

# The role of interleukin-6 and janus kinases in the pathogenesis, and treatment of SARS-CoV-2

## Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a deadly pneumonia caused by an enveloped, single-stranded RNA betacoronavirus belonging to the coronavirus family. Pathophysiologically, SARS-CoV-2 is due to severe hyperinflammatory host response to the coronavirus, resulting in overproduction of cytokines, chemokines, and growth factors by macrophages, such as interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-2, IL-6, IL-8, IL-10, and tumour necrosis factor- $\alpha$ . SARS-CoV-2 is characterized by diffuse alveolar damage due to direct infection of alveolar type II pneumocytes, pulmonary edema, vascular occlusion, interstitial infiltrates, and ventilation/perfusion mismatch, which rapidly progress to hypoxemia, acute respiratory distress syndrome, 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 very severe cases. However, the mortality is exceptionally high even with these therapies. Covid-19 is due to dysregulation, and over-production of cytokines, including IL-1 $\beta$ , IL-6, IL-10, and TNF- $\alpha$ . IL-6 plays a key role in orchestrating the hyperinflammation and the cytokine storm, which leads to acute lung injury, respiratory failure, and multi-organ failure. Interleukin-6 signaling is via the transmembrane IL-6 receptor- $\alpha$  (mIL-6R $\alpha$ ), and the soluble IL-6R $\alpha$ . Tocilizumab, and sarilumab are IL-6R $\alpha$  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 respiratory support. Another therapeutic approach to treat Covid-19 is to target the downstream JAK/STAT pathway which plays a critical role in inciting IL-6 immunopathological effects. Baricitinib and tofacitinib have been granted EUA by the FDA. A systemic review has shown that JAK-inhibitors significantly decrease odd of mortality ( $P < 0.0005$ ), and ICU admission ( $P < 0.0005$ ). Additionally JAKinibs significantly increase odds for patient discharge within 2 weeks  $P < 0.00001$ . 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. Selective JAK inhibitors in addition to usual care are effective in the treatment of patients with Covid-19.

**Keywords:** Covid-19, cytokine storm, interleukin-6, JAK, baricitinib, tofacitinib

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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a life-threatening pneumonia caused by an enveloped, single-stranded RNA betacoronavirus belonging to the coronavirus family.<sup>1</sup> It initially originated from Wuhan, Hubei province, China.<sup>2</sup> SARS-CoV-2 five waves of pandemics have infected over 160 million people worldwide, and have caused the death of about 4.8 million individuals globally.<sup>3</sup> Covid-19 pandemics have had devastating public health, socio-economical, commercial, and industrial consequences, due to lockdowns in many countries.

Approximately 80% of the patients with coronavirus disease 2019 (Covid-19) develop mild illness, whereas 15-30% progress to critical disease with respiratory failure, and multi-organ failure (MOF).<sup>4</sup> About 5% of Covid-19 patients develop hypoxemic respiratory failure requiring invasive mechanical ventilation (IMV).<sup>5</sup> However, the range of possible mortality due to severe Covid-19 is exceptionally high, and very variable. For example, Richardson et al.<sup>6</sup> reported a range of 24.5-96.7%; the ICNARC documented a range of 30.5-84.5%;<sup>7</sup> and

Grasselli and colleagues in Lombardy, Italy have reported a range of 25.6-83.6%.<sup>8,9</sup> The mortality rate is particularly very high in critically ill patients requiring invasive mechanical ventilation (IMV), about 15%.<sup>6</sup> to 75%.<sup>8,9</sup>

The hallmark of SARS-CoV-2 is hyperinflammatory due to host response to the coronavirus, resulting in overproduction of cytokines, chemokines, and growth factors by macrophages (cytokine storm), such as IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-10, IL-17, IL-18, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), GM-CSF, and interferon- $\gamma$  (IFN- $\gamma$ ).<sup>10-12</sup> Several other cytokines, 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, due to coronavirus 2 systemic infection.<sup>13-15</sup> Furthermore, chemoattractant chemokines recruit, and activate inflammatory cells, such as neutrophils, monocytes, and macrophages, which liberate more cytokines, and chemokines, thus amplifying the inflammatory cascade. Table 1 shows the myriad of inflammatory mediators secreted by inflammatory, and immune cells during the cytokine storm, due to Covid-19 infection.

**Table 1** Inflammatory cytokines, chemokines, and growth factors secreted during the cytokine storm, and SARS-CoV-2

Cytokines	Chemokines
Interleukin-1 $\beta$ (IL-1 $\beta$ )	CCL2, CCL3, CCL5, CXCL8, CXCL9, CXCL10
IL-1 $\beta$	Macrophage inflammatory protein-1 (MIP-1 $\alpha$ /CCL3)
IL-1RA	Monocyte chemoattractant protein-1 (MCP-1/CCL2)
IL-2	Interferon gamma-induced protein 10 (IP-10/CXCL10)
IL-6	<b>Growth factors</b>
IL-8 (CXCL8)	Granulocyte colony-stimulating factor (G-CSF)
IL-9	Granulocyte-macrophage colony stimulating factor
IL-10	Platelet-activating factor (PAF) IL-17
IL-17	Fibroblast growth factor (FGF)

Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ )Interferon-gamma (IFN- $\gamma$ )

Overproduction of IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$ , is associated with severe Covid-19 disease, need for IMV or ECMO.<sup>16</sup>, and very poor prognosis.<sup>17-20</sup> Notably, high levels of serum IL-6 are associated with fatal severe SARS-CoV-2 pneumonia.<sup>21,22</sup> Furthermore, high levels of IL-6 are predictive biomarker of severe SARS-CoV-2, and a poor outcome.<sup>23-25</sup> A reference level of IL-6 of 80 pg/ml has been suggested as a more sensitive biomarker than C-reactive protein (CRP), and D-dimers in determining patients who require IMV.<sup>16,22</sup> Additionally, high serum IL-6 levels may be used to categorize patients who are more likely to benefit from targeted IL-6R $\alpha$  antagonists, or JAK inhibitors (JAKinibs).

