DR SARAH CHRISTINA JANE JORGENSEN (Orcid ID : 0000-0003-1333-0378)

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Baricitinib: A review of pharmacology, safety and emerging clinical experience in COVID-19

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AUTHORS

Sarah CJ JORGENSEN[1#]

Christopher LY TSE[2]

Lisa BURRY[1,2]

Linda D DRESSER[2,3]

AFFLIATIONS

1. Department of Pharmacy, Sinai Health System, Toronto, ON, Canada
2. Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada
3. Antimicrobial Stewardship Program, University Health Network, Toronto, ON, Canada

 # CORRESPONDING AUTHOR

Sarah CJ Jorgensen
Department of Pharmacy, Sinai Health System
600 University Ave., Toronto, ON M5G1X5, Canada
sarah.jorgensen@sinaihealth.ca

CONFLICT OF INTEREST

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ABSTRACT

A hyperinflammatory response to SARS-CoV-2 infection, reminiscent of cytokine release syndrome, has been implicated in the pathophysiology of acute respiratory distress syndrome and organ damage in patients with COVID-19. Agents that inhibit components of the pro-inflammatory cascade have garnered interest as potential treatment options with hopes that dampening the pro-inflammatory process may improve clinical outcomes. Baricitinib is a reversible Janus-associated kinase (JAK)-inhibitor that interrupts the signaling of multiple cytokines implicated in COVID-19 immunopathology. It may also have antiviral effects by targeting host factors that viruses rely for cell entry and by suppressing type I interferon driven angiotensin-converting-enzyme-2 up regulation. However, baricitinib's immunosuppressive effects may be detrimental during acute viral infections by delaying viral clearance and increasing vulnerability to secondary opportunistic infections. The lack of reliable biomarkers to monitor patients' immune status as illness evolves complicates deployment of immunosuppressive drugs like baricitinib. Furthermore, baricitinib carries the risk of increased thromboembolic events which is concerning given the proclivity towards a hyper-coagulable state in COVID-19 patients. In this article we review available data on baricitinib with an emphasis on immunosuppressive and antiviral pharmacology, pharmacokinetics, safety and current progress in COVID-19 clinical trials.

INTRODUCTION

Patients with severe acute respiratory syndrome coronavirus (SARS-CoV)-2 disease, (COVID-19), experience a wide spectrum of clinical manifestations and illness severity.[1, 2] Although most symptomatic patients have a relatively mild clinical course, approximately 20% require hospitalization and 20% of those hospitalized will be admitted to the intensive care unit (ICU).[2, 3] In some patients a sudden and rapid clinical deterioration manifesting as acute respiratory distress syndrome and multi-organ failure has been observed around day 7 to 10 of hospitalization.[1, 4] Interestingly, clinical deterioration often occurs when viral titers are declining leading some to postulate that an over

exuberant immune response may be involved in the underlying pathophysiology of organ damage.[5, 6] This theory is supported by the correlation between COVID-19 complications and elevated levels of acute phase reactants, coagulation abnormalities and hypercytokinemia, reminiscent of cytokine release syndrome.[4, 5, 7, 8] A number of agents that inhibit one or more components of the pro-inflammatory cascade are now being investigated in clinical trials with hopes that blunting this process may improve clinical outcomes.[9, 10]

The use of immunosuppressive drugs during an acute viral illness carries the risk delaying viral clearance and increasing vulnerability to secondary opportunistic infections.[9, 11] Coupling these drugs with effective antiviral agents, either sequentially or concurrently, may therefore be essential for positive patient outcomes.[12, 13] Antiviral drug discovery has traditionally focused on designing compounds that target essential viral components, including viral proteases or polymerases.[14] This approach has been successful for chronic viral infections such as HIV and hepatitis C.[15, 16] However, direct-acting antivirals are typically narrow in spectrum, take years or even decades to develop and may have a low barrier to resistance when used as monotherapy.[14] An alternative approach that may be more suitable for emerging viral pathogens is to repurpose approved drugs that target host functions that viruses rely on.[14] This may drastically reduce the costs and time devoted to early drug discovery and these agents may, in theory, have higher barriers to resistance since most resistance is secondary to mutations in the viral genome.[14]

Baricitinib ($C_{16}H_{17}N_7O_2S$, formerly LY3009104) is a small molecule reversible Janus-associated kinase (JAK)-inhibitor approved in over 65 countries for the treatment of adults with moderate to severe rheumatoid arthritis (RA).[1, 5, 17] The JAK/signal transducers and activators of transcription (STAT)-pathway mediates the signaling of multiple cytokines and interrupting this pathway may therefore be an attractive strategy to modulate the immunopathology seen with SARS-CoV-2 infection.[9, 12, 18] Furthermore, many drugs within this class exhibit antiviral effects, albeit often at supra-therapeutic concentrations, by targeting host factors that viruses usurp for cell entry.[12, 19] Baricitinib has the advantage of providing *in vitro* antiviral activity at concentrations achieved with approved dosing.[13, 20]

The purpose of this article is to review available data on baricitinib with an emphasis on immunosuppressive and antiviral pharmacology, pharmacokinetics (PK), safety and current progress in COVID-19 clinical trials.

DATA SOURCES

A literature search of PubMed was conducted on May 10, 2020 and updated on May 22, 2020 using various combinations of the search terms “baricitinib,” “LY3009104,” “Janus-associated kinase inhibitors,” “safety,” “adverse effects,” “infection,” “thrombosis,” “coronavirus,” “COVID-19,” and “severe acute respiratory syndrome coronavirus (SARS-CoV)-2.” Results were limited to English language articles. Articles were selected based on their relevance to baricitinib’s use in COVID-19, pharmacology, PK, and safety. The reference lists of relevant articles were examined to identify sources not captured in the electronic literature search. Additional data were obtained from ClinicalTrials.gov, bioRxiv, medRxiv, the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) drug approval documents.

