CORONAVIRUS

HiJAKing SARS-CoV-2? The potential role of JAK inhibitors in the management of COVID-19

Francesca Romana Spinelli[1], Fabrizio Conti[1], Massimo Gadina[2]*

JAK kinase inhibitors are being investigated as a way of managing cytokine storm in patients with severe COVID-19.

Soon after infection with the novel coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), began to spread around the world, the search for potentially useful drugs—while awaiting development of a vaccine—was on. Once SARS-CoV-2 enters the host, drugs capable of enhancing the innate or adaptive immune response could assist in controlling dissemination of the virus. Later on, in severely ill patients, hyperimmune activation leading to cytokine storm occurs and is a potential target for modulation to prevent further lung damage. Therefore, currently approved drugs used to treat rheumatologic and inflammatory diseases such as biological disease-modifying anti-rheumatic drugs (DMARDs) were immediately considered as candidate agents to be repurposed for use in patients with severe coronavirus disease 2019 (COVID-19).

The angiotensin-converting enzyme 2 (ACE2) transmembrane protein provides the pathway for SARS-CoV-2 to infect epithelial cells. ACE2 is highly expressed on lung alveolar epithelial cells, which explains why the lungs are particularly vulnerable to injury following SARS-CoV-2 infection (*1*). ACE2 also exerts a natural protective effect against acute lung injury mediated by the renin-angiotensin system, and its down-regulation after virus entry contributes to the onset of the acute respiratory distress syndrome (ARDS) (*1*). Clinical features of SARS-CoV-2 pneumonia [coronavirus disease 2019 (COVID-19)] resemble what has been observed in other beta-coronavirus infections with the inflammatory response driven by $CD4^+$ and $CD8^+$ T cells, natural killer (NK) cells, monocytes, and the cytokines that these immune cells secrete. Among 41 patients with laboratory-confirmed COVID-19 pneumonia described by Huang and colleagues (*2*), the 13 patients requiring intensive care showed significantly higher serum levels of many cytokines and chemokines including interleukin-2 (IL-2), IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), tumor necrosis factor–α (TNF-α), and IL-1Ra compared with those who were not in an intensive care unit (ICU). In comparison with healthy controls, they also showed higher serum levels of IL-6, IL-9, IL-13, granulocyte-macrophage CSF (GM-CSF), interferon-γ (IFN-γ), IL-1β, IL-8, and IL-17 (*2*). In a study of 50 patients with COVID-19, Hadjadj *et al*. (*3*) found an increase in peripheral blood of IL-6 and IL-6–induced genes, TNF-α and TNF-α pathway–related genes, as well as IL-10. Therefore, the use of Janus kinase (JAK) inhibitors (JAKi) targeting IL-6 and other cytokines with JAK-dependent signaling is one way to restrain the excessive level of cytokine signaling.

Even if cytokines measured in the serum do not reflect their tissue concentrations, high tissue levels of IFN-γ and other cytokines may account for ACE2 down-regulation contributing to the progression of SARS-CoV-2 pneumonia. IL-1β, IL-6, and IL-12 seem to be the main drivers of the hyperimmune response leading to cytokine storm via activation of T helper 1 (T_H1) and NK cells and release of chemokines, which, in turn, recruit more neutrophils and inflammatory macrophages further enhancing lung inflammation (*4*). In this context, the inflammatory response resembles the cytokine release syndrome (CRS) observed in patients receiving chimeric antigen receptor (CAR) T cell therapy and bispecific T cell–engaging antibodies. In CAR T cell–driven CRS, elevated secretion of IL-6 by monocytes and macrophages is thought to be the main driver of symptoms. The clinical signs of CRS are life-threatening complications—fluid-refractory hypotension and cardiac dysfunction, respiratory failure, coagulopathy, and renal and liver failure—which can be effectively treated with anti-cytokine therapy targeting IL-6–IL-6R signaling. More than 30 clinical trials with the anti–IL-6R antibodies tocilizumab and sarilumab are ongoing, and an open-label pilot study of tocilizumab in hospitalized patients with severe COVID-19 demonstrated improvements in respiratory and laboratory parameters (*5*). Other classes of tyrosine kinase inhibitors such as Bruton's tyrosine kinase (BTK) inhibitors are also being tested for their ability to interfere with the CRS observed in patients with severe COVID-19; preclinical studies and case series have suggested that the BTK inhibitor ibrutinib may provide protection against severe lung injury (*6*).