Interleukin-6 is a master player cytokine responsible for the cytokine storm in severe Covid-19.<sup>26</sup> Overproduction of IL-6, and dysregulation of the IL-6 signaling can result in chronic inflammatory diseases, autoimmune disorders,<sup>27</sup> and even cancer.<sup>28,29</sup> Targeting IL-6, IL-6R, and the downstream signaling kinases, such as Janus kinases (JAKs) is an attractive therapeutic approach to treat chronic inflammatory diseases, such as rheumatoid arthritis,<sup>27</sup> and cytokine release syndromes, including SARS-CoV-2.<sup>30,31</sup>

Currently, there are several monoclonal antibodies (mAb) which have been granted emergency use (EUA) by the Food and Drug Administration (FDA) for the treatment of Covid-19. However, some of the mAb approved for EUA, or in clinical trials do not retain activity against mutated variants of SARS-CoV-2, including the Omicron variant, such as anti-spike protein mAbs.<sup>32-35</sup> There is urgent need to develop novel biologics with sustainable pharmacokinetics, efficacy, and safety. Selective JAK inhibitors by blocking the final IL-6 immunopathological pathway may stand atop of the precision biotherapeutics for the treatment of Covid-19.

## SARS-CoV-2 infection

SARS-CoV-2 is a member of the  $\beta$ -coronavirus genera in the *Coronaviridae* family.<sup>36</sup> It contains a single strand of positive-sense RNA gene which is about 30 kb, and consists of 29,903 base nucleotide pairs.<sup>36</sup> The genome is packed in an envelope protein structure called the nucleocapsid (N). The nucleocapsid is surrounded by a bilayer lipid corona structure, which includes the membrane (M),

envelope (E), and spike proteins (S).<sup>37</sup> On electron microscope, the virion appears like a crown because it has structural spike proteins on its surface that facilitates its entry, proliferation, and infectivity in host cells.<sup>38</sup> The structural components of SARS-CoV-2, including the membrane receptors, nucleocapsid, spike proteins, and enzymes are potential targets for the development novel biotherapeutic agents, and vaccines.

SARS-CoV-2 mainly spreads through the respiratory system by airborne droplets.<sup>39</sup> The virus possesses a unique spike of glycoprotein with high affinity for angiotensin-converting enzyme 2 (ACE2).<sup>40,41</sup>, which it adopts for entering into the cells. The trimers of the spike proteins on the SARS-CoV-2 caspid binds to ACE2 receptors, which triggers clathrin-dependent concomitant endocytosis of SARS-CoV-2 and ACE2.<sup>42</sup> Attachment, fusion and entry of the virus are aided by spike proteins, and type II transmembrane serine protease (TMPRSS2), which enable the virus to translocate into the cell endosomes (43). The novel coronavirus enters into host cells by interaction between the SARS-CoV-2 spike glycoprotein (S) and the N-terminal segment of ACE2 cell membrane enzyme.<sup>43</sup> S protein consists of two functional subunits, including S1 that that bind to the cell surface ACE2, and S2 for fusion of the virion and cell membrane. Before entry, binding requires cleavage of the S protein into S1 and S2, and activation of some of the coronavirus S glycoproteins by different host proteases. The cleaving enzymes include cathepsin L, cathepsin B, factor X, elastase, furin proprotein convertase, and TMPRSS2.<sup>44</sup>

The spike protein is an attractive target for the development of monoclonal antibodies, entry inhibitors, and vaccines for SARS-CoV-2.<sup>45</sup> Currently, there are several entry inhibitors which have been granted EUA by the FDA, and in clinical trials for the treatment of SARS-CoV-2, such as bamlanivimab, etesevimab, and imdevimab.

The ACE2 receptor protein is highly expressed on multiple human cells, such as type II nasal epithelial cells, alveolar cell (AT2), club cells, respiratory epithelial cells, oral, esophageal, and ileal epithelial cells, hepatocytes, proximal tubules in the kidneys, urothelial cells of the bladder, testes, brain, and heart.<sup>46-49</sup> ACE2 is highly expressed on lung alveolar epithelial cells, particularly type II pneumocytes, consequently the lungs are the most vulnerable target for SARS-CoV-2 infection.<sup>50</sup> Upon entry of the virions in the host cells, they replicate recklessly, spread, and create inflammation, and diffuse alveolar damage (DAD) in the entire lung. Although the virus primarily causes severe pneumonia, it is capable of causing infections in most of organs in the human body, which express the ACE2 receptor, or indirectly from the effects of the cytokine storm.

## Cytokine storm in SARS-CoV-2

SARS-CoV-2 infection induces a cytokine release syndrome, also dramatized as the cytokine storm. The initial priming step during infection or inflammation is the recognition of pathogen-associated molecular patterns (PAMPs), and damage-associated patterns (DAMPs) by pattern recognition receptors (PRR) by the host innate system. This initiates inflammatory responses through the production, and over-expression of pro-inflammatory cytokines, and chemokines, which are responsible for multi-organ injury, and ultimately multi-organ dysfunction, and failure.

The cytokine storm is defines as a systemic hyperinflammatory response that can be triggered by a dysregulated, and over-production of cytokines by several activated immune, and inflammatory cells, such as macrophages, monocytes, T cells, B cells, dendritic cells, and natural killer (NK) cells. Table 2 elaborates the immune, inflammatory and structural cell responsible for the cytokine storm. The causes

of the cytokine storm are multifactorial, such as infection, trauma, radiation, and chimeric antigen-receptor T-cell (CAT-T) therapy.<sup>13-15</sup>

**Table 2** Immune and structural cells which secrete cytokines, and chemokines responsible for the cytokine storm

Inflammatory and immune cells	Structural cells
Macrophages	Epithelial cells
Monocytes	Endothelial cells
Mast cells	Astrocytes
Neutrophils	Microglia
Dendritic cells	Neurons
CD4 <sup>+</sup> Th2 lymphocytes	Adipocytes
B cells	Malignant cells
Natural killer T cells	

Several pro-inflammatory cytokines, chemokines, growth factors, and proteases are secreted in large quantities during the SARS-CoV-2 cytokine storm. They include cytokines, such as IL-1 $\beta$ , IL-2, IL-6, IL-19, IL-17, IL-18, TNF- $\alpha$ , IFN- $\gamma$ ; chemokines, including CCL2, CCL3, CXCL9, CXCL10, MCP-1, MIP-1 $\alpha$ , and IP-10; and growth factors, such as GM-CSF, G-CSF.<sup>10-15</sup> The cytokine and chemokine are the major cause of acute lung injury (ALI), acute respiratory distress syndrome (ARDS), and multi-organ dysfunction and failure.<sup>16-20</sup> The levels of cytokines, and in particular, IL-1 $\beta$ , IL-6, and IL-10, and TNF- $\alpha$  correlate with the severity of Covid-19.<sup>21,22</sup> However, IL-6 is the master player in inciting Covid-19 cytokine storm, and progressive fatal disease.

