The role of Janus kinases in the pathogenesis and treatment of covid-19

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is severe pneumonia caused by an enveloped, single-stranded RNA beta coronavirus, belonging to the coronaviridae family. SARS-CoV-2 is due to severe hyperinflammation in response to the coronavirus 2 infection. This results in overproduction of cytokines, chemokines, and growth factors by macrophages, such as interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-10, and tumour necrosis factor-α, which cause lung and multi-organ damage. Covid-19 pneumonia is characterized by diffuse alveolar damage due to direct infection of alveolar type II pneumocytes, pulmonary enema, interstitial infiltrates, microthrombi, and ventilation/perfusion mismatch. Covid-19 is a progressive disease which ultimately results in acute respiratory distress syndrome, respiratory failure, multi-organ failure, and death. The standard of care of Covid-19, includes high-flow nasal oxygen (HFNO), dexamethasone, remdesivir, and mechanical ventilation or extracorporeal membrane oxygenation in severe disease. However, the mortality is exceptionally high even with these therapies. IL-6 plays a key role in orchestrating the hyperinflammation and the cytokine storm, which lead to respiratory failure, and multi-organ failure. Interleukin-6 signalling is via the transmembrane IL-6 receptor-α (mIL-6Rα), and the soluble IL-6Rα. Tocilizumab, and sarilumab are IL-6Rα antagonists, and have been issued an emergency use authorization (EUA) by the FDA. Both biologics are safe, and effective in the treatment of severe Covid-19, particularly in patients requiring HFNO, and mechanical ventilation. Another therapeutic approach to treat Covid-19 is to target the downstream JAK/STAT pathway which plays a critical role in promoting IL-6, and other cytokines in orchestrating acute respiratory distress syndrome. Baricitinib and tofacitinib have been granted EUA by the FDA. Both Janus kinase inhibitors have been shown to significantly decrease odds of mortality, and ICU admission. Additionally, JAK inhibitors significantly increase odds for patients’ discharge within 2 weeks. Tofacitinib has been reported to lead to a lower risk of respiratory failure or death through day 28 than placebo in hospitalized patients with Covid-19. Baricitinib in addition to standard of care, including dexamethasone was associated with reduced mortality in hospitalized adults with Covid-19. Baricitinib is effective in reducing mortality in patients with Covid-19, even in progressive disease stages on mechanical ventilation. Selective JAK inhibitors in addition to usual care are effective in the treatment of hospitalized patients with Covid-19, and in reducing mortality.

Keywords: Covid-19; Cytokine storm; Interleukin-6; Janus kinase; JAKinibs; Baricitinib
Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a life-threatening pneumonia caused by an enveloped, single-stranded RNA beta coronavirus, belonging to the coronaviridae 2B lineage [1]. It originated from Wuhan, Hubei province, China [2], but has spread mercilessly throughout the globe. SARS-CoV-2 waves of pandemics have infected over 160 million people worldwide. The World Health Organization (WHO) has estimated more than 5 535 000 COVID-19 deaths since the beginning of the pandemic in 2020 [3], although this is an underestimate. The coronavirus disease 2019 (Covid-19) pandemics have had severe impact on public health, socio-economical, commercial, and industrial consequences, due to lockdowns in several countries. Approximately 80% of the patients with Covid-19 develop mild illness, usually within 12 days, whereas 15-30% progress to critical disease with acute respiratory distress syndrome (ARDS), respiratory failure, and multi-organ failure (MOF) [4]. About 5% of Covid-19 patients develop hypoxemic respiratory failure requiring invasive mechanical ventilation (IMV) or extracorporeal membrane oxygenation (ECMO) [5]. However, the range of possible mortality due to severe Covid-19 is exceptionally high, and very variable. For example, Richardson et al. [6] have reported a range of 24.5-96.7%; the ICNARC documented a range of 30.5-84.5% [7]; and Grass Elli and colleagues in Lombardy, Italy have reported a range of 25.6-83.6% [8,9]. The mortality rate is particularly high in critically ill patients requiring invasive mechanical ventilation, averaging about 75% [9]. Complications of SARS-CoV-2, such as cardiac failure, pulmonary embolism, stroke, acute renal failure, and MOF contribute to the high mortality. Early institution of the standard of care (SoC) plus IL-6R antagonists, or Janus kinase inhibitors may probably reduce the mortality rate. A comprehensive meta-analysis summarizing data from more than 50 000 patients with COVID-19 receiving IMV reported a mortality rate of 45%, although a large number of the patients were not treated with corticosteroids at the beginning of the pandemic [10]. The immunopathological characteristics of SARS-CoV-2 is hyperinflammation, due to host response to the coronavirus infection. This results in dysregulated overproduction of cytokines, chemokines, and growth factors, principally by monocytes and macrophages (cytokine storm). A myriad of cytokines, such as interleukin-1β (IL-1β), IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, tumour necrosis factor-α (TNF-α), GM-CSF, and interferon-γ (IFN-γ) [11-13]; chemokines, including CCL2, CCL3, CCL5, CXCL8 (IL-8), CXCL9, and CXCL10; proteases; and growth factors are secreted by activated immune, inflammatory, and structural cells during the cytokine storm [14-18]. Infection of alveolar type II pneumocytes, and the systemic effects of pro-inflammatory mediators result in diffuse alveolar damage (DAD), ARDS, respiratory failure, and MOF. Table 1 shows the myriad of inflammatory mediators secreted by inflammatory, and immune cells during severe Covid-19 infection. Overproduction of the key cytokines, in particular IL-1β, IL-6, IL-8, IL-10, and TNF-α, is associated with severe Covid-19 disease, need for IMV or ECMO, and very poor prognosis [18-21]. Noteworthy, high levels of serum IL-6 are associated with fatal severe SARS-CoV-2 pneumonia, and extrapulmonary complications, such as septic
shock, cardiac failure, arrhythmias, acute renal failure, acute liver injury, and thromboembolism [22,23]. Furthermore, high levels of IL-6 are predictive biomarker of severe SARS-CoV-2, poor prognosis, and mortality [13,24-26]. A reference level of IL-6 of 55 pg/ml has been suggested as a more sensitive biomarker than C-reactive protein (CRP), and D-dimers in determining patients who require IMV or ECMO [17,21,24]. Therefore, high serum IL-6 levels may be useful in categorizing patients who are more likely to benefit from targeted IL-6Rα antagonists, or Janus kinase inhibitors.