PHARMACOLOGY AND PHARMACODYNAMICS

Basic and translational science have identified a wide array of subcellular pathways that regulate normal and aberrant immune responses.[18, 21, 22] One of these is the JAK / STAT pathway.[21, 22] The JAK/STAT pathway mediates signal transduction from extracellular stimuli, including cytokines, growth factors and hormones, to the nuclei of cells.[21, 22] Baricitinib exerts its anti-inflammatory effects through reversible JAK inhibition, as shown in Figure 1.[3] Signaling is initiated when cytokines bind to their receptor on the cell membrane.[22] This results in conformational changes that trigger activation of associated JAK complexes. JAK activation in turn leads to autophosphorylation and subsequent increased JAK kinase activity as well as phosphorylation of the intracellular portion of their cognate receptors.[22] Receptor phosphorylation creates a docking site for signaling molecules especially members of the STAT family.[22] Once docked to the receptor, STAT molecules are also phosphorylated by JAKs. The phosphorylated STATs are then released from the receptor, form homo- or hetero-dimers through reciprocal interactions with their newly phosphorylated tyrosine domains, and translocate to the cell nucleus where they bind to specific DNA sequences to activate target gene transcription.[22]

The JAK family is comprised of 4 cytoplasmic protein tyrosine kinases: JAK1, JAK 2, JAK3 and tyrosine kinase 2 (TYK2).[21, 22] Cytokine receptors recruit 2 of the 4 JAKs to the intracellular domain of the signaling complex (i.e. JAK1/JAK2, JAK1/JAK3, JAK1/TYK2, JAK2/TYK2, Figure 1).[18] Inhibition of one or both JAK monomers associated with the cytokine receptor is typically sufficient to interrupt signal transduction.[4] JAK1, JAK2 and TYK2 are expressed throughout the human body whereas JAK3 is primarily expressed by hematopoietic cells in the bone marrow. [4, 21] The various JAK complexes mediate distinct cytokine signaling pathways. For example, innate antiviral responses via type I interferon (IFN) are mediated by JAK1/TYK2 and IFN-gamma signaling is mediated by JAK1/JAK2.[4, 18, 23] IL-6, which has emerged as a strong predictor of poor outcomes in COVID-19, transduces signaling via complexes of JAK1, JAK2 and TYK2.[8, 9]

Baricitinib was designed to selectively inhibit JAK1 and JAK2 with less potency for JAK3. It has been postulated that sparing JAK3 could reduce the immunosuppression associated with pan-JAK inhibition. [1, 4] However as presented in Table 1, baricitinib's purported selectivity is only evident in cell-free assays but not recapitulated in cell-based assays.[4] Baricitinib 50% inhibitory concentrations (IC_{50}) for JAK complexes that mediate signaling for a wide variety of cytokines implicated in COVID-19 immunopathology generally fall below the free C_{max} values achieved with approved dosing (Tables 1 and 2).[1, 3, 4, 13]

ANTIVIRAL ACTIVITY

Baricitinib may also have antiviral activity. [12, 13, 24] It's potential antiviral activity was identified by searching a large repository of structured medical and drug information extracted using machine learning (Benevolent[AI], London, England). Nearly 50 currently approved drugs for variety of indications from oncology to auto-immune disorders were identified by this approach as inhibitors of host enzymes involved in regulating intracellular viral trafficking. Only baricitinib however showed inhibitory activity at clinically achievable serum concentrations.

Many viruses gain entry into human cells by hijacking host-derived membrane trafficking processes; one of the most well studied, is clathrin-mediated endocytosis.[25, 26] Clathrin is an endocytic coat protein that clusters on the inner leaflet of the plasma membrane to form the initial spherical cage-

like vesicle structure involved in endocytosis.[26] Viral internalization via clathrin-mediated endocytosis is shown in Figure 2. The process is initiated when the virus binds to the host cell surface receptor (angiotensin-converting enzyme 2 (ACE2) in the case of SARS-CoV-2).[25, 27] Receptor binding leads to activation of 2 host-derived kinases, AP2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK).[25, 28] AAK1 and GAK in turn phosphorylate and activate key host proteins called adaptor protein complexes (APs).[25, 28] Activated APs bind to the cytoplasmic tail of the cell-surface receptors and recruit clathrins to assemble into a cage-like structure in preparation for endocytosis.[25, 28] Next, the cell surface receptor with bound virus is invaginated into the cage-like structure which pinches off and traffics the virus and associated APs in early endosomes.[25, 28] In addition to clathrin recruitment, APs also interact with the cargo (in this case the virus) to facilitate intracellular transport and regulate *trans*-Golgi network trafficking involved in subsequent stages of the viral lifecycle.[25, 28]

Baricitinib has been shown to inhibit AAK1 and, to a lesser degree, GAK (Table 1) and may thereby impede viral cell entry and internal transport.[12, 13] It is uncertain if compounds need to inhibit both AAK1 and GAK to block SARS-CoV-2 viral cell entry although in murine infection models the combination of both sunitinib (an anticancer drug that inhibits AAK1) and erlotinib (an anticancer drug that inhibits GAK) was required to protect mice from lethal Ebola and dengue virus challenges.[19, 28] It should also be pointed out that, SARS-CoV-1 uses several different endocytic pathways for viral entry [25, 29] and if this is also true for SARS-CoV-2, baricitinib's inhibition of clathrin-mediated endocytosis could be circumvented by use of an alternative pathway.