Baricitinib, a targeted synthetic DMARD approved for rheumatoid arthritis (RA), has been proposed as a possible therapeutic option for COVID-19. Baricitinib is a first-generation JAKi targeting JAK1 and JAK2 enzymatic activity, which was also predicted, based on artificial intelligence algorithms, to inhibit the AP2-associated kinase 1 (AAK1) and the cyclin G–associated kinase (GAK), members of the numb-associated kinase (NAK) family involved in clathrin-mediated endocytosis (*7*). Therefore, mechanistically, baricitinib might impair SARS-CoV-2 endocytosis and the early stages of virus spread while also inhibiting the signaling of several cytokines involved in the pathogenesis of viral pneumonia and the emergence of cytokine storm (Fig. 1). On the other hand, IFN-γ and IL-4—two cytokines whose signaling relies on JAKs—inhibit ACE2 expression in vitro at the transcriptional level, leading to a reduction of infection, replication, and excretion of the related SARS-CoV in E6 Vero cells (*8*).

Several clinical trials were initiated to examine the use of baricitinib or other JAK inhibitors such as ruxolitinib and tofacitinib in patients with COVID-19. Table 1 summarizes the ongoing trials addressing the efficacy of baricitinib. The first of these trials

[1]Sapienza Università di Roma, Dipartimento di Scienze Cliniche, Internistiche, Anestesiologiche e Cardiovascolari—Reumatologia, Rome, Italy. [2]Translational Immunology Section, Office of Science and Technology, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, MD, USA.
*Corresponding author. Email: gadinama@mail.nih.gov

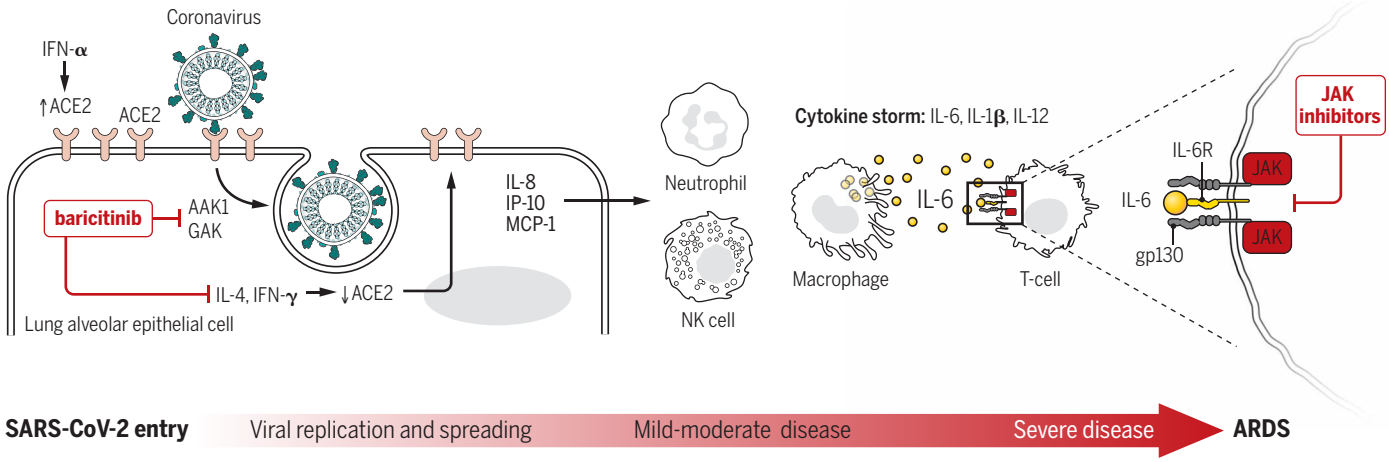

Fig. 1. JAK inhibitors in the pathogenesis of SARS-CoV-2 infection–associated ARDS. Once the SARS-CoV-2 virus has infected alveolar epithelial cells, activation of immune cells and release of chemokines result in recruitment of neutrophils, macrophages, and T_H1 cells enhancing lung inflammation. The hyperimmune response associated with massive cytokine release is responsible for the clinical evolution toward ARDS. Once interstitial pneumonia has developed, the down-regulation of ACE2 expression by epithelial cells has a detrimental effect by contributing to further lung injury. In the later stages of COVID-19 pneumonia, inhibition of multiple cytokines with JAK inhibitors may be helpful. The JAK inhibitor baricitinib may also impair the entry of SARS-CoV-2 by inhibiting the AAK1- and GAK-mediated endocytosis of virus through ACE2.

Table 1. Clinical trials addressing the safety and efficacy of baricitinib in COVID-19 patients. NR, nonrandomized; R, randomized; SA, single-arm; OL, open-label; DB, double-blind.