<table>
<thead>
<tr>
<th>Table 1: Inflammatory cytokines, chemokines, and growth factors secreted during the cytokine storm, and SARS-CoV-2.</th>
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<tbody>
<tr>
<td><strong>Cytokines</strong></td>
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<td>Interleukin-1β (IL-1β)</td>
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<td>IL-1β</td>
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<td>IL-1RA</td>
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<td>Interferon-gamma (IFN-γ)</td>
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<td>Tumor necrosis factor-α (TNF-α)</td>
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<td><strong>Chemokines</strong></td>
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<td>CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10</td>
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<td>Macrophage inflammatory protein-1 (MIP-1α/CCL3)</td>
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<td>Monocyte chemoattractant protein-1 (MCP-1/CCL2)</td>
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<tr>
<td>Interferon gamma-induced protein 10 (IP-10/CXCL10)</td>
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<tr>
<td><strong>Growth factors</strong></td>
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<td>Granulocyte colony-stimulating factor (G-CSF)</td>
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<td>Granulocyte-macrophage colony stimulating factor</td>
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<td>Platelet-derived growth factor (PDGF)</td>
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<td>Hepatocyte growth factor (HGF)</td>
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<td>Vascular endothelial growth factor (VEGF)</td>
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Interleukin-6

Interleukin-6 is a master player cytokine responsible for the cytokine storm in severe Covid-19 [27]. Overproduction of IL-6, and dysregulation of the IL-6 signalling, including the JAK/STAT pathway can result in chronic inflammatory diseases, autoimmune disorders [28], inflammatory bowel disease (IBD) [29], myeloproliferative disorders [30], solid cancers [31], and SARS-CoV-2 [27]. Targeting IL-6, IL-6Rα, and the downstream signalling kinases, such as Janus kinases (JAKs) is a precision therapeutic approach for the treatment of chronic inflammatory diseases, such as rheumatoid arthritis [32]; haematological malignancies [33]; and cytokine release syndromes, including SARS-CoV-2 [34-36]. Currently, there are several monoclonal antibodies (mAbs) which have been granted emergency use (EUA) by the Food and Drug Administration (FDA) for the treatment of Covid-19. They include spike protein entry inhibitors, such as bamlanivimab-etteveimab, casirivimab-imdevimab, and sotrovimab; IL-6R antagonists, including as tocililitamb, and sarilumab; JAK inhibitors, namely baricitinib, and tofacilinib. However, some of the mAbs approved for EUA do not retain activity against mutated variants of SARS-CoV-2, such as the Omicron variant (B.1.1.529). Anti-spike protein mAbs, such as bamlanivimab-etteveimab, and casirivimab-imdevimab have been shown to be less susceptible to the Omicron variant [37-40]. However, sotrovimab has been demonstrated to retain activity against 37 individual key mutations of the Omicron variant, as well as the highly contagious deadly Delta variant (B.1.617.2) [41]. Notwithstanding, spike entry inhibitors are only authorized for the treatment of mild-to-moderate Covid-19, and for post-exposure prophylaxis. There is still unmet need to develop novel versatile biologics, such as JAK inhibitors which have been proven to be effective in several diseases, characterized by hyperinflammation for the treatment of severe Covid-19.
Janus kinases

The discovery of the JAK/STAT signalling pathway has a fascinating and enthralling history [42,43]. The molecular details of JAK/STAT pathway were largely uncovered in a series of ground-breaking studies from the laboratories of James Darnell, George Stark, and Ian Kerr more than 30 years ago [42]. Janus kinases (JAKs) were identified through sequence comparison as a unique class of tyrosine kinases, because they contain both a catalytic domain, and a second kinase-like domain that serves an autoregulatory function of the first kinase. The nickname Janus is a symbolic tribute to the two-faced Roman God of the doorways Janus [42-45], and the name was colloquially shortened to JAK by Andrew Wilks from Australia in 1989 [46]. Janus kinases are a family of intracellular non-receptor tyrosine kinases that play an essential role in the signalling of more than 60 cytokines, type I and II interferons, growth factors, and hormones. JAK signalling via the signal transducer and activators of transcription (STAT) plays important roles in cellular biology, cell growth, differentiation, survival, apoptosis, immunity; and many other physiological functions. However, mutations in JAKs, and dysregulation of the master JAK/STATs signaling pathways contribute to the pathogenesis of chronic inflammatory and autoimmune diseases, viral syndromes, including HIV/AIDS, Ebola, and Covid-19; and cancer [47,48]. JAKs consists of four members, namely JAK1, JAK2, JAK3, ad Tyrosine kinase 2 (TYK2) [49-54], whereas the signal transducer and activators of transcription family comprises of seven members: STAT1, STAT2, STAT3, STAT4, STAT4a, STAT5b, and STAT6 [53,55]. STATs primarily function as transcription factors in the nucleus of cells [56]. The JAK-STAT pathway is one of the most important downstream signalling pathways of cytokine receptors. Following binding of a ligand to its cognate receptor, receptor-associated JAKs are activated. This is followed by activation of STATs by tyrosine phosphorylation by Janus kinases, and dimerization of STATs. Activated STATs subsequently translocate into the nucleus, where they modulate transcription of target genes [56]. Janus kinases are primitive proteins which have been identified in the primeval chordata Ciona and Drosophila [57]. They are relatively large proteins comprising of more than 1,100 amino acids, and molecular masses of 120-140 kDa [58]. Seven distinct JAK homology domains (JH) have been identified (JH1 to JH7), and these form the putative structural domains of the JAK family members [59]. They include the kinase domain (JH1), the inhibitory pseudokinase domain (JH2), the Src homology (SH2) receptor interaction domain (JH2-JH5), and the Band 4.1, ezrin, radixin, myosin (FERM domain (JH6-JH7). The catalytically active kinase domain (JH1) is located at the carboxyl-terminus, and at its amino-terminal site, it is directly followed by the enzymatically inactive pseudokinase domain [60]. JAK1, JAK2, and TYK2 are expressed ubiquitously in mammals, and JAK3 is primarily expressed in haematopoietic cells [58,61]. JAK2 is the most ubiquitous kinase, and it is activated by two third of the ligands, whereas JAK1 and TYK2 although ubiquitously expressed have limited signaling pathways in comparison to JAK2 [62].

Janus kinase 1 is expressed in several tissues, and cells, and can phosphorylate and signal through all the six STATS [63]. It is phosphorylated by four cytokine families namely: (1) cytokines with receptors consisting of the common gamma-chain (γc), such as IL-2, IL-4, IL-7, IL-9, and IL-15 receptors; (2) cytokines with receptors with the glycoprotein 130 (gp130) co-receptor, including the IL-6 family members, IL-6, IL-11, oncostatin M, ciliary neutrophilic factor, leukemia inhibitory factor, and cardiotropin 1; (3) class II cytokines, such as IFN-α, IFN-β, IFN-γ, and IL-10 family cytokine receptors [64]; (4) cytokines whose receptors are homodimers, such as growth hormone, prolactin, erythropoietin (EPO) and thrombopoietin (TPO) [65]. JAK1 mutation is associated with lethal neurological defects [66]. JAK2 can be phosphorylated by members of the
gp130 family, the class II cytokine receptor family, and the IL-3 receptor family (IL-3R, IL-5R, and GM-CSF receptor) [66]. JAK2 also associates with the erythropoietin receptor (EPOR), and the thrombopoietin receptor (TPOR) [67]. Mutations in JAK2 JH2 domain is linked with haematological malignancies [68]. JAK2 deficiency results in embryonic lethality at day 12.5 [69]. JAK3 is involved in signal transduction of the IL-2R, IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R, these receptors are γc receptors with the γ chain [70]. JAK3 deficiency is associated with autosomal recessive severe combined immunodeficiency (SCID) [71,72]. TYK2 was the first discovered member of the JAKs family, and was capable of transducing signals from IFN-α, and IFN-β. It is also involved in signaling of IL-6 [73], IL-10 [74], IL-13 [75], and IL-23 [76]. There are myriads of cytokines, chemokines, growth factors, and hormones which signal via the JAK/STAT pathways, which enable the Janus kinases to regulate several physiological functions. However, dysregulation of the JAK/STAT signaling may be associated with innumerable diseases, haematological disorders, cancer, and weird lethal syndromes [77-80]. Consequently, pan-inhibition of JAKs may be associated with serious adverse events, such as immunodeficiency, opportunistic infections, thromboembolism [81], and malignancy.