## Pathology

The inflammatory cytokines, reactive oxygen species (ROS), and proteases secreted during the cytokine storm play a key role in the pathogenesis of ARDS, and respiratory failure in patients with severe Covid-19. In particular, increased serum IL-6 concentration has been shown to be associated with severe SARS-CoV-2,<sup>51</sup> and mortality, suggesting that IL-6 plays a central role in the pathogenesis of severe Covid-19, and multi-organ failure (MOF).

Pathologically, SARS-CoV-2 is characterized by severe diffuse alveolar damage, pulmonary oedema, proteinaceous exudate, hyaline membranes, patchy inflammatory cellular infiltration, and focal reactive hyperplasia and desquamation of pneumocytes.<sup>52-54</sup> The severe acute lung injury is due to direct infection of alveolar pneumocytes by SARS-CoV-2; and lung parenchymal injury by inflammatory cytokines, proteases, and ROS.<sup>55</sup>

The inflammatory cytokines secreted during SARS-CoV-2, such as lipid mediators, IL-6, and angiotensin 1-7, lead to increased vascular permeability, and pulmonary oedema. Increased alveolar exudates caused by aberrant host immune response to coronavirus 2 contribute to impaired gas exchange, hypoxemia, and increased mortality in patients with severe Covid-19.<sup>56</sup> Severe hypoxemia due to SARS-CoV-2 is also due to haemoglobinopathies, microangiopathy, vascular occlusion due to microemboli, ventilation-perfusion mismatch, and shunts.<sup>57,58</sup> Disease resolution in the lung is variable after recovery, and may include fibrosis, resulting in decrease in total lung capacity, and increase in specific airway resistance (sRaw).

Persistent hypoxemia with oxygen saturation (SaO<sub>2</sub>) < 94%, and PaO<sub>2</sub>/FiO<sub>2</sub> ratio < 300 mm Hg can lead to pulmonary hypertension, and haemodynamic compromise. Furthermore, coronavirus has

tropism for cardiomyocytes, and pericardial serosal cells, and can result in arrhythmias, myocarditis, pericarditis, cardiomyopathy, and heart failure.<sup>59-62</sup> The cardiovascular dysfunction may persist after Covid-19 treatment, such as reduced left and right ventricular function on transthoracic echocardiography, as well as elevated high-sensitivity cardiac troponin I (hs-cTnI), and N-terminal pro-B-type natriuretic peptide (NT-proBNT).<sup>63</sup>

Severe Covid-19 disease is associated with extracardiopulmonary organ damage, such as septic shock, acute kidney injury, renal failure,<sup>64,65</sup> acute liver injury,<sup>66</sup> disseminated intravascular coagulation (DIC), thromboembolism,<sup>67</sup> neurological disorders,<sup>68</sup> and cerebrovascular disease.<sup>69,70</sup>

Laboratory features of Covid-19 include neutrophilia,<sup>22</sup> lymphopenia, thrombocytopenia,<sup>71</sup> raised serum levels of C-reactive protein (CRP), procalcitonin, D-dimer, fibrinogen, ferritin,<sup>65,66</sup> creatinine, and urea.<sup>64,65</sup> There may be residual renal dysfunction, such as minor elevation in creatinine and urea after Covid-19.<sup>63</sup> Impaired liver function results in hypoalbuminaemia, and high concentrations of total serum bilirubin, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and glutamyl transpeptidase.<sup>66</sup>

Abnormal coagulation parameters include high serum levels of fibrinogen, and D-dimer are associated with poor prognosis in patients with novel coronavirus pneumonia,<sup>72</sup> due to higher risk of thrombosis, particularly deep venous thrombosis (DVT), and pulmonary embolism.<sup>72,73</sup> This is because; IL-6 stimulates the coagulation pathway leading to microthrombi in the pulmonary circulation, and venous system, which increase the risks of thrombotic events.<sup>74</sup> Complement-mediated microvascular injury may also contribute to thrombotic complications in critically ill patients in the intensive care units (ICU).<sup>75</sup> Thromboembolism can also occur in the cerebral vasculature, leading to stroke,<sup>70</sup> and portrays a gloomy prognosis.

Interleukin-6 can induce hepatocytes to produce acute phase proteins,<sup>76</sup> such as CRP, serum amyloid A (SAA), fibrinogen, haptoglobin, and 1-antichymotrypsin. Increased serum levels of CRP, and D-dimer are associated with severe SARS-CoV-2, poor outcomes, and need for non-invasive ventilation (NIV) or invasive mechanical ventilation. CRP and D-dimer are useful biomarkers to depict the severity of Covid-19, and can guide therapy, and predict the prognosis in critically ill patients.<sup>77</sup>

Interleukin-6 is a key cytokine in orchestrating the cytokine storm.<sup>78</sup> Overproduction of IL-6, and dysregulation of the IL-6 signaling can result in chronic inflammatory diseases, and autoimmune disorders;<sup>79,80</sup> and even cancer.<sup>81,82</sup> Targeting IL-6, IL-6R, and the signaling kinases, such as Janus kinases (JAKs) is an attractive therapeutic approach to treat chronic inflammatory diseases, such as rheumatoid arthritis;<sup>83</sup> and SARS-CoV-2.<sup>84</sup>

## Interleukin-6

Interleukin-6 is a member of the IL-6 family, which consists of ten polypeptide cytokines with a four-helix structure.<sup>85</sup> It was discovered and cloned by Hirano and colleagues in 1986.<sup>86</sup> IL-6 is a small glycoprotein with a molecular mass of 21-28 kDa, comprising of 212 amino acids, including 28-amino acid signaling peptides.<sup>85,86</sup> It is produced by inflammatory and immune cells, such as macrophages, mast cells, neutrophils, dendritic cells, CD4<sup>+</sup> Th2 lymphocytes, and B cells.<sup>87-90</sup> Additionally, IL-6 is secreted by structural cells, such as epithelial cells, endothelial cells, fibroblasts, adipocytes, astrocytes, neurons, and malignant cells.<sup>90,91</sup>



Several stressful stimuli are known to trigger production and secretion of IL-6, such as microbial products, viral infections, trauma, irradiation, ultraviolet light, reactive oxygen species (ROS), and pro-inflammatory cytokines, such as IL-1 $\beta$ , and TNF- $\alpha$ .<sup>92,93</sup> Additionally, IL-6 production is activated by angiotensin II, which is produced by inflamed blood vessels in a JAK-STAT-dependent signaling pathway.<sup>94</sup> Interleukin-6, and other cytokines, such as IL-1 $\beta$ , IL-2, IL-8, IL-10, IL-17, and TNF- $\alpha$  secreted due to SARS-CoV-2 infection, have emerged as major instigators of hyperinflammation, and the cytokine storm.