National Clinical Trial Number (NCT)	Sponsor	Study design	Regimen dose duration	Patients	Primary endpoints
04320277	Hospital of Prato (Italy)	NR/OL	4 mg/day 14 days	Hospitalized Laboratory-confirmed Mild-moderate pneumonia	% of patients requiring ICU admission
04321993	Nova Scotia Health Authority Dalhousie University (Canada)	NR/OL	2 mg/day 10 days	Hospitalized Laboratory-confirmed Mild-moderate pneumonia	Clinical status at day 15 as assessed on seven-point scale
04340232	University of Colorado Denver (USA)	SA/OL	2 mg/day 14 days	Hospitalized Laboratory-confirmed Mild-moderate pneumonia requiring supportive care	Clinical status at day 15 as assessed on eight-point scale
04346147	Hospital Universitario de Fuenlabrada Madrid (Spain)	R/OL	4 mg/day 7 days	Laboratory-confirmed Pneumonia Symptoms onset <7 days	Time to clinical improvement (increase of two points on eight-point scale)
04345289	Aalborg University Hospital (Denmark)	R/DB	4 mg/day 7 days	Hospitalized Laboratory-confirmed Pneumonia Symptoms onset <10 days	Composite outcome (all-cause mortality or need for invasive mechanical ventilation)

to report results (NCT043202770) was an open-label trial performed at the Hospital of Prato, Italy. This trial recruited hospitalized and laboratory-confirmed patients with COVID-19 with mild-to-moderate pneumonia. Twelve patients received baricitinib (4 mg for 14 days) in addition to standard of care (ritonavir-lopinavir and hydroxychloroquine) and were compared with 12 age- and sex-matched controls who received standard of care alone (*9*). The primary endpoint of the study was the percentage of patients treated with baricitinib requiring intensive care admission; secondary endpoints included the clinical course of the disease and treatment-induced adverse events (*9*). Baricitinib-treated patients achieved significantly greater improvements in clinical signs (fever, cough, and dyspnea), lung function tests, and C-reactive protein (CRP) values. None of the patients in the baricitinib arm was admitted in intensive care compared with 33% of the controls; moreover, after 2 weeks, 7 of 12 (58%) of the baricitinib-treated patients compared with 1 of 12 (8%) of controls were discharged (*9*). Despite these initial positive findings, there are several JAKi-associated issues to be considered. JAKi administration is known to

result in an increased risk of varicella zoster infection reactivation in patients with RA, which may cause concerns about the inhibition of antiviral cytokines—mainly type I IFN. However, SARS-CoV-2 takes advantage of an escape mechanism to block the IFN regulatory pathway at an early step; the highly pathogenic beta coronaviruses encode viral proteins that potently antagonize type I IFN production or degrade type I IFN mRNA; Chu *et al.* (*10*) showed that SARS-CoV-2 did not significantly trigger the expression of IFN and IFN-related genes in human lung tissues infected with SARS-CoV-2. Moreover, the type I IFN response is high in patients with mild to moderate COVID-19, but it is profoundly reduced in critically ill patients that display low plasma levels of IFN-α and IFN-β and downregulation of IFN-stimulated genes (*3*).

The preliminary clinical evidence that oral administration of baricitinib provides a benefit in COVID-19 (*8*) could be due to impairments in viral entry and/or by controlling the CRS-like excessive inflammation occurring in these patients (*5*). If the latter is a major component of the therapeutic efficacy, it will be important to assess circulating levels of these cytokines, or at least CRP levels, which are known to correlate with IL-6, before and after the initiation of the therapy. In light of the ability of JAKi to inhibit multiple cytokines, it would be expected that patients with the highest serum levels of IL-6 and other inflammatory cytokines would be the ones that would benefit the most from this class of drugs. Furthermore, the incidence of SARS-CoV-2 infection among patients with RA treated with maintenance baricitinib, compared with patients with RA treated with conventional synthetic or biological DMARDs, may provide clues as to whether the antiviral effect suggested by Stebbing and colleagues (*7*) provides pre- and early post-exposure protection to those receiving baricitinib.

Although public health officials work to resolve this global health threat as quickly as possible, physicians and patients want to be confident that any repurposed drugs adopted for widespread use in patients infected by SARS-CoV-2 have acceptable safety profiles. The reduction of NK cells detected during baricitinib and tofacitinib treatment suggests that use of these drugs in patients with COVID-19 should be limited in its duration. Another possible concern for using JAKi in COVID-19 is the thromboembolic risk associated with this class of drugs. At the later critical stages of the disease, most patients with COVID-19 develop coagulation abnormalities, which are associated with a poorer prognosis. The mechanisms explaining the coagulopathy are not fully clear, with direct effects of SARS-CoV-2 on endothelial cells, effects of the cytokine storm, and anti-phospholipid antibodies being potential contributors. In this scenario, any drugs with the potential of increasing thrombotic risk should be used cautiously. Within the next few weeks and months, the clinical approach to treating patients with COVID-19 will surely be refined, guided by the results from ongoing prospective, randomized trials and the role for JAKi as well as other small molecules that have the potential to interfere with the cytokine cascade driving CRS.

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