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JAK inhibitors and COVID-19

Gabriel Levy ,[1,2,3] Paola Guglielmelli,[4,5] Peter Langmuir,[6] Stefan N Constantinescu [1,2,3,7]

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[1]Signal Transduction and Molecular Hematology, Ludwig Institute for Cancer Research, Brussels, Belgium
[2]Signal Transduction on Molecular Hematology, de Duve Institute, Université Catholique de Louvain, Bruxelles, Belgium
[3]WELBIO, Walloon Excellence in Life Sciences and Biotechnology, Brussels, Belgium
[4]Department of Clinical and Experimental Medicine, University of Florence, Firenze, Italy
[5]Center of Research and Innovation for Myeloproliferative Neoplasms (CRIMM), Azienda Ospedaliero Universitaria Careggi, Firenze, Italy
[6]Oncology Targeted Therapeutics, Incyte Corp, Wilmington, Delaware, USA
[7]Nuffield Department of Medicine, Oxford University, Ludwig Institute for Cancer Research, Oxford, UK

Correspondence to
Dr Stefan N Constantinescu; stefan.constantinescu@uclouvain.be

ABSTRACT

During SARS-CoV-2 infection, the innate immune response can be inhibited or delayed, and the subsequent persistent viral replication can induce emergency signals that may culminate in a cytokine storm contributing to the severe evolution of COVID-19. Cytokines are key regulators of the immune response and virus clearance, and, as such, are linked to the—possibly altered—response to the SARS-CoV-2. They act via a family of more than 40 transmembrane receptors that are coupled to one or several of the 4 Janus kinases (JAKs) coded by the human genome, namely JAK1, JAK2, JAK3, and TYK2. Once activated, JAKs act on pathways for either survival, proliferation, differentiation, immune regulation or, in the case of type I interferons, antiviral and antiproliferative effects. Studies of graft-versus-host and systemic rheumatic diseases indicated that JAK inhibitors (JAKi) exert immunosuppressive effects that are non-redundant with those of corticotherapy. Therefore, they hold the potential to cut-off pathological reactions in COVID-19. Significant clinical experience already exists with several JAKi in COVID-19, such as baricitinib, ruxolitinib, tofacitinib, and nezulcitinib, which were suggested by a meta-analysis (Patoulias *et al*.) to exert a benefit in terms of risk reduction concerning major outcomes when added to standard of care in patients with COVID-19. Yet, only baricitinib is recommended in first line for severe COVID-19 treatment by the WHO, as it is the only JAKi that has proven efficient to reduce mortality in individual randomized clinical trials (RCT), especially the Adaptive COVID-19 Treatment Trial (ACTT-2) and COV-BARRIER phase 3 trials. As for secondary effects of JAKi treatment, the main caution with baricitinib consists in the induced immunosuppression as long-term side effects should not be an issue in patients treated for COVID-19.
We discuss whether a class effect of JAKi may be emerging in COVID-19 treatment, although at the moment the convincing data are for baricitinib only. Given the key role of JAK1 in both type I IFN action and signaling by cytokines involved in pathogenic effects, establishing the precise timing of treatment will be very important in future trials, along with the control of viral replication by associating antiviral molecules.

INTRODUCTION

Cytokine receptors and the Janus kinase-Signal Transducers and Activators of Transcription pathway

Cytokines are alpha-helical proteins of 160–170 aminoacids that are secreted and act on target cells as a function of expression and exposure on their surface of specific receptors. They are fundamentally required for blood formation and regulation of the immune response. In blood formation, on commitment to differentiation of hematopoietic stem cells (HSCs), lineage specific cytokines regulate the survival, proliferation and differentiation of progenitors and the final blood levels.[1]