An additional antiviral mechanism related to baricitinib's inhibitory effect on IFN signaling has been proposed.[24] As noted above, IFN responses are essential host antiviral defenses but recent work has revealed that type I IFN and to a lesser extent type II IFN up-regulate ACE2 expression in multiple human cell lines including upper airway epithelial cells and primary bronchial cells.[30] Suppressing type I IFN antiviral response could, in theory, decrease ACE2 expression and thereby interfere with the ability of SARS-CoV-2 to infect neighboring cells.[30] However, ACE2 is also counter-regulatory to the renin-angiotensin-aldosterone-system (RAAS) and has a protective effect against RAAS-related organ damage, including acute lung injury.[31] One of SARS-CoV-2's key virulence factors is its ability to down-regulated ACE2 expression after cell entry, thereby thwarting ACE2 lung protective effects.[31] It is conceivable that baricitinib's suppression of type I IFN signaling could amplify ACE2 down-

regulation, further diminishing its protective effects. The net effect of IFN suppression (beneficial versus detrimental) in the setting of COVID-19 might depend on the underlying immune status of the patient and the stage of infection.[30]

PHARMACOKINETICS

Table 2 summarizes pertinent baricitinib PK parameters which were derived from single and multiple-dose studies in healthy adult volunteers and RA patients.[1, 3, 32] After oral administration baricitinib is rapidly absorbed reaching peak plasma concentrations within 60 minutes.[1, 3] The absolute bioavailability is 79% and food has minimal impact on PK parameters.[1, 3] Baricitinib exhibits linear dose proportional PK following single oral doses between 1 mg and 20 mg with minimal accumulation for up to 28 days.[3, 32] Both C_{max} and area under the concentration time curve over 24 hours (AUC_{24}) values increase approximately 60% and 75% in patients with RA compared to healthy subjects, respectively and inter-individual variability is higher in RA patients.[1, 3] Exposure is also increased greater than 2-fold in those with moderate to severe renal impairment and end stage renal disease (ESRD). Exposure in patients with COVID-19 or other acute viral infections has not been reported at this time (acute infection at baseline was a contraindication for all RA clinical trials).[2] As shown in Table 1 and 2, baricitinib free C_{max} values with 4 mg once daily dosing exceed IC_{50} values for inhibition of cytokine-induced JAK/STAT signaling in cell-free and cell-based assays and concentrations also exceed the dissociation constant (K_d) for AAK1 but supratherapeutic levels may be required to inhibit GAK.[1, 4, 13] Additionally, PK modeling of 4mg once daily dosing showed that there is a 12 hour window when baricitinib serum levels fall below IC_{50} values for JAK complexes.[1] The clinical implications of this in the setting of COVID-19-related cytokine storm are unclear.

Plasma protein binding for baricitinib is 50% and is not concentration dependent. The mean volume of distribution is 1.1 L/kg, suggesting moderate distribution into tissues.[1, 3] Epithelial lining fluid concentrations have not been reported.

Baricitinib is primarily cleared by renal elimination through both filtration and active secretion.[1, 3] Approximately 75% is excreted in the urine (69% unchanged) and 20% in the feces (15% unchanged).[1, 3] The half-life is 6 to 9 hours in healthy volunteers but increases to 12 hours in RA patients and 19 hours in subjects with severe renal impairment or ESRD.[1, 3] Baricitinib is effectively

dialyzed with a mean clearance by hemodialysis of 6 L/h.[3] The impact of continuous renal replacement therapy and extracorporeal membrane oxygenation on baricitinib PK have not been described at this time. In population PK analyses, body weight did not have a clinically meaningful impact on baricitinib clearance, however obese RA patients have been reported to have lower response rates.[3, 33] As discussed in the DRUG INTERACTIONS section, baricitinib is a substrate of several drug transporters which impact absorption, distribution and elimination.[3]

Only a small fraction (6%) of baricitinib is metabolized, predominantly by CYP3A4, and there is no clinically relevant difference in baricitinib exposure in patients with moderate hepatic function (Child-Pugh B).[3]

Baricitinib PK has been evaluated in a small number of pediatric patients (n=18, mean age 12.5 years, weight 9.2 kg – 84.3 kg) who received the drug through a compassionate use program for rare Mendelian autoinflammatory diseases.[34] Weight and renal function significantly influenced volume of distribution and clearance respectively, suggesting the need for weight and renal function based dosing. Importantly the half-life of baricitinib was significantly shorter in children, especially among those weighing less than 40 kg, and the authors of this study recommended twice daily to four times daily dosing in children depending on renal function.[34]

PK parameters in pregnant or breastfeeding women have not been reported at this time. It is not known if baricitinib crosses the placenta in humans. Skeletal malformations and developmental toxicity have been observed in the offspring of pregnant rats exposed to supra-therapeutic doses of baricitinib.[4] Effects on fertility in animals have been inconsistent.[4]

DRUG-DRUG INTERACTIONS

Baricitinib is not an inhibitor or inducer of CYP450 enzymes or drug transporters (P-glycoprotein, BCRP, OATP1B1, OATP1B3, OCT 1-3, MATE-1, MATE2-K) at clinically relevant concentrations.[1, 3] Although a small fraction (6%) of baricitinib is metabolized by CYP3A4, co-administration with ketoconazole (a strong CYP3A4 inhibitor) or rifampin (a strong CYP3A4 inducer) did not have a clinically meaningful impact on baricitinib PK.[1, 3]

As noted in the PK section, baricitinib is a substrate of several drug transporters (P-glycoprotein, BCRP, MATE2-K, OAT3).[1, 3] Co-administration with cyclosporine (P-glycoprotein inhibitor) did not result in clinically relevant changes to baricitinib PK however, co-administration with probenecid (a strong OAT3 inhibitor) lead to decreased renal clearance and a ~2-fold increase in AUC.[1, 3] Dose reduction is recommended in patients taking strong OAT3-inhibitors (see DOSAGE AND ADMINISTRATION section).[1, 3] Based on PK modeling, less potent OAT3 inhibitors such as ibuprofen and diclofenac are expected to have minimal impact on baricitinib PK.[3] Studies examining the impact of BCRP or MATEK-2 inhibitors have not been reported at this time. Increased gastric pH and the use of proton-pump inhibitors does not alter overall exposure to baricitinib although the time to peak plasma concentrations was prolonged to 2 hours with concomitant administration of omeprazole.[3] No signal of QT_c interval prolongation has been observed with baricitinib doses up to 40 mg in healthy volunteers. [2, 34]

