and Lopinavir/Ritonavir</li> <li>Hydroxychloroquine 200 mg/12 hours and Lopinavir/Ritonavir 200/50 mg/12 hours</li> <li>Hydroxychloroquine and Imatinib</li> <li>Hydroxychloroquine tablet 200 mg/12 hours and Imatinib tablet 400 mg/24 hours.</li> <li>Hydroxychloroquine and Baricitinib</li> <li>A single tablet of Hydroxychloroquine 200 mg/12 hours and Baricitinib 4 mg/24 hours.</li> </ul>	<ul style="list-style-type: none"> <li>Time to clinical improvement</li> </ul>

(Continued)

**Table 3.** (Continued).

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04321993	<ul style="list-style-type: none"> <li>• Non-Randomized</li> <li>• Parallel assignment</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 800</li> </ul>	<ul style="list-style-type: none"> <li>• Baricitinib</li> </ul> <p>Baricitinib- 2 mg orally for 0–10 days.</p>	<ul style="list-style-type: none"> <li>• Clinical status of patients at day 15.</li> </ul>
NCT04320277	<ul style="list-style-type: none"> <li>• Non-Randomized</li> <li>• Cross-over assignment</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 200</li> </ul>	<ul style="list-style-type: none"> <li>• Baricitinib and Lopinavir/Ritonavir</li> </ul> <p>Baricitinib- 4 mg/day orally + ritonavir for 2 weeks.</p>	<ul style="list-style-type: none"> <li>• Percentage of ICU admission in patients and compared to controls.</li> </ul>
NCT04399798	<ul style="list-style-type: none"> <li>• Single group assignment</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 13</li> </ul>	<ul style="list-style-type: none"> <li>• Baricitinib</li> </ul> <p>Baricitinib-4 mg/day for 7 days.</p>	<ul style="list-style-type: none"> <li>• Absence of moderate to severe oxygenation impairment.</li> </ul>
NCT04366206	<ul style="list-style-type: none"> <li>• Prospective</li> <li>• Cohort</li> <li>• Observational</li> <li>• n = 143</li> </ul>	<ul style="list-style-type: none"> <li>• Patients are either subjected to treatment or the identified risk factor.</li> <li>• Patients are not exposed to treatment or risk factor.</li> </ul>	<ul style="list-style-type: none"> <li>• Composite of death and mechanical ventilation.</li> </ul>
NCT04365764	<ul style="list-style-type: none"> <li>• Cross-sectional</li> <li>• Case control</li> <li>• Observational</li> <li>• n = 400</li> </ul>	<ul style="list-style-type: none"> <li>• Patients who are given treatment.</li> <li>• Patients are not given treatment.</li> </ul>	<ul style="list-style-type: none"> <li>• Composite of death and mechanical ventilation.</li> </ul>