Cytokines act via specific cytokine receptors. The human genome codes for over 40 cytokine receptors. They all signal via Janus kinases (JAKs), initially called Just Another Kinases, that are appended non-covalently to their cytosolic tails. Four JAKs are coded by the human genome, namely JAK1, JAK2, JAK3 and TYK2. Activated receptors induce via JAKs the activation of Signal Transducers and Activators of Transcription (STATs). There are 7 STATs coded by the human genome. Several receptors use the same JAKs and sharing of JAKs allows specific signals by the different receptors and cytokines, but different outputs.[1] The cytokine receptor superfamily is divided in type 1 and type 2 families (figure 1) and this distinction is derived from different sequence features, such as conserved WSXWS motifs in the extracellular domains and boxes 1 and 2 motifs in the cytosolic domains for type 1 (figure 2A).[1] The type 1 family consists of homodimeric receptors (for Epo, Tpo, GCSF, Growth Hormone, Prolactin), heterodimeric receptors (for IL-3, IL-5, GM-CSF), using JAK2, hetero-oligomeric receptors, using JAK1 and to some extent JAK2 and TYK2 (represented by the IL6 family receptors),[2] and finally the gamma chain using receptors (namely those for IL-2, IL-7, IL-9, IL-15, IL-21) (figure 1) which are composed of a specific chain bound to JAK1 and a common chain shared by all these receptors (the common gamma-chain or IL-2R subunit gamma), bound to JAK3. The type 2 family consists of receptors for type I interferon (bound to JAK1 and TYK2) (figure 3),[3] for type II interferon or IFN-gamma (bound to JAK1 and JAK2) (figure 3)[4] and receptors for IL-10, IL-20, IL-22 to IL-24 and others, using JAK1 or JAK2 and TYK2. The fundamental mechanism

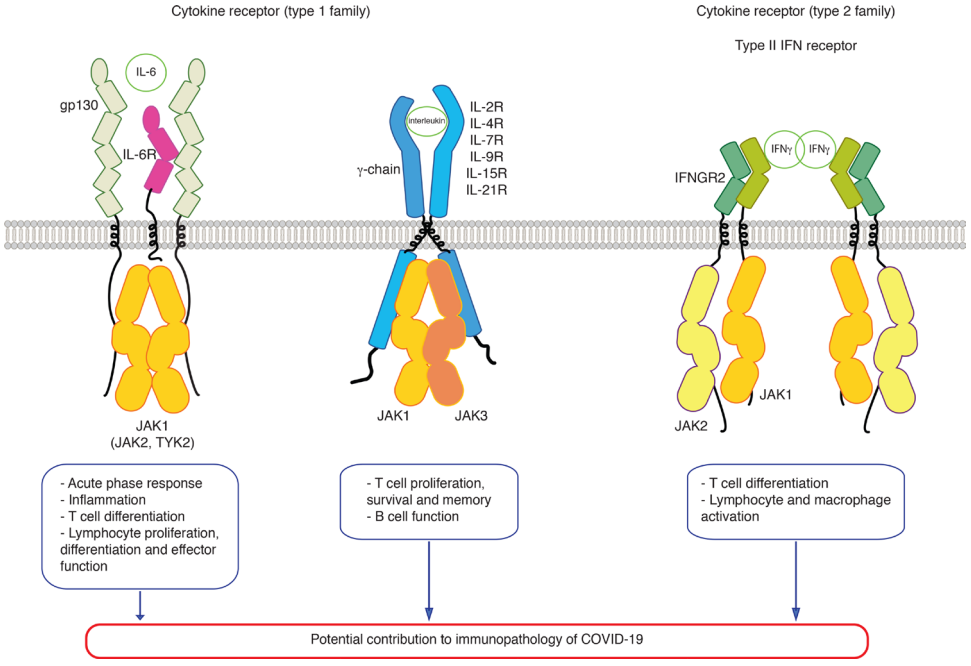

Figure 1 JAK-dependent cytokine receptors signaling involved in response to SARS-CoV-2 infection and potentially in COVID-19 immunopathology. EC, extracellular; IC, intracellular; IFN, interferon; JAK, Janus kinase.