CLINICAL EXPERIENCE FOR COVID-19

Baricitinib is under investigation in multiple ongoing clinical studies (Table 3), including the second iteration of the National Institute of Allergy and Infectious Diseases (NIAID) Adaptive COVID-19 Treatment Trial (ACTT-2).[27-29] ACTT-2 is an adaptive, randomized, double-blind, active-controlled multinational study.[27, 29] Hospitalized patients with laboratory confirmed SARS-CoV-2 infection and one of the following are eligible for enrolment: infiltrates on chest imaging, an oxygen saturation ≤ 94% on room air, need for supplemental oxygen or need for mechanical ventilation.[29] The primary endpoint is time to recovery within 28 days after randomization using a 3-point ordinal scale.[29] In the first iteration of the study (ACTT-1), patients were randomized to the antiviral drug, remdesivir, or placebo.[33] Preliminary results were recently published after enrolling over 1000 patients: the median time to recovery was significantly shorter in the remdesivir group (11 days vs. 15 days, hazard ratio 1.32; 95% confidence interval 1.12 – 1.55).[33] Moving forward in ACTT-2, all patients will receive remdesivir and additionally be randomized to baricitinib 4 mg daily or placebo for up to 14 days.[27]

The off-label use of baricitinib in patients with COVID-19 was recently reported in a small before-after study of patients at centers in the Northern Italian province of Prato.[35] This study included consecutive patients hospitalized between March 16 and 30, 2020 with moderate COVID-19 defined as a positive SAR-CoV-2 real-time polymerase chain reaction (RT-PCR) nasopharyngeal or

oropharyngeal swab, evidence of pneumonia on chest imaging and 3 of fever, cough, myalgia or fatigue. Patients (n=12) were treated with lopinavir/ritonavir (250 mg twice daily) plus baricitinib (4 mg daily) for 14 days. Those with thrombophlebitis, latent tuberculosis and pregnant or breastfeeding women were excluded. An equal number of patients with moderate COVID-19 admitted in the week preceding this period served as the control group. All patients in the control group received lopinavir/ritonavir (250 mg twice daily) plus hydroxychloroquine (400 mg daily) for 14 days. [35]

Overall, recorded demographics, comorbidities and baseline signs and symptoms were similar in the 2 groups.[35] The median oxygen saturation was 91-92% and none of the patients resided in the ICU at enrolment. At 2 weeks, most clinical and laboratory parameters had normalized in the baricitinib group, no patients required ICU admission and 7 (58%) were discharged home. In the control group, there was no significant improvement in most clinical and laboratory parameters, 4 (33%) required ICU admission and 1 (8%) was discharged home. With regards to safety, no new bacterial, viral or opportunistic infections were reported in either group. Baricitinib (and lopinavir/ritonavir) was stopped in 1 patient after 10 days due to increased transaminases.[35] Platelets increased from a median of 203 x 10^9/L at baseline to 354 x 10^9/L at day 14 in patients who received baricitinib ($p = 0.018$). There was no change in platelets over 2 weeks in the control group (see SAFETY section for further discussion on increased platelets associated with baricitinib).[35]

The authors of this report rightly acknowledge its main weaknesses including the lack of a randomized control group and the small sample size.[35] The use of a historical control group in an emerging infectious disease is fraught with limitations due to rapidly evolving knowledge and patterns of care. In addition, the use of concomitant antiviral and adjunctive agents complicates interpretation. The small sample size and short duration of follow-up do not allow a meaningful assessment of safety. Finally, although the authors report that antibiotics were only used when bacterial infection was suspected, it is unclear if any were in fact administered; this information is important when interpreting rates of secondary infections.

SAFETY

Pooled data from 3492 baricitinib exposed patients (7860 patient-years) enrolled in Phase 2 and 3 RA clinical trials together with long-term extensions of these studies in the baricitinib development

program has been used to characterize baricitinib's safety profile.[2, 23] One caveat to these analyses is that patients in the placebo or baricitinib 2 mg/day arms of many studies were allowed to crossover to the 4 mg/day group after week 16 which complicates interpretation and raises the possibility that some risks in the 4 mg/day group may be overestimated. Furthermore, as discussed below, although many adverse effects appeared to be dose related, far fewer patients were exposed to 2 mg/day so there is more uncertainty in relative risk estimates. In ongoing COVID-19 studies, the duration of baricitinib therapy is typically 7 to 14 days. Safety data by contrast is derived from patients who received baricitinib for months and many adverse effects manifested after prolonged exposures. Finally, all trials excluded patients with acute infections at baseline limiting generalizability for COVID-19 patients.

The most common side effects with baricitinib are upper respiratory tract infection (14-22%), headache (11-24%) and nasopharyngitis (11-18%).[2] In addition, dose-related changes in multiple laboratory parameters have been observed in patients treated with baricitinib.[2, 23] Many of these have been reported with other JAK-inhibitors and include rapid and sustained decreases in neutrophil and lymphocyte counts, decreases in hemoglobin, small increases in creatinine (< 0.1 mg/dL), increases in lipid parameters, elevations in liver enzymes and bilirubin and increases in creatine phosphokinase (CPK).[18, 36] Decreases in lymphocyte counts have been associated with higher rates of treatment emergent infections among RA patients in clinical trials.[2, 34] Lymphopenia is one of the most prominent laboratory abnormalities in COVID-19 patients and lower lymphocyte counts have been associated with more severe disease.[37, 38] In addition to being quantitatively reduced, lymphocytes from SARS-CoV-2 infected patients also show functional exhaustion and decreased functional diversity.[39] The consequences of exacerbating this immunophenotype with baricitinib requires further study.