### Interleukin-6 signaling

Interleukin-6 signaling is mediated via its receptor IL-6R $\alpha$ , which exists in two isoforms, the transmembrane IL-6R $\alpha$  (mIL-6R $\alpha$ ) which has a molecular mass of 80 kDa, and the soluble IL-6R $\alpha$  (sIL-6R $\alpha$ ) with a molecular mass of 50-55 kDa.<sup>95</sup> Signaling using mIL-6R $\alpha$  is called the classic signaling, and via sIL-6R $\alpha$  is termed the trans-signaling pathway.<sup>96</sup> Effector cells lacking the mIL-6R $\alpha$  can signal via the sIL-6R $\alpha$  (trans-signaling). The trans-signaling allows IL-6 to modulate an additional broad spectrum of cells functions.

Soluble IL-6R $\alpha$  is produced by proteolytic cleavage of mIL-6R $\alpha$  by a disintegrin and metalloproteinase domain containing protein 10 (ADAM10).<sup>97</sup> The gp130 protein which is essential for signaling is expressed ubiquitously in many cells in the human body, and acts as a signal-transducing co-receptor.<sup>98</sup> This allows IL-6 to exert its biological and immunological effects in several tissues, and organs, such as lung, kidney, gut, heart, brain, thyroid glands, testes, and bones.

In the classic pathway, IL-6 binds to membrane-bound IL-6 receptor, and the complex of IL-6/mIL-6R $\alpha$  associates with gp130, and dimerizes before initiating signaling.<sup>98,99</sup> The intracellular signaling is mediated by Janus kinases (JAKs) constitutively associated with the cytoplasmic portion of gp130 protein. This is followed by activation of signal of transducers and activators of transcription (STATs), which after phosphorylation by Janus kinases dimerize and translocate into the nucleus.<sup>100</sup> STATs (STAT1, and STAT3) act as transcription factors in the nucleus, and translate the biological, and immunopathophysiological effects of IL-6, such as production of the cytokines responsible for hyperinflammation, and many other cellular functions.

The gp130 co-receptor mainly signals via JAK1, and STAT3.<sup>101</sup>, but other IL-6 family members can signal via JAK1 and STAT1. Interleukin-6 can also signal via the JAK-MAPK (mitogen-activated protein kinase) pathway.<sup>102</sup> Additionally, IL-6 can activate the C/EBP $\beta$  transcription factor (also known as NF-IL6).<sup>103</sup> All these signaling pathways lead to production of pro-inflammatory cytokines, and chemokines responsible for ALI, respiratory failure, and MOF.

### Immunology of interleukin-6

Interleukin-6 was originally clones as a factor acting on B cells to induce immunoglobulin (IgG) production, and was termed B cell stimulating factor 2 (BSF-2).<sup>104</sup> IL-6 act directly on plasma cells to promote antibody production, such as IgG, LgM, and IgA, and indirectly by promoting Bc16-dependent follicular CD4 T (Tfh) cell differentiation in conjunction with IL-21 (105-107). In addition, IL-6 signaling via STAT3 is essential for *in vivo* plasma cell survival, and immunoglobulin production.<sup>108</sup> Apart from its incriminated multiple pathologies, IL-6 play an important role in humoral immunity.

Interleukin-6 induces differentiation of IL-4-producing T helper (Th2) cells, and inhibits the differentiation of IFN $\gamma$ -producing Th1

cells, thus polarizing the innate immune system towards Th2 high.<sup>109</sup> IL-4 plays an important role in the pathogenesis of eosinophilic airway diseases, several autoimmune diseases, neuroendocrine disorders, bowel diseases, cardiovascular diseases, and cancer. It induces the  $\epsilon$  isotype switch, and switching of B cell immunoglobulin production from IgM to IgE.<sup>110-112</sup> Additionally, IL-4 enhances IgE-mediated responses by upregulating IgE receptors on various immune cells, such as the high-affinity IgE receptors (Fc $\epsilon$ RI) on mast cells and basophils, and the low-affinity IgE receptors (Fc $\epsilon$ RII; CD23) on lymphocytes and mononuclear cells.<sup>111,112</sup>

Interleukin-6 plays an important role in the differentiation of CD4<sup>+</sup> positive T cell subset.<sup>113</sup> IL-6 in conjunction with TGF- $\beta$ , or IL-1 $\beta$  via JAK/STAT signaling, drive naïve CD4<sup>+</sup> positive T cells to the Th17 lineage, which consist of cells that produce IL-17 family cytokines, such as IL-17A, IL-17F, and IL-25.<sup>114</sup> ROR-related orphan receptor gamma t (ROR $\gamma$ t) is the master transcription factor for IL-6 and TGF- $\beta$ -induced Th17 differentiation.<sup>115</sup> Conversely, IL-6 inhibits the TGF- $\beta$ -induced differentiation of regulatory T (Treg) cells.<sup>116</sup>

Furthermore, IL-6 up-regulates IL-23R expression via STAT3 dependent pathway. Increased expression of IL-23R activity promotes differentiation of pathogenic potential Th17 cells (116). Finally, IL-23R is essential for the terminal differentiation of Th17 cells *in vivo*, which produce Th17 cytokines, such as IL-17A (synonymous to IL-17), and IL-17F.<sup>117</sup> IL-17 and TGF- $\beta$  promote ROR $\gamma$ t expression, and regulates transcription of Th17 genes.<sup>118</sup>

IL-17 and IL-17F play a key role in inducing the expression of chemokines, cytokines, and growth factors, such as IL-1 $\beta$ , IL-6, IL-8, TNF- $\alpha$ , G-CSF, GM-CSF, and TGF- $\beta$ .<sup>119-121</sup> The chemoattractant mediators, such as CXCL8 (IL-8), leukotriene B4 (LTB4), platelet activating factor (PAF), and thromboxanes (TXB2) promote neutrophil recruitment, activation, and degranulation resulting in neutrophilic inflammation.<sup>122</sup>