Jak-stat signaling

The first step in the JAK/STAT signaling is initiated when a specific cytokine binds to the surface of its target transmembrane receptor, which causes receptor dimerization. The receptors contain intracellular domains which are constitutively associated with members of JAKs family of tyrosine kinases [59,82-84]. In the resting state, JAKs are inactive. They are activated by transphosphorylation when the cytokine binds to its receptor [85]. Activated JAKs phosphorylate the intracellular tails of the receptor on specific tyrosines, which in turn act as docking sites for the Src Homology 2 (SH2) domain of the STAT proteins [86,87]. The receptor-localized STATs on the tyrosine docking sites are phosphorylated by JAKs [88], which cause them to dissociate from the receptor [89], dimerize and translocate into the nucleus in an import α-5 dependent manner via Ran nuclear import pathway. In the nucleus STATs bind to specific DNA sequences either to activate or suppress transcription of effector genes, which are responsible for the production of cytokines, chemokines, and growth factors [86,90,91]. The JAK/STAT signaling is tightly regulated, and is switched off by a number of proteins that attenuate cytokine signaling at multiple levels of the pathway. They include the suppressors of cytokine signaling (SOCS) family which are negative feedback inhibitors of the cytokine signaling [92,93]. SOCS proteins in conjunction with STAT-dependent operons switch off the signaling [94]. The JAK-STAT pathway can also be regulated by the protein inhibitors of activated STATs (PIASs), and protein tyrosine phosphatases (PTPs) [93]. In principle, each cytokine binds to a specific receptor, this induces activation of specific JAK(s), and STAT(s). Several cytokine receptors play an important role in the pathogenesis of autoimmune, and chronic inflammatory diseases through dysregulation of the JAK-STAT pathway, especially T cell mediated diseases, and cancer progression [95-97]. Therefore, targeting and inhibiting the JAK-STAT pathway with monoclonal antibodies is an attractive opportunity for precision treatment of autoimmune diseases [98,99], cancer [95,96], and SARS-CoV-2. Currently, there are several biologics (mAbs), targeting the JAK-STAT axis that are in clinical trials, although only few JAK inhibitors (JAKinibs) are approved or authorized by the FDA for the treatment of various diseases, including Covid-19. Interleukin-6 signals via JAK1/3-STAT3, and to a lesser extent through JAK3/STAT3. Currently, there are three biologics which have been approved or granted an EUA by the FDA for the treatment of chronic inflammatory, and autoimmune diseases, which inhibits downstream IL-6/JAKs signaling (IL-6/JAK axis), such as ruxolitinib [100], baricitinib [101], and tofacitinib [102].
orally administered JAKinibs have now been repurposed for the treatment of SARS-CoV-2, and fabulously seem to be effective, and are safe and well tolerated by patients.

**TREATMENT OF SARS-cov-2**

Treatment of SARS-CoV-2 is challenging because there are no specific effective antiviral agents for the treatment of the disease, and arrest progression to ARDS, and hypoxemic respiratory failure. Artificial intelligence (AI) technologies have permitted repurposing of some antivirals and mAbs which are effective in the treatment of chronic inflammatory, autoimmune diseases, and other viral syndromes, such as AIDS, and Ebola for the treatment of SARS-CoV-2. Notably, some of the antivirals, and biologies have been able to shorten the duration of the severe illness, and reduce mortality. The repurposed biotherapeutic agents have resulted in early hospital discharge, prevention of invasive mechanical ventilation, and reduction in mortality in hospitalized patients with Covid-19.

**Non-invasive ventilation**

In patients with severe Covid-19 requiring increasing oxygen supplementation, non-invasive mechanical ventilation (NIV), and continuous positive airway pressure (CPAP) provide potentially attractive strategies for preventing intubation, and mechanical ventilation [103], and in reducing mortality. Management of SARS-CoV-2 include proper nursing care in a prone position, which has been documented to improve oxygen saturation (SaO2), and partial pressure of arterial oxygen (PaO2) [104,105]. High-flow nasal oxygenation via a nasal cannula is the most recommended initial treatment of severe SARS-CoV-2. This can be delivered through high-flow nasal cannula up to 60 L/min of nearly 100% oxygen [106]. The recommended target SaO2 is 92-96% in adults with severe Covid-19, using supplemental oxygen as needed [107]. HFNO decreases the requirement of endotracheal intubation, and IMV in patients with ARDS. It is effective and safe in mild-to-moderate Covid-19, and even in some patients with moderate-to-severe SARS-CoV-2 (108). The use of HFNO is supported by several studies, and reviews which have documented its beneficial outcomes [109,110]. The high-flow Nasal Cannula in Severe COVID-19 with Acute Hypoxemic Respiratory Failure (HiFlo-Covid) trial [109] conducted in three hospitals in Columbia, South America, compared HFNO with conventional oxygen therapy in 220 adult patients with Covid-19. The study showed that HFNO reduced both the need for tracheal intubation (hazard ratio, 0.62; 95% confidence interval (CI), 0.39-0.96), and shortened the clinical recovery time [109]. The HENIVOT clinical trial compared two modes of non-invasive respiratory strategies, such as NIV via a helmet (with pressure support) versus HFNO [110]. In a total of 110 patient with COVID-19 recruited from four intensive care units, there was no significant difference for the primary outcome of days free of respiratory support between the two groups. However, fewer patients in the helmet NIV group required tracheal intubation compared to the HFNO group (odds ratio, 0.41; 95% CI, 0.18-0.98) [110]. The results from the two studies indicate that O2 is vital with or without pressure support in acutely hypoxemic patients with SARS-CoV-2 [111,112]. In patients with more severe hypoxemia due to pulmonary oedema, and atelectasis, the use of continuous positive airway pressure is recommended to increase the total lung capacity (TLC) by recruitment of collapsed lung units. Provision of low positive end-expiratory pressure (PEEP) of 2-5 cm H2O which does not induce alveolar over-distension, and barotrauma is required for the effective delivery of oxygen, and in improving the PaO2 [113,114]. Conventionally, CPAP is delivered at pressure levels between 5 and 15 cm H2O [113,114]. CPAP has been shown to reduce the need for intubation, and invasive mechanical ventilation, thus reducing the high mortality rate associated with IMV. A large randomized clinical trial consisting of 1273 patients has
shown that in acute hypoxic respiratory failure and Covid-19, an initial strategy of CPAP significantly reduced tracheal intubation or mortality compared with conventional oxygen therapy (36.3% versus 44.4%, \( P = 0.03 \)) [103]. The decrease in the incidence of the primary outcome with CPAP was attributable to a significant decrease in the need for tracheal intubation, and invasive mechanical ventilation [103]. Tracheal intubation and IMV are associated with high mortality in Covid-19 patients in the intensive care units (ICU) [6-9].