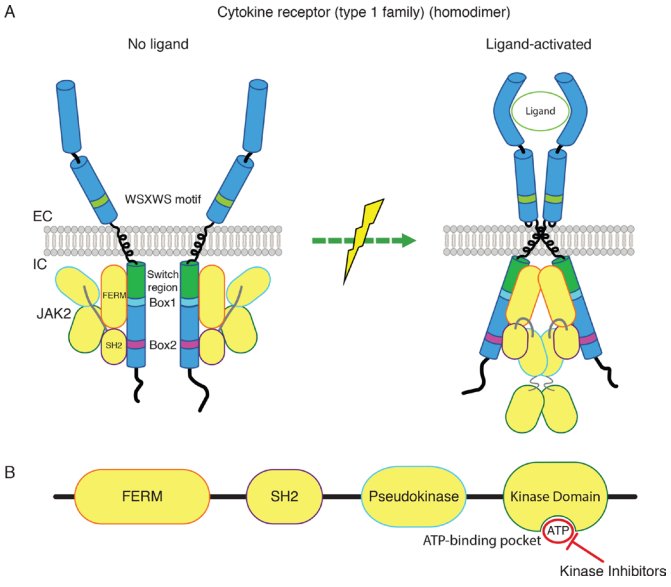

Figure 2 Homodimeric cytokine receptor signaling via JAK2. (A) Homodimeric cytokine receptors and conformational changes on activation by ligands. JAK2 binds to boxes 1 and 2 motifs of the cytosolic domains of receptors, via the FERM and SH2 domains, respectively. The region separating the end of the transmembrane domain and the Box 1 is denoted 'switch region' and is required for ligand-activation of receptors and JAK2. (B) JAK2 domain structure and the activating mutations in myeloproliferative neoplasms (V617F and insertions and deletions mutating K539) shown as red stars. FERM, SH2, pseudokinase and kinase domains are shown from the NH2- to the COOH-terminus. kinase inhibitors currently in use act on the ATP-binding pocket of the kinase domain and inhibit both mutated and wild type forms of JAK2. EC, extracellular; IC, intracellular; IFN, interferon; JAK, Janus kinase.

of activation of both type I and type II cytokine receptors is represented by cytokine-induced dimerization/oligomerization of receptor subunits, which brings the JAKs appended non-covalently to cytosolic domains into such relative proximity and conformation that they can activate the appanded JAKs and trigger the signaling cascade.[5–8] This is starting with tyrosine phosphorylation of receptor tails, which then become attraction sites for SH2 containing signaling molecules and adaptors. The major substrate of JAKs is represented by the family of STATs.[9] The choice of one of several STAT molecules is made as a function of which JAK and which sequence sites are tyrosine phosphorylated in receptors. In addition, adaptors linking cytokine receptors with ras-MAP-kinase and PI-3'-kinase-Akt are also attracted to receptors on JAK activation.[1]

It is important to stress two major features of cytokine receptor function: (1) in the absence of cytokines these receptors are completely inactive, unlike other receptor types which maintain a certain level of basal activity; (2) activation of JAKs is absolutely required for all signaling downstream of these receptors. In addition, JAKs are key to stabilize and chaperone cytokine receptors to the cell surface.[10–13] JAKs bind to cytokine receptors' cytosolic domains, namely the FERM domain binds to the Box 1 and the region between boxes 1 and 2, while the SH2 domain binds to Box 2[14–16] (figures 2A and 3). Box 1 is a proline-rich short sequence that is conserved in most type 1 cytokine receptors (such as EpoR or TpoR), while Box 2 is a sequence composed of hydrophobic and negatively charged residues.[17] Interestingly, the region of the cytosolic tails located between the transmembrane domain and Box 1 is crucial for activation of JAKs, not for their binding; this is why this region of 10–14 aminoacids rich in