The significance of modest increases in lipid parameters has been difficult to predict; major cardiac events have occurred in a small number of patients in RA trials, most commonly in extension phases after week 52 but a clear a link with lipid parameters has not been reported. Patients with preexisting cardiovascular diseases are at increased risk of the most severe COVID-19 complications.[32, 40] Furthermore, myocardial injury has been observed in nearly 30% of hospitalized patients with COVID-19 and is significantly associated with higher short term mortality.[32, 40] However, in this

setting, the underlying pathogenesis of myocardial injury may be related to the pro-inflammatory response to infection [40] and countering this with baricitinib could conceivably be protective.

Although increases in liver enzymes and bilirubin have been reported with baricitinib, no cases of liver injury satisfying Hy's law have occurred.[2, 34] Thirteen patients were withdrawn from studies due to liver function test abnormalities (vs. 1 withdrawal with placebo) and patients with transaminase elevations at baseline (> 1.5 x the upper limit of normal) have been excluded from all studies.[2, 34] Many patients who experienced liver function test abnormalities were receiving concomitant hepatotoxic drugs (i.e. methotrexate or isoniazid). In case series, between 2% and 11% of patients with COVID-19 had chronic liver comorbidities and 14% to 53% had elevated transaminases during the course of the disease (reviewed in [40]). Furthermore, higher rates of liver dysfunction have been correlated with more severe COVID-19.[40] Hepatotoxic drug effects may be difficult to detect in these circumstances and clinicians may need to maintain a high index of suspicion.

In the clinical trials program, CPK elevations were not associated with muscle pain or rhabdomyolysis.[2, 34] However, a recent report describes 2 RA patients who developed unexplained lower and/or upper extremity muscle pain and joint swelling coupled with moderate CPK elevations following the initiation of baricitinib.[41] In both cases, clinical and biochemical resolution occurred rapidly following baricitinib discontinuation.[41] The mechanism behind baricitinib-associated CPK elevations has not been widely studied although experimental evidence supports the theory that certain pro-inflammatory cytokines may block differentiation of myoblasts into mature myocytes.[42] CPK increases observed with JAK-inihibitors may therefore represent recovery of muscle development and CPK expression. [42]

Increased CPK is correlated has been with mortality in COVID-19 [4] and rhabdomyolysis has been reported as a late complication.[17] The interaction between possible baricitinib-associated CPK elevations and those secondary to COVID-19 requires further study.

Increased platelet counts is a unique baricitinib effect and has not been observed with other JAK-inhibitors.[2, 36, 43] In fact, small decreases in platelets and occasional thrombocytopenia occur 2 other JAK-inhibitors, tofacitinib and upadacitinib.[36, 43] With baricitinib, platelet counts increase rapidly after initiation and peak around week 2 (mean increase 50 x 10^9/L).[2, 23] Thereafter they decline and stabilize but remain above placebo and comparators for the duration of therapy. Thrombocytosis

appears to be dose related but still occurs with the 2 mg/day dose. No clear temporal or quantitative association between platelet increases and thromboembolic events (discussed below) has been established.[2, 23] The etiology is not known although the prevailing theory, based on animal experiments, implicates selective JAK2 inhibition in increased circulating thrombopoietin (TPO, the hormone that stimulates megakaryopoiesis and platelet production) levels. TPO signals are transduced by JAK2. Knockout of the *Jak*2 gene in hematopoietic stem cells (HSCs) results in thrombocytopenia in mice.[44] In contrast deletion of *Jak*2 or the TPO receptor gene in megakaryocytes and mature platelets results in thrombocytosis.[23, 45, 46] Megakaryocytes and mature platelets are responsible for internalizing and degrading circulating TPO by a JAK2 dependent mechanism.[23, 45, 46] Thus it is possible that predominant JAK2 inhibition at the level of megakaryocytes and mature platelets may lead to increased circulating TPO resulting in the increased platelet counts seen with baricitinib. JAK-inhibitors that are less selective for JAK2 may act mainly on JAK2 signaling at the level of HSCs to decrease platelet production.[23] Early case series from Wuhan, China suggested thrombocytopenia was a prominent feature of severe COVID-19.[47] For unclear reasons, later studies and those from other regions have shown normal or even elevated platelet counts in COVID-19 patients. [48, 49] The impact of thrombocytosis secondary to baricitinib in the setting the COVID-19 coagulopathy is difficult to predict.

Besides common side effects and changes in laboratory parameters, baricitinib has been associated with serious adverse effects including infections, thrombosis, malignancy, gastrointestinal perforations, and major cardiovascular events.[2, 23] Adverse effects of particular relevance to COVID-19 patients are infection and thrombosis and are expanded upon below.

Overall the incidence of serious and opportunistic infections in RA patients treated with JAK-inhibitors is comparable to other biological DMARDs, however the risk of viral infections, specifically herpes zoster virus (HZV) reactivation, appears to be higher with JAK-inhibitors.[23] HZV reactivation rates are approximately 1.5 to 2-fold higher among RA patients taking JAK-inhibitors (3.2 – 4.0 cases / 100 patient years) compared to the general RA population. [2, 11, 23] Other factors associated with decreased cell-mediated immunity, such as older age and concomitant steroid use, amplify this risk.[23] The incidence of HZV and other infections were numerically higher with baricitinib 4mg/day versus 2 mg/day.[23] Type I IFNs orchestrate a critical antiviral defense via the JAK/STAT pathway and their inhibition by baricitinib is thought to be responsible for HSV reactivation.[9, 23] Critically ill patients

with COVID-19 demonstrate an impaired type I IFN response and the degree of impairment has been correlated with higher viral loads and poor outcomes.[50, 51] Interestingly, type I IFN deficiency was associated with an exacerbated inflammatory response with markedly elevated levels of IL-6 and tumor necrosis factor (TNF)-α. These data suggest timing of baricitinib initiation may be important to both avoid amplifying impaired innate immunity and suppress a harmful hyperinflammatory response. An additional concern with baricitinib use in COVID-19 is its inhibition of signaling from mediators of immune restoration (i.e. IL-2 and IL-7) which may make patients more vulnerable to nosocomial infections.[9] Although rates of co- or secondary infections in COVID-19 patients have been low,[52, 53] little is known about incidence with the use of immunosuppressive drugs.