**Invasive mechanical ventilation**

Critically ill patients with Covid-19 may require intubation and invasive mechanical ventilation. However, the decision to place patients with Covid-19 on a ventilator is not clear, and neither are the outcomes in terms of mortality [115]. Moreover, placing patients in the intensive care units on IMV may be influences by socio-economic factors, availability of ICU facilities, and Physicians skilled in ICU management, and clinical practices. Dr. Henry Lassen in 1952 during the great polio epidemic “claimed that American had put their patients in the respirators far too early – certainly they would not have been ventilated in Copenhagen. It’s no wonder they survived, he claimed because they didn’t need treatment in the first place’” [116]. However, despite IMV, the mortality rate is high, and very variable depending on the medical centres. Richardson et al. [6] in New York City, USA, have reported a mortality rate of 24.5%-96.7%, mean 88.9%, whereas Grusselle and colleagues in Lombardy, Italy have documented a mortality rate of 25.6-83.8% [8]. A meta-analysis summarizing data from more than 50 000 patients with COVID-19 receiving IMV reported a mortality of only 45%, although a large number of these patients were not treated with corticosteroids at the beginning of the pandemic [10]. Still more, about 5% of the patients with severe Covid-19 require extracorporeal membrane oxygenation [117]. However, some reports on the use of ECMO have been associated with prohibitively high mortality rates [118,119]. Recently, Barbaro et al. [120] have shown that in 2020, in 4812 patients with COVID-19 who received ECMO across 349 centres from 41 countries, the mortality rate ranged from 51.9% in centres which started ECMO before May 1, 2020 (group A), and 58.9% in centres which started ECMO after May 1, 2020 (group B). Ramanathan et al. [121] in a meta-analysis of 24 observational studies with 1896 patients reported a pooled in-hospital mortality rate of 35.7% in patients who received mostly venovenous ECMO. Despite ECMO, the mortality rate in severe Covid-19 remains alarmingly high.

**Standard of care**

Most guidelines recommend treatment of severe Covid-19 with low-to-moderate dose dexamethasone plus remdesivir or lopinavir-ritonavir (Kaletra). Early use of corticosteroids has been life-saving in patients with severe SARS-CoV-2 requiring HFNO and IMV or ECMO. The beneficial effects of glucocorticoids are additive to antivirals, anti-spike monoclonal antibody, IL-6Ra antagonists, and Janus kinase inhibitors therapies.

**Corticosteroids**

Corticosteroids are the cornerstone in the management of Covid-19. Several clinical trials have demonstrated the efficacy of corticosteroid in improving the clinical outcomes, and in reducing mortality in critically ill patients with Covid-19 [122-125]. The RECOVERY trial has showed that moderate dose of dexamethasone (6 mg for 10 days) reduced mortality in hospitalized patients with Covid-19 and respiratory failure, who required therapy with supplemental oxygen or IMV [125]. Furthermore, a systemic review and meta-analysis comprising of 44 credited studies, and 20, 197 patients, has confirmed the beneficial effects of corticosteroids on short-term mortality, and in reducing the need for mechanical ventilation [126].
formulation of corticosteroids at equivalent dose to dexamethasone 6 mg, such as prednisone 40 mg, methylprednisolone 32 mg, and hydrocortisone 132 mg, are equally effective in reducing the mortality rate in hospitalized patients with severe SARS-CoV-2 [122-124]. Some inhaled corticosteroids, including caledonite have been shown to impair the replication of SARS-CoV-2 [127], and downregulate expression of receptors for the entry of the virus into host cells [128,129]. However, systemic corticosteroids are associated with serious adverse events, such as delayed viral clearance, and opportunistic bacterial infections [130-132], particularly when co-administered with IL-6Rα antagonists, such as tocilizumab, or JAK inhibitors, including tofacitinib [132].

Remdesivir

Remdesivir (Veklury) is an analogy inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp), which is essential for viral replication. It is an adenosine nucleotide prodrug that is metabolized to the pharmacologically active nucleoside triphosphate (RdRp)e metabolite after distribution into cells [133,134]. Remdesivir was developed by Gilead Science for the treatment of Ebolaviruses in 2016 [135]. It has been demonstrated to have in vitro activity against SARS-CoV-2 [136], and has been repositioned and repurposed for the treatment of Covid-19. Remdesivir in combination with corticosteroid is very effective in the treatment of severe SARS-CoV-2. It is effective in shortening the period to recovery, and in reducing the need for IMV or ECMO, and mortality [137-139]. Remdesivir is also effective in combination with tocilizumab [140], and baricitinib [141] in the treatment of hospitalized patients with Covid-19The positive results from randomized clinical trials on the efficacy and safety of intravenous remdesivir [142-144], resulted in the intravenous issue an EUA on May 1, 2020, to permit the use of remdesivir for treatment of COVID-19 in adult and paediatric patients (aged ≥12 years and weighing ≥40 kg), with suspected or laboratory confirmed Covid-19 [145]. Remdesivir has also received full or conditional approval in several countries. It is administered via an intravenous injection with a loading dose on day 1 (200 mg in adults, adjusted for body weight in paediatric patients) followed by a daily dose maintenance dose (100 mg in adults) for up to 10 days. Remdesivir should be administered early in the course of Covid-19 before the novel virus destroys the alveolar air sacs, and the alveolar-capillary membranes required for the diffusion of HFNO. Moreover, alveolar type II pneumocytes secrete surfactant which keeps the alveoli dry, and maintains lung compliance, preventing atelectasis, and ARDS which are common in Covid-19. Treatment with remdesivir monotherapy does not reduce the requirement for IMV, and mortality substantially. The National Institutes of Health COVID-19 Treatment Guidelines Panel, and the IDSA recommend that remdesivir be administered with anti-inflammatory agents, such as corticosteroids, or immunotherapeutic agents, including IL-6Rα antagonists, or JAK inhibitors [146-148].

Interleukin-6 antagonists

Tocilizumab (Actemra) is a recombinant humanized monoclonal antibody that inhibits binding of IL-6 to its transmembrane, and soluble IL-6 receptors, thus blocking the downstream JAK1/SATAT3 signaling pathway. This results in inhibition of overproduction of several pro-inflammatory cytokines, including IL-6, responsible for hyperinflammation, acute lung injury, and ARDS in patients with severe Covid-19. Several observational and retrospective studies have shown that tocilizumab improves clinical outcomes, and reduces need for IMV, and death in hospitalized patients with severe Covid-19 [149-152]. Similarly, a large randomized trial (REMAP-CAP), which enrolled 803 patients, demonstrated that treatment with tocilizumab improved survival in patients with SARS-CoV-2 [153].
The RECOVERY clinical trial in the United Kingdom evaluates the efficacy and safety of tocilizumab in 4116 hospitalized patients with severe Covid-19. About 3385 (82%) of the patients were receiving corticosteroids [154]. In patients with hypoxia, and systemic inflammation, tocilizumab improved the clinical outcomes, and survival regardless of the amount of respiratory support [154]. Patients who received tocilizumab were more likely to be discharged from hospital within 28 days compared to those who received usual care (57% versus 50%; rate ratio 1.22; 1.12-1.33; P < 0.0001). In a sub-group of patients not receiving mechanical ventilation at baseline, patients allocated tocilizumab were less likely to reach the composite endpoint of mechanical ventilation or death (35% versus 42%; risk ratio 0.84; 95% CI 0.77-0.92; P < 0.0001). The mortality rate in 2022 patients allocated tocilizumab was 31%, and in 2094 patients who received the usual care was 35% within 28 days (rate ratio 0.85; 95CI 0.76-0.94; P = 0.0028) [154]. Actemra was granted an EUA by the FDA for the treatment of adults and paediatric patients (2 years of age or older) who are receiving systemic corticosteroids, and requiring NIV or IMV, or ECMO [155]. The National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel recommends add-on tocilizumab 8 mg/kg (up to 800 mg) administered as a single intravenous (IV) dose. It is recommended that tocilizumab be administered in combination with corticosteroids or remdesivir, or a spike entry inhibitor, such as bamlanivimab-etesevimab, casirivimab-imdevimab, and sotrovimab. However, co-administration of tocilizumab with another IL-6Ra antagonist, such as sarilumab, or with JAK inhibitors, including baricitinib, and tofacitinib is associated with increased risk of opportunistic infections, and helminths infestation [156]. Table 2 shows antivirals, and monoclonal antibodies approved for EUA, and in clinical trials for the treatment of SARS-CoV-2.