Activated neutrophils can produce an oxidative burst.<sup>123</sup>, releasing multiple proteases, cytokines, chemokines, lipid mediators, and reactive oxygen species which lead to inflammation and tissue injury. Neutrophil-derived inflammatory mediators, include proteases, such as neutrophil elastase, cathepsin G,<sup>124</sup> and metalloproteases.<sup>125</sup>; and lipid mediators, including LTB4, LTC4, PAF, TBX2.<sup>126</sup>; ROS.<sup>127,128</sup>, and myeloperoxide.<sup>129</sup> orchestrate tissue injury. In patients with Covid-19, they contribute to ALI, respiratory failure, and MOF. The robust inflammatory response due to activation of neutrophils, play a key role in the pathophysiology of Covid-19-related ARDS.<sup>130</sup>

In summary, IL-6 plays a very important role in the pathophysiology of severe SARS-CoV-2. High serum levels of IL-6 are associated with severe disease, worse radiological picture, necessity for mechanical ventilation, and high mortality.<sup>131,132</sup>

### Janus kinases signaling

The discovery of the JAK/STAT signaling pathway has a fascinating and mesmerising history.<sup>133,134</sup> 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.<sup>133</sup> 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, hence the symbolic tribute to the two-faced Roman God.<sup>133-135</sup> JAK was taken from the two-faced God of doorways, Janus.<sup>136</sup>, and the name was colloquially shortened to JAK by Andrew Wilks from Australia in 1989.<sup>137</sup>

Janus kinase are a family of intracellular non-receptor tyrosine kinases that play an essential role in the signaling of more than 60 cytokines, type I/II interferons, and growth factors that have been implicated in the pathogenesis of inflammatory diseases.<sup>138,139</sup> JAKs consists of four members, namely JAK1, JAK2, JAK3, and Tyrosine kinase 2 (TYK2).<sup>140-145</sup>, whereas the signal transducer and activators of transcription (STAT) family comprises of seven members: STAT1, STAT2, STAT3, STAT4, STAT4a, STAT5b, and STAT6.<sup>146</sup> STATs primarily function as transcription factors.<sup>147</sup>

The JAK-STAT pathway is one of the most important downstream signaling 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.<sup>148</sup>

JAKs are ancestral proteins which have been identified in the primitive chordata *Ciona*, and *Drosophila*.<sup>17</sup> They are relatively large proteins composed of more than 1,100 amino acids, and molecular masses of 120-140 kDa.<sup>149</sup> Seven distinct JAK homology regions (JH) have been identified (JH1 to JH7), and these form the putative structural domains of the JAK family members.<sup>150</sup> They include the kinase domain (JH1), the inhibitory pseudokinase domain (JH2), the Src homology (SH2) receptor interaction domain (JH2-JH5), and the 4.1 ezrin radixin moesin (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.<sup>151</sup>

JAK1, JAK2, and TYK2 are expressed ubiquitously in mammals, and JAK3 is primarily expressed in haematopoietic cells.<sup>149,152</sup> 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.<sup>153</sup>

Type II cytokine-like receptors for IL-10, IL-19, and IL-22, and the gp130 subunit sharing receptor for IL6 and IL-11 primarily signal via JAK1, but also associate with JAK2, and JAK3.<sup>154,155</sup> Receptors for hormone-like cytokines, including growth hormone, prolactin; erythropoietin, thrombopoietin; and cytokine receptors involved in hematopoietic cell development, such as IL-3, and granulocyte-macrophage colony-stimulating factor (GM-CSF) use JAK2 (155,156) JAKs play a key role in cell growth, development, survival, and differentiation of immune cells.<sup>144,151</sup>

### Jak-stat signaling

The first step in the JAK/STAT signaling is initiated when a specific cytokine binds to the surface of its target cognate transmembrane receptor, which causes receptor dimerization. The receptors contain intracellular domains which are constitutively associated with members of JAKs family of tyrosine kinases.<sup>140,157-159</sup> In resting state, JAKs are inactive. They are activated by transphosphorylation when the cytokine binds to its receptor.<sup>160</sup> 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.<sup>161,162</sup> The receptor-localized STATs on the tyrosine docking sites are phosphorylated by JAKs.<sup>163</sup>, which cause them to dissociate from the receptor.<sup>164</sup>, dimerize and translocate into the nucleus in an import  $\alpha$ -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.<sup>161,165,166</sup>

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.<sup>167,168</sup> SOCS proteins in conjunction with STAT-dependent operons switch off the signaling.<sup>169</sup> The JAK-STAT pathway can also be regulated by the protein inhibitors of activated STATs (PIASs), and protein tyrosine phosphatases (PTPs).<sup>168</sup> 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.<sup>170-172</sup> Therefore, targeting and inhibiting the JAK-STAT pathway with monoclonal antibodies (mAb) is an attractive opportunity for precision treatment of autoimmune diseases.<sup>173,174</sup>, cancer.<sup>170,171</sup>, and SARS-CoV-2. Currently, there are several biologics (mAb), targeting the JAK-STAT axis that are in clinical trials, although only few JAK inhibitors (JAKinib) are approved 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 few 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.<sup>175</sup>, baricitinib.<sup>176</sup>, and tofacitinib.<sup>177</sup> These JAKinibs have now been repurposed for the treatment of SARS-CoV-2, and splendidly seem to be effective and safe.

### Treatment of SARS-COV-2

Treatment of SARS-CoV-2 is difficult because there are no specific effective targeted antiviral agents for the treatment of the disease, and arrest progression to respiratory failure. Due to the impact of the Covid-19, some biologics which are effective in the treatment of chronic inflammatory, autoimmune diseases, and other viral syndromes, such as Ebola, have been repurposed for the treatment of SARS-CoV-2. Nevertheless, some of the antivirals, and biologics have been able to shorten the duration of the severe illness. 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.