With regards to thrombosis, there was a numerical imbalance in both arterial and venous thromboembolic events (VTE) not favoring baricitinib treated patients in pooled safety data, primarily with 4 mg/day.[2, 34] Five VTEs occurred in patients receiving baricitinib 4 mg/day during the first 16 weeks of therapy (compared to zero in the baricitinib 2 mg/day and placebo groups) and additional events continued to accumulate in both the 4 mg/day and 2 mg/day groups with extended follow-up. In total 39 VTE have been reported with baricitinib in the clinical trials program (34 at 4 mg/day and 5 at 2 mg/day) compared to none with placebo (VTE incidence rates 0.6 / 100 patient year and 0.4 / 100 patient year for 4 mg/day and 2 mg/day, respectively). Twenty-nine arterial thrombotic events have also been reported in patients who received baricitinib (incidence rates 0.5 / 100 patient year and 0.3 / 100 patient year for 4 mg/day and 2 mg/day respectively) versus 1 event with placebo.[2, 34] It should be noted that in population-based observational studies, VTE rates among individuals with RA on DMARDs range from 0.68 to 1.63 /100 patient years, in line with what was observed in the baricitinib RCTs, however differences in study designs and patient populations make such comparisons problematic.[2, 34] Furthermore, an increased incidence of thromboembolic events was also recently reported with higher doses of tofacitinib, another JAK-inhibitor used for RA.[54] Thrombotic events and other dose-related adverse effects coupled with the absence of a clear efficacy benefit in RA with the 4 mg/day versus 2 mg/day dose were the primary reasons behind the FDAs failure to approve the manufacturer's first submission in 2017.[2, 34] Baricitinib was approved one year later but only at the lower 2 mg/day dose.[2, 34] Health Canada has similarly only approved the 2 mg/day dose.[17] Four mg/day has been approved in some European and Asian countries however.[1] (see DOSAGE AND ADMINISTRATION section).

The coagulation system is closely linked to inflammation through the innate immune system and patients with COVID-19 appear to have an increased proclivity towards immunothrombosis.[49, 55] Common coagulation abnormalities include elevations in D-dimer and fibrinogen and prolonged prothrombin time.[49, 55] Published series also describe what appears to be a higher than expected incidence of VTE.[49, 55] Baricitinib's inhibition of inflammatory mediators that also drive immunothrombosis could have collateral benefits of reducing hypercoagulability; it is equally plausible however that baricitinib's pro-thrombotic tendencies could be detrimental. Moving forward, thorough baseline risk assessment and use of the minimally effect dose will be important in minimizing iatrogenic harm. Suggested monitoring parameters for patients receiving baricitinib are shown in Table 4.

DOSAGE AND ADMINISTRATION

When used for RA, baricitinib is taken once daily by mouth with or without food. The recommended starting dose in Europe is 4 mg/day with the option to decrease to 2 mg/day when RA signs and symptoms are controlled.[1] In Canada and US, only 2 mg/day is approved.[2, 17] As shown in Table 3, both 2 mg/day and 4 mg/day are being tested in clinical trials. Recommendations for dosage reductions vary by country. The EMA recommends a 50% dose reduction in the following patients: age ≥ 75 years, a history of chronic or recurrent infections, creatinine clearance (CrCl) between 30 mL/min and 60 mL/min, and concomitant use of a strong OAT3-inhibitor.[1] According to current prescribing information, baricitinib should not be initiated and therapy should be interrupted for the following laboratory parameters: absolute lymphocyte count < 0.5 x 10^9/L, absolute neutrophil count < 1 x 10^9/L and hemoglobin < 8 g/mL.[1, 2, 17] Baricitinib is contraindicated in patients with CrCl < 30 mL/min.[1, 2, 17] Baricitinib is only available as a film-coated, immediate release tablet.[1] There is no published data on the stability and bioavailability of crushed/dissolved tablets or extemporaneously compounded suspensions at this time.

DISCUSSION

A growing body of evidence suggests that the host immune response to SARS-Cov-2 infection may be critically import in determining outcomes.[8, 37-39, 47, 50] This has bolstered enthusiasm about

treatment strategies aimed at attenuating both pathogen virulence and the pro-inflammatory phenotype seen in the many critically ill patients with COVID-19.[5, 9, 12, 13, 20, 56] As detailed in this review, baricitinib pairs immunosuppressive properties with antiviral activity making it a logical candidate for further evaluation in COVID-19 clinical trials .[9, 12, 13, 20]

It is unlikely that a single treatment strategy will help all patients with COVID-19 or have the same effect in an individual patient as illness evolves over time. For many years, an uncontrolled pro-inflammatory response was thought to be the driver of poor outcomes in sepsis.[57, 58] On the basis of this theory and supportive pre-clinical data, multiple immunosuppressive agents were investigated in sepsis but with uniformly disappointing results.[57-62] We now know that anti-inflammatory mediators, which invoke a state of immunoparalysis, also contribute to poor outcomes by impairing the host's ability to clear infection and increasing vulnerability to secondary opportunistic infections.[57] Our understanding of the pathogenesis of and immune response to COVID-19 is rapidly evolving and, like sepsis, relative immunodeficiency also appears to be at play.[9, 37, 39, 50] At this time we do not have a reliable way to gauge whether the over-ruling response is pro or anti-inflammatory and this complicates deployment of immunosuppressive drugs like baricitinib. If given to the wrong patient (i.e. a patient with a predominantly immunosuppressed phenotype) or at the wrong time during the illness, these drugs could cause harm by inhibiting the cytokines required for viral clearance (type-I IFNs) or immune restoration (IL-2, IL-7).