### Janus kinase inhibitors

Janus kinase inhibitors (JAKinibs) are monoclonal antibody, which inhibit signaling of type I and II cytokines, thus preventing their downstream immunopathological effects. They have proven efficacy and safety in the treatment of chronic inflammatory diseases [157], such as rheumatoid arthritis [158], psoriasis [159], and myeloproliferative disorders, [160], including polycythaemia vera [161]. Additionally, several Janus kinase inhibitors are in clinical trials for the treatment of several diseases, such as alopecia areata, atopic dermatitis [162], inflammatory bowel disease [163], haematological malignancies [164], and various cancers [165]. The deadly Covid-19 pandemics, and none availability of specific antiviral agents for the treatment of the catastrophic disease, has resulted in repositioning and repurposing second-generation JAKinibs for the treatment of SARS-CoV-2 [166]. Janus kinase inhibitors are effective in controlling the cytokine storm [167], due to high levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α, in patients with severe Covid-19 [166]. JAKinibs act by inhibiting one or more Janus kinase proteins. Multiple cytokines implicated in the pathogenesis of Covid-19, including IL-2, IL-6, IL-10, and IL-17 signals through the JAK-STAT pathway, and are potentially inhibited by Janus kinase inhibitors [168]. Noteworthy, angiotensin II (Ang II) mediates its pathophysiological effects via the JAK-STAT signaling pathway, resulting in vasoconstriction, hypertension, and chronic tissue injury [169]. Thus, inhibiting IL-6, Ang II, and JAK-STAT signaling may be very effective in dampening hyperinflammation, and in the treatment of severe SARS-CoV-2. The cytokines implicated in the cytokine storm, and SARS-CoV-2, including IL-6 utilizes different combinations of JAKs and STATs. Typically, beneficial cytokines recruit JAK1, and JAK3, whereas the pathogenic cytokines utilize JAK2. IL-6 signals via JAK1 and JAK3, and to a lesser extent JAK2, with downstream activation of STAT3. This signaling pathway is associated with more immunopathological effects, and is
The role of Janus kinases in the pathogenesis and treatment of Covid-19

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the most precise target for the treatment of Covid-19 [170]. Pan-inhibition of the IL-6 and JAKs signaling pathway might not result in the expected benefit outcomes [170]. Schiff et al. [171] have suggested targeting JAK2 downstream of IL-6, and GM-CSF to ameliorate hyperinflammation; and sparing JAK1/JAK3 downstream of IL-2, IL-6, IL-21, IFN-1, and IFN-γ involved in viral clearance. Janus kinase inhibitors possess key pharmacological features for a potentially successful repurposing. They have favourable pharmacokinetic profile, and multifunctional pharmacodynamics by exerting dual anti-inflammatory and antiviral effects. JAK inhibit the effects of several pro-inflammatory cytokines, and growth factors, and have antiviral activity by impeding host cellular endocytosis of SARS-CoV-2 (172,173). JAKinibs inhibit the entry of SARS-CoV-2 into type II alveolar pneumocytes (172), thus preventing diffuse alveolar damage, ARDS, and respiratory failure. They are also convenient to administer because they are orally administered, and have short half-lives. Currently, there are three JAK inhibitors which have been granted emergency use authorization by the Food and Drug Administration, including baricitinib [174], ruxolitinib [175], and tofacitinib [176]. Baricitinib and ruxolitinib predominantly inhibit JAK1 and JAK2 [174,175], whereas, tofacitinib inhibits JAK1 and JAK3 [176]. Table 3 shows the approved Janus kinase inhibitors, and in clinical trials for the treatment of Covid-19, and other diseases.

Table 2: Monoclonal antibodies in clinical trials for the treatment of SARS-CoV-2.

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Target</th>
<th>Dosage</th>
<th>FDA status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra 2021</td>
<td>IL-1α, IL-1β</td>
<td>200 mg, 100 mg Q6h</td>
<td>EUA</td>
</tr>
<tr>
<td>Canakinumab 2020</td>
<td>IL-1β</td>
<td>459-750 mg infusion</td>
<td>Phase 3</td>
</tr>
<tr>
<td>Tocilizumab 2020</td>
<td>IL-6R</td>
<td>8 mg/kg (Max 800 mg) EUA 2021</td>
<td></td>
</tr>
<tr>
<td>Sarilumab 2020</td>
<td>IL-6R</td>
<td>400 mg in 100 ml saline Phase 2</td>
<td></td>
</tr>
<tr>
<td>Baricitinib 2020</td>
<td>JAK1, JAK2</td>
<td>1-4 mg PO OD x 14 days EUA</td>
<td></td>
</tr>
<tr>
<td>Tofacitinib 2021</td>
<td>JAK1, JAK3</td>
<td>10 mg PO BD x 14 days EUA 2021</td>
<td></td>
</tr>
<tr>
<td>Ruxolitinib 2021</td>
<td>JAK1, JAK2</td>
<td>5 mg PO BD Phase 3</td>
<td></td>
</tr>
<tr>
<td>Bamlanivimab 2021</td>
<td>Spike protein</td>
<td>700 mg IV single dose EUA</td>
<td></td>
</tr>
<tr>
<td>Etesevimab 2021</td>
<td>Spike protein</td>
<td>1.4 g IV single dose EUA</td>
<td></td>
</tr>
<tr>
<td>Casirivimab 2021</td>
<td>Spike protein</td>
<td>600 mg IV OD EUA</td>
<td></td>
</tr>
<tr>
<td>Imdevimab 2021</td>
<td>Spike protein</td>
<td>600 mg IV OD EUA</td>
<td></td>
</tr>
<tr>
<td>Sotrovimab 2021</td>
<td>SARS-CoV-1/2 epitope 500 mg IV infusion</td>
<td>EUA 2021</td>
<td></td>
</tr>
<tr>
<td>Tixagevimab 2021</td>
<td>SARS-CoV-2 epitopes 273 ng/ml or 147 ng/ml</td>
<td>EUA 2021</td>
<td></td>
</tr>
<tr>
<td>Cilgavimab 2021</td>
<td>SARS-CoV-2 epitopes 273 ng/ml or 147 ng/ml EUA 2021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mavrilimumab 2/3</td>
<td>GM-CSFRα 6 mg/kg IV infusion</td>
<td>Phase</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BD, twice daily; EUA, Emergency Use Authorization; FDA, Food and Drug Administration; GM-CSF, Granulocyte-macrophage colony stimulating factor; IV, Intravenous; JAK, Janus kinase; OD, once daily. Spike protein inhibitor and epitope inhibitors are given in combination for Covid-19 prophylactic treatment, e.g., tixagevimab co-packed with cilgavimab (273 ng/ml of 147 ng/ml).
Table 3. Janus kinases inhibitors in clinical trials for the treatment of Covid-19, and other diseases