**Table 4.** Summary of active clinical trials employing Tofacitinib in COVID-19 patients.

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04415151	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Parallel assignment</li> <li>• Interventional</li> <li>• n = 60</li> </ul>	<ul style="list-style-type: none"> <li>• Tofacitinib</li> </ul> <p>Tofacitinib- orally 10 mg (B.I.D) followed by 5 mg (B.I.D) for 0–14 days.</p>	<ul style="list-style-type: none"> <li>• Improvement in clinical results of COVID-19 cases.</li> </ul>
NCT04469114	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Multicentred</li> <li>• Double-blind</li> <li>• Placebo-controlled</li> <li>• Interventional</li> <li>• n = 260</li> </ul>	<ul style="list-style-type: none"> <li>• Placebo</li> <li>• Tofacitinib</li> </ul> <p>Tofacitinib- 10 mg orally 2x/day for 14 days or up to discharge from hospital</p> <p>Placebo</p> <p>Matching placebo treatment.</p>	<ul style="list-style-type: none"> <li>• Death or respiratory failure at day 28.</li> </ul>
NCT04390061	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Multicentred</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 116</li> </ul>	<ul style="list-style-type: none"> <li>• Tofacitinib + Hydroxychloroquine</li> </ul> <p>Tofacitinib- 10 mg cp 2/day + Hydroxychloroquine-200 mg cp 3/day both for 0–14 days.</p> <ul style="list-style-type: none"> <li>• Hydroxychloroquine</li> </ul> <p>Hydroxychloroquine-200 mg cp 3/day both for 0–14 days.</p>	<ul style="list-style-type: none"> <li>• Patients maintaining PaO<sub>2</sub>/FIO<sub>2</sub> &gt; 150 via mechanical ventilation</li> </ul>
NCT04332042	<ul style="list-style-type: none"> <li>• Prospective cohort study</li> <li>• Single group assignment</li> <li>• Open-label</li> <li>• Interventional</li> <li>• n = 50</li> </ul>	<ul style="list-style-type: none"> <li>• Tofacitinib</li> </ul> <p>Tofacitinib- 10 mg 2/day for 0–14 days.</p>	<ul style="list-style-type: none"> <li>• Patients requiring the use of mechanical ventilation for PaO<sub>2</sub>/FIO<sub>2</sub> &gt; 150</li> </ul>

**Table 5.** Active clinical trial employing Pacritinib in COVID-19 patients.

Clinical Trials Identifier	Study Parameters	Intervention groups	Outcomes
NCT04415151	<ul style="list-style-type: none"> <li>• Randomized</li> <li>• Double-blinded</li> <li>• Placebo-control</li> <li>• Multicentered</li> <li>• Interventional</li> <li>• n = 358</li> </ul>	<ul style="list-style-type: none"> <li>• Pacritinib + SOC</li> <li>• Placebo + Standard of care</li> </ul>	<ul style="list-style-type: none"> <li>• Patients progressing to Intermittent Mandatory Ventilation (IMV) and/or Extracorporeal membrane oxygenation (ECMO)</li> <li>• Death of patients along 28 days post-randomization.</li> </ul>
		Pacritinib-400 mg 1/day (QD) at day1, then 200 mg 2x/day (BID) from Day 2–14 + SOC.  Day1- 4 capsules (QD), Day 2–14- 2 capsules 2x/day (BID) + SOC.	

were interventional and included Tofacitinib as the sole drug in the therapeutic regimen except (NCT04390061) where hydroxychloroquine was compared as shown in Table 4.

The single regimen study of Pacritinib (NCT04404361) is undergoing phase III trials. It is a randomization study that is designed in a double-blinded setup, having a placebo-control, and is carried out at multiple sites. Its main aim is to ascertain the effectiveness and safety of Pacritinib in COVID-19 either having or not having cancer as elaborated in Table 5.

## 10. Conclusion

Inflammation majorly contributes to the worsening of COVID-19. The JAK/STAT pathway is involved in the cytokine and chemokine signaling that regulates inflammation in organisms. This is attributed to the ability of cytokines receptors to phosphorylate JAK and then STAT that ultimately binds to the DNA in the nucleus translating for inflammatory mediators.

With the onset of SARS-CoV-2 infection, its nsp3 facilitates STAT phosphorylation causing the unraveling of the inflammatory cascade leaving markers like CRP, ESR, PCT, SAA, serum ferritin, and NLR that determine disease severity. Thus, it becomes essential to introduce the JAK/STAT inhibitors that act via SOCS, PIAS, and PTP proteins that seem to be operative in counteracting the inflammatory actions of the SARS-CoV-2. Their use has been associated with adverse reactions like GI toxicity, diarrhea, dizziness, nasopharyngitis, elevation in ALT, AST levels, angioedema, pulmonary embolism, yet WHO, NIH, ICMR, NIAID have supported several clinical trials that are in progress to ascertain its safety and efficacy in COVID-19 patients.

The JAK/STAT signaling inhibition looks to be a promising approach to reduce inflammation in COVID-19 patients yet falls short of concrete conclusions due to a lack of understanding of mechanism occurring at molecular levels thus warranting further studies.

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