Baricitinib's associated with thromboembolic events is equally concerning in the context of treating patients with COVID-19. Markers of systemic coagulation activation have been widely reported in patients with COVID-19 and a more pronounced prothrombotic state has been correlated with a more severe disease course and poor outcomes.[4, 47, 48] These patients also have multiple thrombotic risk factors related to critical illness and the supportive care they receive. The ability to detect a thrombotic safety signal related to baricitinib may be challenging in patients with COVID-19 since pulmonary embolism symptoms overlap with symptoms of COVID-19 and imaging may not be feasible.

CONCLUSIONS

This review highlights the current challenges faced when balancing potential risks and benefits of immunotherapies for patients with COVID-19. Moving forward, it is incumbent on researchers to develop and validate reliable tools to classify and monitor the overall immune status of patients with COVID-19 to help guide appropriate use of drugs like baricitinib.

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Table 1: Anti-inflammatory and antiviral activity of baricitinib (adapted from [4, 13, 20]

JAK enzymes (cell-free)	**Baricitinib mean IC_{50} (nM)**	**JAK enzyme pair (cell-based)[a]**	**Baricitinib mean IC_{50} (nM)**
JAK1	5.9	JAK1/JAK2	32.8
JAK2	5.7	JAK1/JAK3	55.4
JAK3	>400	JAK1/TYK2	71.6
TYK2	53	JAK2/TYK2	69.0
NAK enzymes (cell-free)	**Baricitinib K_d (nM)**	**NAK enzymes (cell-based)[b]**	**Baricitinib K_d (nM)**
AAK1	17	AAK1	34
GAK	136	GAK	272

a. Across multiple cell-types including B-cells, CD^{4+} T-cells, CD^{8+} T-cells, Natural killer cells and monocytes

b. Not directly measure; calculated based on ratio of cell-based to cell-free inhibition of JAK enzymes [13]

AAK1: AP2-associated protein kinase 1; GAK: cyclin G-associated kinase; JAK: Janus-associated kinase; NAK: numb-associated kinase; TYK2: tyrosine kinase 2

Table 2: Pharmacokinetic parameters of baricitinib 4 mg orally once daily (adapted from [1, 3])

Parameter	**Value**	
$C_{max, ss}$[a]	Total	Free
	53.4 ng/mL 143.8 nM[b]	26.7 ng/mL 71.9 nM[b]
$C_{min, ss}$[a]	Total	Free
	6.9 ng/mL 18.6 nM[b]	3.5 ng/mL 9.3 nM[b]
AUC_{24}[a]	477.6 ng*h/mL 1285.9 nM[b]	
Bioavailability	79%	
V_d	75.7 L	
Free fraction	50%	
T ½ Healthy subjects RA patients	 6 – 9 hours 12 hours	

a. Concentrations from studies in patients with RA

b. Calculated based on molecular mass 371.42 [1]

AUC: area under the concentration time curve; $C_{max, ss}$: maximal concentration at steady state; $C_{min, ss}$: minimum concentration at steady state; JAK: Janus-associated kinase; RA: rheumatoid arthritis; T ½: half-life; V_d: volume of distribution

Table 3: Ongoing clinical studies registered on ClinicalTrials.gov of baricitinib for COVID-19 (adapted from [27, 54])

ClinicalTrials.gov Identifier	Study design	Intervention/ treatment of interest	Location	Primary outcome	Target sample size	Sponsor
NCT04280705	Adaptive, randomized, multicenter, double-blind, placebo-controlled	• Remdesivir IV 200 mg day 1 then 100 mg days 2 – 10 x 10 days PLUS one of: • Baricitinib 4 mg PO OD x 14 days • Placebo x 14 days	Multinational	Time to recovery through day 29 according to 3-point ordinal scale	1000	National Institute of Allergy and Infectious Diseases (NIAID)

NCT04340232	Prospective, single arm, single-center, open-label	• Baricitinib 2 mg PO OD x 14 days	USA	Grade 3 or 4 adverse events	80	University of Colorado
NCT04390464	Randomized, multicenter, parallel assignment, open label	• Baricitinib 4 mg PO OD x 14 days • Ravulizumab IV (weight-based dosing) on day 1 • Standard of care	UK	Time to composite endpoint up to day 14 defined as 1 of: death, mechanical ventilation, ECMO, CV support or renal failure	1167	Cambridge University Hospitals NHS Foundation Trust
NCT04362943	Retrospective, observational, single-center	• Baricitinib • Anakinra	Spain	All-cause mortality	576	Complejo Hospitalario Universitario de

	cohort study					Albacete
NCT04346147	Randomized, single-center, parallel assignment, open-label	• Hydroxychloroquine 200 mg PO BID x 7 days PLUS one of: • Baricitinib 4 mg PO OD x 7 days Lopinavir/ritonavir • 200/50 mg PO OD x 7 days • Imatinib 400 mg PO OD x 7 days	Spain	Time to clinical improvement on 7-point ordinal scale	165	Hospital Universitario de Fuenlabrada
NCT04320277	Non-randomized, before-after, single-center	• Lopinavir/ritonavir 200/50 mg PO OD x 7 days PLUS Baricitinib 4 mg PO OD x 14 days	Italy	ICU transfer	200	Hospital of Prato