<table>
<thead>
<tr>
<th>Biologic</th>
<th>JAK/ JTK Target</th>
<th>Disease Approved/clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baricitinib</td>
<td>JAK1, JAK2</td>
<td>Rheumatoid arthritis (RA), PA, PV, AD</td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>JAK1, JAK3, JAK2</td>
<td>RA, psoriatic arthritis, PV, IBD</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>JAK1, JAK2</td>
<td>Myelofibrosis, polycythemia vera, GVHD</td>
</tr>
<tr>
<td>Fedratinib</td>
<td>JAK2</td>
<td>Myelofibrosis</td>
</tr>
<tr>
<td>Filgotinib</td>
<td>JAK1</td>
<td>Rheumatoid arthritis, IBD</td>
</tr>
<tr>
<td>Momelotinib</td>
<td>JAK1, JAK2</td>
<td>Myelofibrosis, ovarian cancer</td>
</tr>
<tr>
<td>Pacritinib</td>
<td>JAK2</td>
<td>Myelofibrosis, AML, colorectal cancer</td>
</tr>
<tr>
<td>Peficitinib</td>
<td>JAK1, JAK2, JAK3</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Olcalitinib</td>
<td>JAK1, JAK2</td>
<td>Leukaemia</td>
</tr>
<tr>
<td>Gandotinib</td>
<td>JAK2</td>
<td>Myeloproliferative disorders</td>
</tr>
<tr>
<td>Upadacitinib</td>
<td>JAK1, JAK3</td>
<td>Rheumatoid arthritis, IBD</td>
</tr>
<tr>
<td>Solicitinib</td>
<td>JAK1</td>
<td>Psoriasis</td>
</tr>
</tbody>
</table>

Abbreviations: AD, atopic dermatitis; AML, acute myeloid leukemia; IBD, inflammatory bowel disease; JKA, Janus kinase; PA, psoriasis arthritis; RA, rheumatoid arthritis. All of the above JAKinibs are in various phases of development for the treatment of Covid-19.

Table 4: Janus kinases inhibitors in clinical trials for the treatment of Covid-19, and other diseases.

<table>
<thead>
<tr>
<th>Biologic</th>
<th>JAK/ JTK Target</th>
<th>Disease Approved/clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baricitinib</td>
<td>JAK1, JAK2</td>
<td>Rheumatoid arthritis (RA), PA, PV, AD</td>
</tr>
<tr>
<td>Tofacitinib</td>
<td>JAK1, JAK3, JAK2</td>
<td>RA, psoriatic arthritis, PV, IBD</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>JAK1, JAK2</td>
<td>Myelofibrosis, polycythemia vera, GVHD</td>
</tr>
<tr>
<td>Fedratinib</td>
<td>JAK2</td>
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</tr>
<tr>
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<td>JAK1</td>
<td>Rheumatoid arthritis, IBD</td>
</tr>
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<td>JAK2</td>
<td>Myelofibrosis, AML, colorectal cancer</td>
</tr>
<tr>
<td>Peficitinib</td>
<td>JAK1, JAK2, JAK3</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Olcalitinib</td>
<td>JAK1, JAK2</td>
<td>Leukaemia</td>
</tr>
<tr>
<td>Gandotinib</td>
<td>JAK2</td>
<td>Myeloproliferative disorders</td>
</tr>
<tr>
<td>Upadacitinib</td>
<td>JAK1, JAK3</td>
<td>Rheumatoid arthritis, IBD</td>
</tr>
<tr>
<td>Solicitinib</td>
<td>JAK1</td>
<td>Psoriasis</td>
</tr>
</tbody>
</table>

Abbreviations: AD, atopic dermatitis; AML, acute myeloid leukemia; IBD, inflammatory bowel disease; GVHD, graft versus host disease; JKA, Janus kinase; PA, psoriasis arthritis; RA, rheumatoid arthritis. All of the above JAKinibs are in various phases of development for the treatment of Covid-19.

Ruxolitinib

Ruxolitinib (INCB018424; Jakavi®; Incyte Corporation) is a potent and selective inhibitor of JAK1, and JAK2, with modest to marked selectivity against tyrosine kinase 2 (TYK2), and JAK3, respectively. It inhibits JAK1/2 kinases activities, thus preventing phosphorylation, and activation of STAT3, and STAT3 translocation into the nucleus [177]. Currently, ruxolitinib is approved in the European Union (EU), and the USA for the treatment of primary myelofibrosis (PMF), polycythemia vera (PV), post-essential thrombocythemia myelofibrosis, and acute graft-versus host disease [178]. It is very successful in the treatment of myeloproliferative disorders, which are characterized by hyperinflammation due to overproduction of cytokines, such as IL-1β, IL-6, IL-8, IL-12, TNF-α, INF-γ, G-CSF, GM-CSF; and growth factors including TGFβ, FGF,
PDGF, and VEGF [179]. Ruxolitinib is a potential Janus kinase inhibitor because it interferes with the JAK/STAT signaling pathway of some of the cytokines, and growth factors implicated in the pathogenesis of SARS-CoV-2. Ruxolitinib inhibits the IL-6/JAK/STAT3 pathway, thus reducing circulating levels and activity of IL-6 the key cytokine in the cytokine storm [180]. Additionally, ruxolitinib may act through antiviral activity by impairing replication through interference with senescence regulatory pathways [181]. Thus, Jakavi® has the potential to reduce hyperinflammation, and Covid-19 associated severe acute lung injury, hypoxemia, ARDS, and respiratory failure [179]. Few small randomized controlled trials have documented beneficial and safety of ruxolitinib in hospitalized patients with Covid-19 [182,183]. Cao et al. [183] have shown that treatment with ruxolitinib in 22 out of 43 patients with Covid-19 resulted in a faster recovery time, and significant improvement in chest computed tomographs compared with placebo. In another multicentre matched study conducted in Moscow, Russia in 146 hospitalized patients (NCT0433359, CINC424A2001M), it was reported that treatment with ruxolitinib was associated with case-fatality rate similar to dexamethasone treatment (14.6% versus 13.0%; P = 0.35) [184]. The median time of discharge without oxygen support requirement was also not different between the ruxolitinib group and the placebo arm (13 versus 11 days). A subgroup analysis, however, revealed a reduced case-fatality rate in ruxolitinib-treated patients with a high fever (≥38.5 °C) [184]. The Phase 3 RUXCOVID clinical trial [NCT04362137] was a randomized, double-blind, placebo-controlled study that enrolled 211 patients 12 years and older to assess the efficacy and safety of ruxolitinib in severe Covid-19 [185]. Patients were randomized to receive either ruxolitinib 5 mg twice daily plus standard of care (SoC), or ruxolitinib 15 mg twice daily plus SoC or placebo plus SoC. Initial data showed that there was no reduction in the proportion of patients receiving ruxolitinib plus SoC who experienced severe complication, such as respiratory failure requiring mechanical ventilation or admission to ICU, or death by Day 29, compared to SoC treatment (12% versus 11.8% [OR: 0.91 {95% CI: 0.48-1.73, P = 0.769}]. In addition, there was no clinically relevant benefit observed among secondary and exploratory endpoints, including mortality rate by Day 29, and the time of recovery. Ruxolitinib was generally well tolerated and had no significant adverse events [185]. Despite not achieving the primary endpoint in the overall population, Incyte (Nasdaq: NCY), the producer and sponsor of the study, said that statistically significant improvement in mortality was achieved in the USA population (N = 191; 91%) [186]. In the American patients, the 5 mg treatment group had a 46.7% mortality rate versus 69.1% for placebo (95% CI: 0.57-0.948, P = 0.0189), whereas the 15 mg group had a 47.1% mortality rate versus 66.7% for placebo group (95% CI: 0.188-0.974, P = 0.215). Additionally, a post-hoc analysis of the overall study population that pooled both 5 mg and 15 mg ruxolitinib groups together versus placebo, showed a statistically significant improvement in mortality of 53.6% versus 70.7% (95% CI: 0.219-0.996, P = 0.0244) [186]. Phase 3 clinical trial results have been disappointing for ruxolitinib, a JAK inhibitor which has a noble successful record in the treatment of myeloproliferative disorders. However, ruxolitinib should be used with caution because of the likelihood of the development of Polyomavirus (JC-Virus and BK-Virus) related fatal encephalopathy, and meningitis during treatment [187,188]. Ruxolitinib has also been associated with reactivation of Cytomegalovirus (CMV), Varicella-Zoster virus (VZV), and Epstein-Barr virus (EBV); and the development of gastric ulcer, and meningoencephalitis [189,190]. Reactivation of viral infections, such as EBV is linked with secondary diseases, such as lymphoproliferative disorders (191).