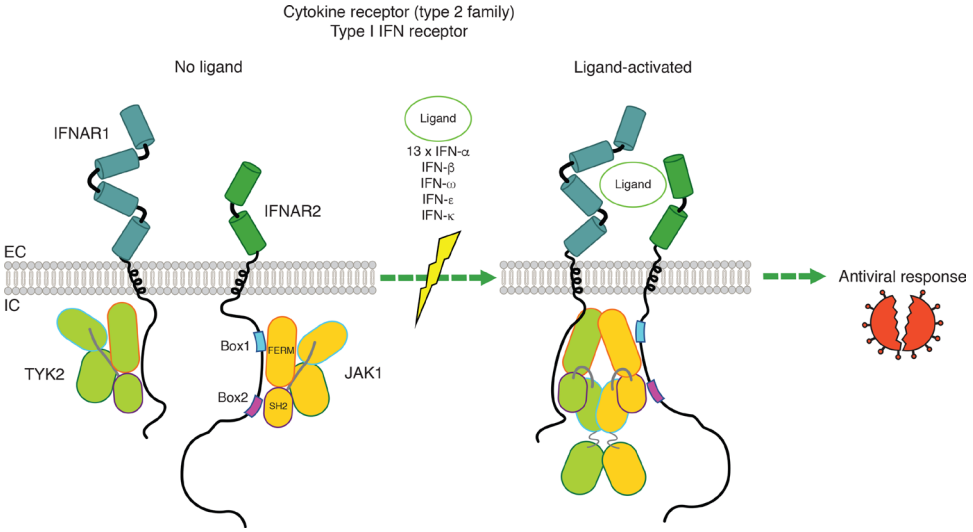

Figure 3 Signaling by heterodimeric type I interferon (IFN) receptor and changes of conformation on activation of the IFN receptor by its ligands. JAK1 binds to boxes 1 and 2 motifs of the cytosolic domain of ifnar2 respectively via the FERM and SH2 domains. Tyk2 binds to the cytosolic domain of IFNAR1. EC, extracellular; IC, intracellular; IFN, interferon; IL, intereukin; JAK, Janus kinase.

positively charged residues[18] has been denoted as « switch » region (figure 2A).[5 10] These principles are correct for all type 1 cytokine receptors and are also globally correct for type 2 cytokine receptor, except that for the latter the boxes 1 and 2 are less well defined.[1]

SARS-CoV-2 and the immune system

Coronaviruses are enveloped spherical viruses with a diameter of 130–160 nm, with single stranded RNA unsegmented and positive polarity. They bind to the ACE2 receptor on target cells for entry and infection through envelope structures containing the S protein. SARS-CoV-2 belongs to the *Coronavirinae* subfamily *Betacoronavirus* genus and S*arbecovirus* subgenus. SARS-CoV belongs to the same subgenus *Sarbecovirus*, while the Middle Eastern respiratory syndrome (MERS) virus belongs to another subgenus, *Merbecovirus*.[19] SARS-CoV-2 entry, decapsidation, replication and virus release follow the path depicted in SARS-CoV and has been the subject of an extensive literature.

An impressive body of work has been accomplished in a very short time describing the roles of individual SARS-CoV-2 proteins in infection, replication and countering the immune system.[20] With extensive sequencing and epidemiology studies, the different variants of the virus are being studied in detail with respect to infectivity, cytopathic effect[21] and sensitivity to vaccine.[22 23]

Persistence of virus in the body,[24] especially in the gut,[25] given the enterocyte replication of the virus[26] or only of transcripts from short integrated viral RNAs[27] may contribute to memory response and may impact disease and other immune stimulations. Furthermore, long-term replication in the context of immunosuppression may promote selection for mutants of SARS-CoV-2.[28–30]

Immunopathology in COVID-19 and previously described SARS CoV and MERS infections

Experience with severe SARS-CoV-1 and MERS pointed to a major feature of these infections, which is to induce a delayed cytokine storm following an initially insufficient induction and action of type I interferons (IFN).[31 32] The same has been clearly observed with SARS-CoV-2 which induces a biphasic disease consisting first of a flu-like phase, followed by a pulmonary and systemic disease, which pathological cytokine action and inflammation may lead to acute respiratory distress syndrome (ARDS).