		• Antiviral and/or hydroxychloroquine				
NCT04373044	Prospective, single-arm, two-center, open-label	• Baricitinib 4 mg PO OD x 14 days PLUS one of the following at the treating physician's discretion: • Hydroxychloroquine Lopinavir/ritonavir • Remdesivir (doses not reported)	USA	Death or mechanical ventilation at day 14	59	University of Southern California
NCT04321993	Non-randomized, multi-center, parallel	• Baricitinib 2 mg PO OD x 10 days Hydroxychloroquine 400 mg PO BID x	Canada	Clinical improvement on 7-point ordinal scale at day 15	1000	Lisa Barrett

	assignment, open label	10 days • Lopinavir/ritonavir 500/100 mg PO BID x 10 days				
NCT04345289	Adaptive, multicenter, randomized, double-blind, placebo-controlled	• Baricitinib 4 mg PO OD x 7 days • Convalescent plasma 600 mL IV x 1 dose • Sarilumab 200 mg SC x 1 dose • Hydroxychloroquine 600 mg PO OD x 7 days • Placebo	Denmark	All-cause mortality of need for mechanical ventilation at day 28	1500	Thomas Benfield

BID: twice daily; CV: cardiovascular; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit; IV: intravenous; OD: once daily; PO: orally; SC: subcutaneous; UK: United Kingdom; USA: United States of America

Table 4: Laboratory and clinical monitoring parameters while receiving baricitinib[10, 43, 63, 64]

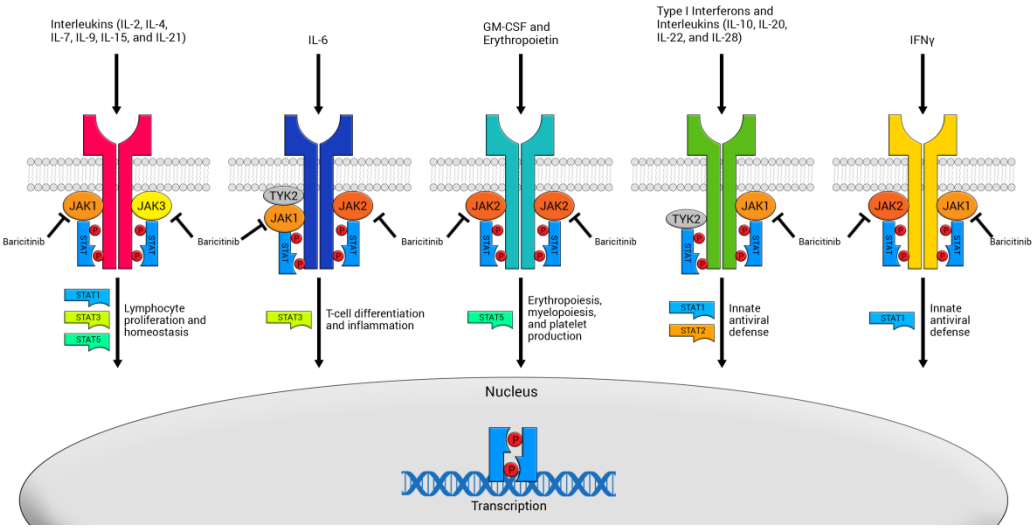
- Serum creatinine
- Absolute lymphocyte count[a]
- Absolute neutrophil count[b]
- Hemoglobin[c]
- Platelets
- ALT
- AST
- Bilirubin
- CPK
- LDL / HDL (if prolonged use)
- Signs and symptoms of infection
- Signs and symptoms of thromboembolic events

a. When used for RA it is recommended to interrupt therapy when the absolute lymphocyte count falls below 500 cells/mm^3

b. When used for RA it is recommended to interrupt therapy when the absolute neutrophil count falls below 1000 cells/mm^3

c. When used for RA it is recommended to interrupt therapy when hemoglobin falls below 8 g/dL

ALT: alanine aminotransferase; AST: aspartate transaminase; CPK: creatine phosphokinase; HDL: high density lipoprotein; LDL: low density lipoprotein



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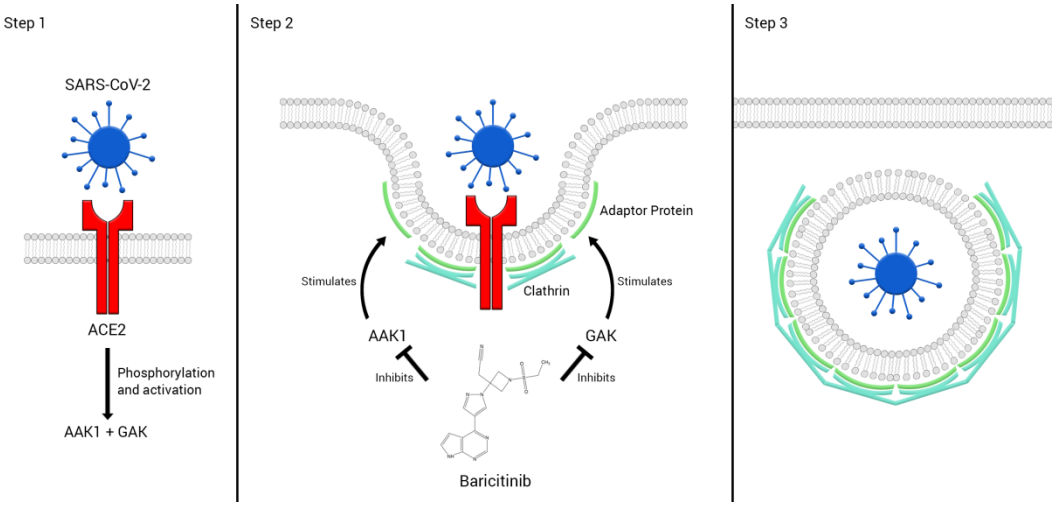

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