Management of SARS-CoV-2 include proper nursing care in a prone position, which has been documented to improve oxygen saturation (SaO<sub>2</sub>), and partial pressure of arterial oxygen (PaO<sub>2</sub>).<sup>178,179</sup> High-flow nasal oxygenation (HFNO) via a nasal cannula is the most recommended initial treatment of SARS-CoV-2. This can be delivered through high-flow nasal cannula up to 60 L/min of nearly 100% oxygen.<sup>180</sup> The recommended target SaO<sub>2</sub> is 92-96% in adults with severe Covid-19, using supplemental oxygen as needed.<sup>181</sup> 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.<sup>182</sup>

Provision of low positive end-expiratory pressure (PEEP) of 2-5 cm H<sub>2</sub>O which does not induce alveolar over-distension, and barotrauma is required for the effective delivery of oxygen, and in improving the PaO<sub>2</sub>.<sup>183-185</sup> In patients with more severe hypoxemia due to pulmonary oedema, and atelectasis, the use of continuous positive airway pressure (CPAP) is recommended to increase the total

lung capacity (TLC) by recruitment of collapsed lung units.<sup>185</sup> CPAP is usually delivered at pressure levels between 5 and 15 cm H<sub>2</sub>O.<sup>186-188</sup>

Critically ill patients with Covid-19 require respiratory support or extracorporeal membrane oxygenation (ECMO). The rate of invasive mechanical ventilation among patients admitted to intensive care units for severe Covid-19 range from 29.1% in one Chinese study,<sup>189</sup> to 89.9% in a USA study.<sup>6</sup> The decision to place patients with Covid-19 on a ventilator is not clear, and neither are the outcomes in terms of mortality.<sup>190</sup> Despite IMV, the mortality rate is high and very variable depending on the centres. Richardson et al.<sup>6</sup> have reported a mortality rate of 24.5%-96.7%, mean 88.9%, whereas Grasselle and colleagues have documented a mortality rate of 25.6-83.8%.<sup>8</sup> Furthermore, about 5% of the patients with severe Covid-19 require ECMO.<sup>191</sup>; however, the fatality rate is still soberly high.

The standard of care therapies for severe Covid-19 which has been recommended by the World Health Organization (WHO), and the National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel include low dose corticosteroids, and remdesivir, or lotinovir/ritonavir (Kaletra). The guidelines recommend dexamethasone (DEX) 6 mg intravenously (IV), once daily (OD), plus remdesivir (RDV) 200 mg IV start dose, then 100 mg IV for 4 days or until hospital discharge. Other centres also use add-on lopinavir/ritonavir (400/100 mg) twice daily orally. If dexamethasone is not available, an alternative corticosteroid with similar potency as DEX 6 mg, such as prednisone 40 mg, methylprednisolone 32 mg or hydrocortisone 160 mg IV once daily.<sup>192-194</sup> can be used.

Several clinical trials have evaluated the efficacy and safety of corticosteroid therapy in critically ill patients with Covid-19. The most impressive results are from the RECOVERY trial which 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.<sup>195</sup> Similarly, a comprehensive systemic review and meta-analysis comprising of 44 credited studies, and 20, 197 patients, has confirmed a beneficial effect of corticosteroids on short-term mortality, and reduction in the need for mechanical ventilation.<sup>196</sup>

Furthermore, some inhaled corticosteroids, including ciclesonide have been shown to impair the replication of SARS-CoV-2.<sup>197</sup>, and downregulate expression of receptors for the entry of the virus into host cells.<sup>198,199</sup> Nevertheless, systemic corticosteroids are associated with serious adverse events, such as delayed viral clearance, and opportunistic infections.<sup>196,200-202</sup>, particularly when co-administered with IL-6R antagonists.

Remdesivir is an analog 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. Although it was initially developed for the treatment of Ebola, it has been demonstrated to have in vitro activity against SARS-CoV-2.<sup>203</sup> 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.<sup>204-206</sup> Remdesivir is also effective in combination with tocilizumab,<sup>207</sup> and baricitinib<sup>208</sup> in the treatment of hospitalized patients with Covid-19.

The positive results from comprehensive randomized clinical trials on the efficacy and safety of intravenous remdesivir.<sup>209-211</sup>, resulted in the FDA to issue an EUA on May 1, 2020, to permit the use of remdesivir for treatment of COVID-19 in adult and pediatric patients

(aged  $\geq 12$  years and weighing  $\geq 40$  kg, with suspected or laboratory confirmed Covid-19.<sup>212</sup> Remdesivir has also received full or conditional approval in several countries.

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 which is 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-6R antagonists, or JAKinibs.<sup>213-215</sup>

The NHI COVID-19 Treatment Guidelines Panel recommends add-on IL-6R antagonists, including tocilizumab 8 mg/kg (up to 800 mg) administered as a single IV dose. Alternatively, sarilumab can be used in prefilled syringe of 400 mg in 100 cc 0.9% NaCl. Tocilizumab (Actemra), and sarilumab (Kevzara) should be given in patients on dexamethasone who have rapidly increasing oxygen requirement, and systemic inflammation.

Tocilizumab 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 a myriad of pro-inflammatory cytokines, including IL-6 itself, responsible for the cytokine storm, and the pathogenesis of SARS-CoV-2.

Observational studies have shown that tocilizumab improves clinical outcomes, and reduces mortality in hospitalized patients with Covid-19.<sup>216,217</sup> Similarly, a large randomized trial (REMAP-CAP), which enrolled 803 patients, demonstrated that treatment with tocilizumab improved survival in patients with SARS-CoV-2.<sup>218</sup>

The RECOVERY clinical trial in Great Britain was one of the largest study to evaluate the efficacy and safety of tocilizumab in 4116 hospitalized patients with severe Covid-19; and 3385 (82%) patients were receiving corticosteroids.<sup>219</sup> In patients with hypoxia, and systemic inflammation, tocilizumab improved the clinical outcomes, and survival regardless of the amount of respiratory support, and additional benefits from systemic corticosteroids.<sup>219</sup> Patient 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;  $P = 0.0028$ ).<sup>219</sup>

Actemra was granted an EUA by the FDA for the treatment of adults and pediatric patients (2 years of age or older) who are receiving systemic corticosteroids, and requiring NIV or IMV, or ECMO.<sup>220</sup> It is recommended that tocilizumab be administered in combination with corticosteroids or remdesivir, or a spike entry inhibitor, including bamlanivimab, casirivimab, or etesevimab.<sup>116</sup> Co-administration of tocilizumab with another IL-6R antagonist, such as sarilumab, or with JAK inhibitors, including baricitinib, and Tofacitinib is associated with increased risk of opportunist infections, and helminths infestation.<sup>221</sup>



Despite all the innovative therapies including antivirals, spike entry inhibitors, IL-6R antagonists, IMV, and ECMO, the novel coronavirus 2019 has evaded the entire therapeutic armoury. The remaining salvation is to attack the bull (IL-6) by the horn (JAK/STAT

signaling), by utilizing JAKinibs in the treatment of Covid-19. Table 3 elaborates antivirals, and monoclonal antibodies in clinical trials for the treatment of SARS-CoV-2.