**Tofacitinib**

Tofacitinib (Xeljanz®) preferentially inhibits JAK1 and JAK3, and to a lesser extent JAK2,
and TYK2 [192]. Tofacitinib inhibits the common gamma-chain (γc) subunit, and the glycoprotein130 (gp130) co-receptor of cytokines which signals via the JAK/STAT pathway in T cells. Thus, it interferes with helper type 1 (Th1), and Th2 lymphocyte differentiation, and impairs production of Th17 inflammatory cells. Tofacitinib, therefore, suppresses the production of cytokines which signals using the γc chain, such as IL-6, IL-11, IL-12, TNF-α, IFN-γ; and Th17-dependent cytokines, including IL-17, IL-17F, IL-25, and IL-23 [193-195]. Additionally, tofacitinib decreases proliferation of Th17 helper T cells, which produce IL-17 and IL-17F, family member cytokines implicated in the cytokine storm. Tofacitinib has been shown to inhibit production of interferon in vitro [196-198], this could promote secondary viral and bacterial infections and further complicate Covid-19 course [132]. Therefore, is should be used with caution when co-administered with other immunosuppressive therapies, such as corticosteroids, and JAK inhibitors.

Tocilizumab is effective in the treatment of chronic inflammatory diseases, and it is approved by the FDA for the treatment rheumatoid arthritis, polyarticular course juvenile idiopathic arthritis [199,200], psoriatic arthritis [200], psoriasis [200,201], and IBD [200,202]. Tofacitinib inhibits pro-inflammatory IL-6/JAK1/STAT3 signaling pathway which is important in the progression of severe pneumonia, respiratory failure, and MOF in patients with SARS-CoV-2. Tofacitinib has been shown to significantly reduce the risk of respiratory failure or death in hospitalized patients with Covid-19. It has also been shown to be safe and well tolerated by the patients [203,204]. In a single-centre retrospective observational study of 269 patients, with 138 (51.3%) who received tofacitinib, Hayek et al. [203] found that adding tofacitinib to dexamethasone seemed to have potential benefit of improving survival when compared with dexamethasone alone [203]. Treatment with tofacitinib resulted in 70% reduced odds of dying compared to dexamethasone treatment alone (AOR: 0.03; 85% CI: 0.21-0.76; P = 0.01). Guimaraes et al. [204] randomized 289 patients (1:1) from 15 hospitals across São Paulo, Brazil, of whom 89.3% were receiving corticosteroids. Half of the patients received tofacitinib 10 mg twice daily orally for 14 days, and the other half received placebo. The cumulative incidence of respiratory failure or death through day 28 was 18.1% in the tofacitinib group, and 29.0% in the placebo arm (risk ratio, 0.63%; 95% confidence interval (CI), 0.41-0.97; P = 0.04) [204]. There were fewer deaths in the tofacitinib group (2.8%) compared to (5.5%) in the placebo group (hazard ratio, 0.49; 95% CI, 0.15-1.63). Tofacitinib was safe and well tolerated. Serious side effects occurred in 20 patients (14.1%) in the tofacitinib group, and in 17 (12.0%) in the placebo arm [204]. The incidence of serious infection was 3.5% in the tofacitinib group, and 4.2% in the placebo group. These results demonstrate that tofacitinib is effective and safe in hospitalized patients with Covid-19, particularly when co-administered with HFNO, corticosteroids, and remdesivir.

**Baricitinib**

Baricitinib (Olumiant) is a JAK1/JAK2 inhibitor with moderate activity against TYK2, and minimal activity against JAK3 (205,206). It suppresses the IL-6/JAK/STAT signaling pathway, and production of pro-inflammatory cytokine, such as IL-1β, IL-1Ra, IL-4, IL-6, IL-10, IL-17, IL-13, IL-7, TNF-α, IFN-γ, GM-CSF, FGF, MCP-1, MCP-1β, and IP-10 [207]; and chemokines including CXCL9, CXCL10, and CXCL112 [208] implicated in the cytokine storm, and in the pathogenesis of SARS-CoV-2 [209]. Olumiant if the most studied Janus kinase inhibitor in clinical trials, and has unique anti-inflammatory, and antiviral properties. Entry of SARS-CoV-2 into host cells depends on S1 spike protein on its surface which adopts the angiotensin converting enzyme II (ACE II) as a cognitive receptor to facilitate viral entry into pneumocytes by clathrin-mediated endocytosis. The ACE receptor is regulated by several kinases, such as AP2-associated protein
kinase-1 (AAK1), and cyclin G-associated kinase (GAK) which mediate endocytosis of the SARS-CoV-2/ACE II complex. Baricitinib inhibits the entry, and assembly of SARS-CoV-2 into target cells by disrupting AAK1 signaling. Thus, preventing endocytosis, and replication of SARS-CoV-2 in host cells [210-213]. Baricitinib has been shown to reduce the levels of the cytokines that signals via the JAK/STAT pathway, such as IL-1β, IL-2, IL-6, IL-17, TNF-α, and GM-CSF implicated in the pathogenesis of chronic inflammation, and SARS-CoV-2 [213-215]. Therefore, baricitinib has the strategic potential for the treatment of severe SARS-CoV-2. Furthermore, baricitinib inhibits macrophage activation and production of cytokines and chemokines, critical for inflammation, and neutrophil recruitment [216].