In a standard viral infection, the viral genome and proteins induce, via Toll-like receptors, activation of the transcription factors required for rapid and sustained synthesis of type I IFNs alpha (produced by leucocytes, 17 subtypes) and beta (fibroblasts), epsilon (produced by reproductive tract epithelium), kappa (produced by epidermal keratinocytes) and omega (produced by leucocytes and is divergent from IFNs alpha and beta). There are 13 forms of IFN-alpha and 1 IFN-omega, which is closely related phylogenetically. IFN-epsilon and kappa are less homologous.[33 34] All human 17 individual type I IFNs bind to the same receptor,[35 36] namely the type I IFN receptor (heterodimer constituted of one IFNAR2 binding subunit, linked to JAK1, and one IFNAR1 signaling subunit, linked to TYK2) (figure 3),[3] which induce, via a specific complex composed of STAT1, STAT2 and IRF9, genes that code for proteins that mediate an antiviral state in the neighboring cells.[37]

These IFNs are secreted by the initially infected cells and act on neighboring cells, inducing an antiviral state. As a consequence, the neighboring cells are protected and the antigen presenting cells can induce the adaptive immune response, composed of B cells that under the help of T cells will generate the plasmocytes that secrete

neutralizing antibodies and also the formation of $CD8^+$ cytotoxic T cells that will recognize and kill infected cells. Patients that exhibit anti-type I IFN antibodies or genetic defects in IFN induction mechanisms evolve towards a severe disease.[38 39] Remarkably, autoantibodies against type I IFNs with neutralizing capacity are present in approximately 4% of uninfected individuals over 70 years old and are responsible for 20% of COVID-19 deaths.[40] Furthermore, X-linked recessive TLR7 deficiency has been reported in ~1% of men under 60 years old with life-threatening COVID-19.[41] Indeed, human TLR7 and plasmacytoid dendritic cells expressing TLR7 are essential for protective innate type I IFN immunity against SARS-CoV-2 in the respiratory tract. Finally, in a large study investigating genetic mechanisms of critical illness in COVID-19 by a genome-wide association study in 2244 critically ill patients with COVID-19 from 208 UK intensive care units, low expression of IFNAR2 or high expression of TYK2, are associated with life-threatening disease.[42] These data clearly indicate that signaling by type I IFN and possibly other JAK-STAT pathway components are critical for COVID-19.

Type II IFN (IFN-gamma) (figure 1) is secreted by T and NK cells and plays a major role in T cell immunity.[43] Type III IFN (IFN-lambda) is produced by epithelial cells at the local level and may function as an entry barrier for several viruses. IFN-lambda utilizes a distinct receptor system (IL-28R or IFN-lambda receptor alpha and IL-10 receptor beta, coupled also to JAK1 and TYK2, respectively).[44] It is possible that defects in induction and action of IFN-lambda are also involved in COVID-19.

The presence of a very large genome with many non-structural proteins allows coronaviruses to target many of the proteins of the innate immunity machinery and especially the mechanisms of type I IFN induction and then signaling in target cells.[31 45] This initial insufficient response allows rapid amplification of virus RNA which then reaches a threshold where it may induce the cytokine storm that usually appears 6–8 days after infection. The precise molecular link between high viral loads and aberrant cytokine/chemokine induction leading to the cytokine storm remains to be determined. Appearance of the cytokine storm is correlated with a decrease in lymphocytes, eosinophils, increase in D-dimers and importantly with an emergency myelopoiesis that generates insufficiently differentiated or skewed monocytic and granulocytic cells.

The SARS-CoV-2 infection impacts both lymphopoiesis and myelopoiesis. COVID-19 patients exhibit a decrease in the number of plasmacytoid and myeloid dendritic cells, different monocyte distribution subsets and activation patterns, as well as neutrophil phenotypic alterations.[46] During COVID-19, defects in myelopoiesis occur with profound alterations of the myeloid compartment.[46–48] In mild forms of COVID-19, inflammatory monocytes (HLA-DRhiCD11chiCD14+) with an IFN-stimulated gene signature are elevated.[48] In severe forms, $HLA\text{-}DR^{Low}$ monocytes are present along with neutrophil precursors and dysfunctional mature neutrophils.[48] Using single cell RNA sequencing of purified cell populations from COVID-19 patients it was demonstrated that while $HLA\text{-}DR^{Low}$ classical monocytes accumulate during severe disease, non-classical $CD14^{Low}CD16^{High}$ monocytes disappear.[47] In severe cases, classical monocytes release high levels of S100A8/S100A9, also called calprotectin.[47] In addition, immunosuppressive $CD10^{Low}CD101^{-}CXCR4^{+/-}$ neutrophils are produced, that migrate to the lung. High levels of S100A8/S100A9 and altered frequency of non-classical monocytes are becoming markers of severe disease.[47] However, the relationship between classic and non-classic monocytes is more complex.[49]

These changes in the myeloid and lymphoid compartments are a pivotal reason for further pathological mechanisms mediated by these cells at the level of lung pneumocytes and endothelial cells. It is important to emphasize that all step-by-step mechanistic details of these chains of events are still worked out, but what is certain is that while corticosteroid therapy is very useful, it is not sufficient to interrupt the immunopathology of severe of COVID-19.