**Table 3** Monoclonal antibodies in clinical trials for the treatment of SARS-CoV-2

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

**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)

### Janus kinase inhibitors

Janus kinase inhibitors (JAKinibs) are monoclonal antibody that inhibit type I and II cytokine receptors. They are effective in the treatment of chronic inflammatory diseases,<sup>222</sup> such as rheumatoid arthritis,<sup>223</sup> psoriasis,<sup>224</sup> and myeloproliferative disorders,<sup>225</sup> including polycythemia vera.<sup>226</sup> Additionally, JAKinibs are in clinical trials for the treatment of several diseases, such as alopecia areata, atopic dermatitis,<sup>227</sup> inflammatory bowel diseases (IBD),<sup>228</sup> haematological malignancies,<sup>229</sup> and various cancers.<sup>230</sup> Due to the emergency of Covid-19 pandemics, and none availability of specific antiviral agents for the treatment of the catastrophic disease, second-generation JAKinibs have been repurposed for the treatment of SARS-CoV-2.<sup>231</sup>

Janus kinase inhibitors are effective in controlling hyperinflammation due to the cytokine storm,<sup>232</sup> characterized by high levels of IL-1 $\beta$ , IL-6, IL-8, IL-10, and TNF- $\alpha$ , in patients with severe Covid-19.<sup>231</sup> 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 potential inhibitory targets for JAKinibs.<sup>233</sup>

Noteworthy, angiotensin II (Ang II) also mediates its pathophysiological effects via the JAK-STAT signaling pathway, resulting in vasoconstriction, hypertension, and chronic tissue injury.<sup>234</sup> Thus, inhibiting IL-6, Ang II, and JAK-STAT signaling may be very effective in dampening the cytokines storm, and in the treatment of SARS-CoV-2. Of course, this may have a price tag. It might lead to susceptibility to systemic infections due to blockade

of multiple cytokine signaling pathways, including the master JAK-STAT pleiotropic pathways.

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 "pathogenic" cytokines employ 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 most precise target for the treatment of Covid-19.<sup>235</sup> Pan-inhibition of the IL-6 and JAKs signaling pathway might not result in the expected benefit outcomes.<sup>235</sup> Schett et al.<sup>236</sup> 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- $\gamma$  involved in viral clearance.

Janus kinase inhibitors have a purposed advantage over other immunotherapy strategies, because they exert dual anti-inflammatory effects. They inhibit the effects of several pro-inflammatory cytokines, and growth factors, and have anti-viral activity by impeding host cellular endocytosis of SARS-CoV-2 (237,238). JAKinibs inhibit the entry of SARS-CoV-2 into type II alveolar epithelial cells (237), and prevent alveolar injury, and diffuse alveolar damage. 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,<sup>239</sup> ruxolitinib,<sup>240</sup> and tofacitinib.<sup>241</sup> Baricitinib

and ruxolitinib predominantly inhibit JAK1 and JAK2,<sup>239,240</sup> whereas, tofacitinib inhibits JAK1 and JAK3.<sup>241</sup> JAK inhibitors have been demonstrated to be effective and safe in treating patients with chronic inflammatory diseases, haematological malignancies, dermatological disorders, and cytokine release syndromes, including SARS-CoV-2. Table 4 shows the Janus kinase inhibitors approved, and in clinical trials for the treatment of Covid-19, and other diseases.

**Table 4** Janus kinase inhibitors in clinical trials for the treatment of Covid-19, and other diseases

Biologic	JAK Target	Disease Approved/clinical trials
Baricitinib	JAK1, JAK2	Rheumatoid arthritis (RA), PA, PV, AD
Tofacitinib	JAK1, JAK3, JAK2	RA, psoriatic arthritis, PV, IBD
Ruxolitinib	JAK1, JAK2	Myelofibrosis, polycythemia vera, GVHD
Fedratinib	JAK2	Myelofibrosis
Filgotinib	JAK1	Rheumatoid arthritis, IBD
Momelotinib	JAK1, JAK2	Myelofibrosis, ovarian cancer
Pacritinib	JAK2	Myelofibrosis, AML, colorectal cancer
Peficitinib	JAK1, JAK2, JAK3	Rheumatoid arthritis
Oclacitinib	JAK1, JAK2	Leukaemia
Gandotinib	JAK2	Myeloproliferative disorders
Upadacitinib	JAK1, JAK3	Rheumatoid arthritis, IBD
Solcitinib	JAK1	Psoriasis

**Abbreviations:** AD, atopic dermatitis; AML, acute myeloid leukemia; IBD, inflammatory bowel disease; JAK, 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

### Tofacitinib

Tofacitinib is a selective Janus kinase inhibitor of JAK1 and JAK3, with partial functional activity against JAK2. It blocks JAK1-STAT3 signaling, thus inhibiting transcription of genes responsible for production of cytokines implicated in the cytokine storm, and SARS-CoV-2.<sup>242-244</sup> Tofacitinib also modulates the action of interferons, and IL-6,<sup>245-247</sup>; and decreases proliferation of Th17 helper T cells, which produce IL-17 and IL-17F, another family member cytokines implicated in the cytokine storm.<sup>248</sup>

Tofacitinib is very effective in the treatment of chronic inflammatory diseases, and it is approved by the FDA for the treatment of rheumatoid arthritis, polyarticular course juvenile idiopathic arthritis,<sup>249,250</sup> psoriatic arthritis,<sup>250</sup> psoriasis,<sup>250,251</sup> and inflammatory bowel disease.<sup>250,252,253</sup> Tofacitinib inhibits pro-inflammatory signaling that is important in the pathogenesis and 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.<sup>241</sup> Guimarães et al.<sup>241</sup> randomized 289 patients (1:1) from 15 hospitals across Sao 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, 0.6.3%; 95% confidence interval (CI), 0.41 to 0.97; P = 0.04).<sup>241</sup>