Another advantage of baricitinib over other mAbs is a favourable pharmacokinetic, and pharmacodynamic properties, such as low plasma protein binding affinity, minimal interaction with hepatic cytochrome enzymes, and drug transporters. Therefore, giving baricitinib the potential for combination treatment with direct-acting antivirals, such as remdesivir [216], lopinavir/ritonavir, or novel spike protein entry inhibitors. Olumiant has minimal effects in inducing hepatic cytochrome enzymes, which may interfere with the bioavailability and half-lives of co-administered antivirals, or other biologics. The co-administration of antivirals with baricitinib could reduce viral replication, viral loads, and infectivity, as well as reducing unwanted host inflammatory reaction from the cytokine storm [217]. Baricitinib was approved by the FDA, and the European Medicines Agency (EMA) in 2018 for the treatment of adult patients with rheumatoid arthritis [218]. It is also indicated for the treatment of pruritus and eczema in patients with moderate-to-severe atopic dermatitis (AD) [219]. Baricitinib is being evaluated in several clinical trials for the treatment of autoimmune diseases, such as juvenile idiopathic arthritis, and systemic lupus erythobates [220]; and dermatological disorders, including atopic dermatitis [260], alopecia areata [221]; and inflammatory bowel diseases [222]. The application of bioinformatics tools, and Artificial Intelligence (AI) technologies on baricitinib-treated models have engineered repurposing baricitinib for the treatment of SARS-CoV-2 [223-225]. Several clinical trials have shown the efficacy of baricitinib in dampening the cytokine storm, and in preventing DAD, ARDS, respiratory failure, and death in patients with Covid-19 [225-227]. Bronte et al. [227] have reported a significant reduction of IL-1β, IL-6, and TNF-α plasma levels in patients with Covid-19 treated with baricitinib. Several small uncontrolled, non-randomized clinical trials, observational studies, and systemic meta-analyses have documented the efficacy and safety of baricitinib in the treatment of hospitalized adult patients with Covid-19 [228-232]. In a meta-analysis, baricitinib has been reported to decrease the use of invasive mechanical ventilation, and the risk of death. However, baricitinib had marginal benefit on the rate of admission to the intensive care unit, and in preventing ARDS [232]. Recently, a retrospective study in 2 academic medical centres in Metropolitan Anthens, Greece including 369 patients, has shown that patients treated with baricitinib and SoC had significantly lower incidence of admission to ICU compared with SoC alone (29.7% versus 44.8%; P = 0.03) [233]. Similarly, the mortality rate was significantly lower in the combination treatment compared to the usual care (14.7% versus 26.6%; P = 0.005) [233]. The ACTT-2 (NCT04401579) double-blind, randomized, placebo-controlled trial evaluated baricitinib plus remdesivir in 1033 adults with Covid-19. All the patients received remdesivir (≤10 days) and ether baricitinib 4 mg once daily (≤14 days) or placebo [234]. Patients receiving baricitinib plus remdesivir had 30% higher odds of improvement in clinical status at day 15 (odds ratio, 1.3; 95% CI, 1.0-1.6). Patients receiving HFNO or non-invasive ventilation at enrolment had a time of recovery of 10 days in the 515 patients receiving combination treatment versus 18 days in the control group (rate ratio
for recovery, 1.51; 95% CI, 1.10-2.08). The 28-day mortality was 5.1% in the combination group and 7.8% in the control arm (hazard ratio for death, 0.65; 95% CI, 0.39-1.09) [234]. Serious adverse events were less frequent in the combination group than in the control group (16.0% versus 21.0%; difference, -5.0 percentage points; 95% CI, -9.8 to -0.3; P = 0.003). Surprisingly, there were fewer new infections in the patients who received baricitinib (5.9% versus 11.2%; difference, -5.3 percentage points; 95% CI, -8.7 to -1.9; P = 0.003). The study showed that baricitinib plus remdesivir was superior to remdesivir alone in reducing the recovery time, and in significantly reducing the mortality rate, particularly in patients receiving HFNO or non-invasive ventilation [234]. The COV-BARRIER randomised, double blind, parallel group, placebo-controlled phase 3 trial (NCT04421027) evaluated the effectiveness of baricitinib in 1525 adult hospitalized patients with Covid-10 [235]. Approximately 79% of the patients were receiving systemic corticosteroids, and about 19% were receiving remdesivir. Although the initial results showed that the disease progression was not significantly reduced, treatment with baricitinib plus the standard of care, including dexamethasone, significantly reduced mortality in hospitalized patients with SARS-CoV-2 [235]. Progression to HFNO, non-invasive ventilation or invasive mechanical ventilation, or death occurred in 28% of the patients receiving baricitinib compared to 30% in the placebo group (a nonsignificant between-group difference). However, 28-day mortality was 8% with baricitinib and 13% with placebo, a 38% relative reduction in mortality. The benefit of baricitinib was most evident among patients requiring HFNO or non-invasive ventilation at baseline. The Jaki nib was safe, and the incidence of serious adverse events, such as serious infection, and thromboembolism were similar in both groups [235]. Based largely on the results of the COV-BARRIER trial, the National Institutes of Health, and the Infectious Diseases Society of America (IDSA) recommended baricitinib plus dexamethasone in select patients with severe Covid-19 [236,237]. The feared adverse event of JAK inhibitors is thrombosis and risk of thromboembolism, including deep venous thrombosis, pulmonary embolism, myocardial infarction, and thrombotic or haemorrhagic stroke [238-240]. This may be compounded by the hypercoagulability state due to the thrombotic effects of IL-6, and SARS-CoV-2 [239-243]. Levi et al. [244] recommend vigilance to the potentially increased thrombotic risk with JAKinibs use, given the hypercoagulability of COVID-19. They recommend prophylactic antithrombotic regimens in all hospitalized patients with SARS-CoV-2, because thrombotic risk is a wider problem in Covid-19 [244].

Other serious, speculative complications of treatment with JAKinibs are gastrointestinal perforation [245,246], and malignancies, including lung cancer, breast cancer, pancreatic cancer, and melanoma [247-249]. The US Food and Administration issued an Emergency Use Authorization for the use of baricitinib to treat Covid-19 in hospitalized adults and paediatric patients aged 2 years and older requiring supplemental oxygen, non-invasive mechanical ventilation, or ECMO [250]. Baricitinib is also recommended for the treatment of Covid-19 in several countries. It is administered orally at 4 mg daily, or 2 mg daily in patients with a glomerular filtration rate of less than 60 ml per minute, or in order to minimize the side effects. The dosage is adjusted for weight in children, and adolescents. The triplet of dexamethasone, remdesivir, plus baricitinib seems to be very effective in the treatment of Covid-19, particularly when administered early before intubation, and IMV or ECMO. In the treatment of severe Covid-19, an antiviral agent should be supplemented with an anti-inflammatory agent with broad immunomodulatory activities, such as JAK inhibitors, e.g., baricitinib. Nevertheless, not forgetting high-flow nasal O₂.
Conclusion

Severe acute respiratory syndrome coronavirus 2 is a life-threatening pneumonia caused by an enveloped, single-stranded RNA beta coronavirus belonging to the coronaviridae lineage. Immunopathologically it is characterized by dysregulated overproduction of cytokines, such as IL-1β, IL-6, IL-8, TNF-α; and chemokines, including CCL3, CCL5, CXCL8, CXCL9, and CXCL10. SARS-CoV-2 has predilection to infect alveolar type II pneumocytes, which result in diffuse alveolar damage, and acute respiratory distress syndrome. The inflammatory cytokines and chemokines induced by SARS-CoV-2 infection orchestrate the inflammatory cascade, and acute lung injury, leading to hypoxemic respiratory failure, and multi-organ failure. The standard of care of Covid-19 include high-flow nasal oxygen, corticosteroids, remdesivir, and IMV or ECMO in severe cases. IL-6 receptor antagonists, and spike protein entry inhibitors have been repurposed for the treatment of Covid-19, but have not met the expected clinical outcomes. The mortality rate due to Covid-19 is prohibitively high despite these innovative therapies. Janus kinase inhibitors, such as baricitinib and tofacitinib have dual antiviral, and anti-inflammatory activities. They have been shown to significantly reduce the need for ICU admission, IMV, and mortality in hospitalized patients with SARS-CoV-2. The combination of corticosteroids, remdesivir, and baricitinib is highly effective in the treatment of Covid-19, and is recommended by the National Institutes of Health for the treatment of severe Covid-19.

Conflict of interest

The author declares that the publication was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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