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 to 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.<sup>241</sup> 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 is a JAK1/JAK2 inhibitor with moderate activity against TYK2, and minimal activity against JAK3 (254,255). It suppresses the IL-6-JAK/STAT signaling pathway, and production of pro-inflammatory cytokines, such as IL-1 $\beta$ , IL-1Ra, IL-4, IL-6, IL-10, IL-17, IL-13, IL-7, TNF- $\alpha$ , IFN- $\gamma$ , GM-CSF, FGF, MCP-1, MCP-1 $\beta$ , and IP-10.<sup>256</sup>; and chemokines including CXCL9, CXCL10, and CXCL11.<sup>257</sup> implicated in the cytokine storm, and in the pathogenesis of SARS-CoV-2.<sup>258</sup>

Baricitinib was approved by the FDA, and the European Medicines Agency (EMA) in 2018 for the treatment of adult patients with rheumatoid arthritis.<sup>259</sup> It is also indicated for the treatment of pruritus and eczema in patients with moderate-to-severe atopic dermatitis (AD).<sup>260</sup> Baricitinib is being investigated in several clinical trials for the treatment of autoimmune diseases, such as juvenile idiopathic arthritis, and systemic lupus erythematosus.<sup>261</sup>; and dermatological disorders, including AD,<sup>260</sup> alopecia areata,<sup>262</sup> and inflammatory bowel disease.<sup>252,253,263</sup>

Some Janus kinase inhibitors have the potential to inhibit entry of SARS-CoV-2 into airway epithelial cells, and type 2 alveolar pneumocytes. Baricitinib binds to AP2-associated protein kinase 1 (AAK1), and cyclic G-associated kinase (GAK), members of the numb-associated kinase (NAK) family which is believed to facilitate propagation of coronavirus into epithelial cells.<sup>264</sup> Baricitinib has high affinity for alveolar type 2 cell-associated protein kinase 1, a key regulator of clathrin-mediated viral endocytosis, and theoretically has the capacity to prevent the entry of SARS-CoV-2 into pneumocytes.<sup>238</sup>, and in preventing DAD. Furthermore, transcriptomic analysis of baricitinib-treated model reveals a significant downregulation of ACE2, and TMPRSS2 in patients treated with baricitinib, thus slowing the entry of SARS-CoV-2 into target cells.<sup>265</sup> Moreover, through binding to numb-associated kinases, baricitinib further inhibits AAK1, and GAK-mediated endocytosis of the virus-ACE complex, thus leading to a reduction in the viral load.<sup>266</sup> Noteworthy, baricitinib at therapeutically clinically-relevant levels has been shown to reduce infectivity of Covid-19 in human primary liver spheroid model.<sup>267</sup>

Another advantage of baricitinib over other JAKinibs is the favourable pharmacokinetic properties, such as low plasma protein binding affinity, minimal interaction with cytochrome enzymes, and drug transporters. Therefore, giving the biologic a greater chance for potential combination of baricitinib with other medications, such as remdesivir.<sup>265</sup>

The application of bioinformatics tools, and Artificial Intelligence (AI) algorithms on baricitinib-treated models have engineered repurposing baricitinib for the treatment of SARS-CoV-2.<sup>238,265</sup> Several clinical trials have shown the effectiveness of baricitinib in preventing the cytokine storm, acute lung injury, respiratory failure, and death in patients with Covid-19.<sup>266-268</sup> Bronte et al.<sup>268</sup> have reported a significant reduction of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  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.<sup>269-273</sup> 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.<sup>273</sup>

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 ( $\leq 10$  days) and either baricitinib 4 mg once daily ( $\leq 14$  days) or placebo.<sup>208</sup> Patients receiving baricitinib plus remdesivir had a 30% higher odds of improvement in clinical status at day 15 (odds ratio, 1.3; 95% CI, 1.0 to 1.6). Patients receiving HFNO or noninvasive 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 to 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 to 1.09).<sup>208</sup> 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 noninvasive ventilation.<sup>208</sup>

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-19.<sup>221</sup> 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. Progression to HFNO, noninvasive 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 noninvasive ventilation at baseline. The JAKinib was safe, and the incidence of serious adverse events, such as serious infection, and thromboembolism were similar in both groups.<sup>221</sup>

Based largely on the results of the COV-BARRIER trial, the National Institutes of Health, and the Infectious Diseases Society of America (IDSA) recommend baricitinib plus dexamethasone in select patients with severe Covid-19.<sup>213,214</sup>

The common adverse event of JAK inhibitors is thrombosis and risk of thromboembolism, including DVT, pulmonary embolism, myocardial infarction, and thrombotic or haemorrhagic stroke.<sup>274-275</sup> This may be compounded by the hypercoagulability state due to the thrombotic effects of IL-6, and SARS-CoV-2.<sup>275-279</sup> Levi et al.<sup>280</sup> 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.<sup>280</sup>

Other feared, but speculative complications of treatment with JAKinibs are gastrointestinal perforation,<sup>281,282</sup> and malignancy.<sup>283,284</sup> However, JAK1 and JAK2 play an important role in IFN signaling and therefore cancer immunoediting.<sup>284</sup>

Despite the risks of thrombotic events, and malignancy, baricitinib is the only JAK inhibitor in combination with remdesivir approved for an EUA by the FDA for the treatment of hospitalized patients with Covid-19, who require oxygen supplement.<sup>285</sup> Baricitinib is also recommended for the treatment of Covid-19 in several countries. The triplet of dexamethasone, remdesivir, plus baricitinib seems very effective in the treatment of Covid-19, particularly when administered early before intubation, and IMV or ECMO.

## Conclusion

Covid-19 is characterized by dysregulated overproduction of cytokines, such as IL-1 $\beta$ , IL-6, IL-10, TNF- $\alpha$ , and chemokines, including CCL3, CCL5, CXCL8, CXCL9, and CXCL10. SARS-CoV-2 infection of alveolar type II pneumocytes, and the effects of pro-inflammatory mediators lead to severe DAD, respiratory failure, and MOF. The standard of care of Covid-19 include HFNO, corticosteroids, remdesivir; and IMV or ECMO in severe cases. IL-6R antagonists, and entry inhibitors have also been used for the treatment of Covid-19, but have not met the expected beneficial outcomes. The mortality rate due to Covid-19 is extremely high despite these innovative therapies. JAKinibs, such as baricitinib have dual anti-viral, and anti-inflammatory activities, and 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 NIH.

## 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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