During a controlled viral infection, early innate mechanisms executed by monocytes, NK cells and cytokines act to induce late adaptive immunity.[50] Viral load peaks during the descending phase of the early innate response and coincides with the peak of development of adaptive response, then both viral load and intensity of adaptive response decrease, with virus becoming undetectable, while the adaptive response develops the long-term memory protection. In an uncontrolled infection there is persistent or delayed innate response with cytotoxicity, lymphopenia and immunosuppression. Mediators of toxicity during the delayed pathological immune response are IL-6, IL-1 and several other cytokines. Attempts to control these events using IL-6 or IL-1 blockers and corticosteroid therapy have led the U.S. Food and Drug Administration (FDA), European Medicines Agency (EMA) and WHO to indicate IL-6R blocker tocilizumab (and sarilumab)[51–55] and IL-1 blocker anakinra (EMA only)[56 57] in certain severe cases of COVID-19. These strategies would need to be compared with the use of JAK inhibitors (JAKi), but at present direct comparisons have not been performed.

Rationale for inhibiting JAKs in COVID-19

There are two major reasons why JAKs became prime targets of the pharmaceutical industry for inhibition. The first is that, in 2005, a unique somatic acquired *JAK2* mutation (*JAK2* V617F) was discovered to be responsible for 70% of a large group of myeloid cancers called myeloproliferative neoplasms (MPNs).[58–61] This was a surprizing finding. Within MPNs, 95% of Polycythemia Vera (PV) and >60% of Essential Thrombocythemia (ET) and Primary Myelofibrosis (MF) were linked to this acquired mutations. 3% of PV are harboring a different set of JAK2 mutations in exon 12, where insertions or deletions lead to mutation of K539 in the linker between

SH2 and pseudokinase domains, also inducing JAK2 persistent activation.[62] The rest of ET and MF patients that do not harbor *JAK2* V617F exhibit mutations that induce unusual modes of activation of TpoR that lead to persistent JAK2 activation.[63] One is represented by mutations in TpoR (*MPL*) itself,[64–67] the other is frameshifting mutations in calreticulin (*CALR*)[68 69] that endow mutated CALR proteins the ability to bind and activate the same TpoR in the absence of ligand.[70–73] Thus, for this very large group of diseases with increasing prevalence (0.4–0.7/1000, 5 times higher than chronic myeloid leukemia induced by the BCR-Abelson kinase (ABL) fusion protein) the cause is persistent activation of JAK2 in clonal HSCs, which gives a major advantage to progenitors of red blood cells, platelets and granulocytes, leading to clonal expansion of these lineages in the absence of cytokines.

In the same time with the identification of *JAK2* V617F it was shown that homologous mutations activate constitutively *JAK1* (V658F) and *TYK2* (V678F)[66] and these and other activating mutations were identified in T-acute lymphoblastoid leukemia (ALL), in B-ALL and several other conditions.[74 75] Overall, these findings provided an impetus to search for potent inhibitors of JAKs.

The second reason why JAKs became prime targets for the pharmaceutical industry is represented by excessive activation of cytokines and their receptors in autoimmune diseases. The prime targets were members of the IL-2, IL-4, IL-7, IL-9, IL-15, IL-21 and especially the two JAK proteins associated with subunits of this group, namely JAK1 and JAK3.[76] Inhibitors were thus seeked that could inhibit JAK1 and JAK3, or only JAK3. Such inhibitors were discovered and it was found that only inhibiting JAK3 exerts a much weaker effect than inhibiting JAK1 and JAK3, implying that JAK1 is the dominant JAK,[77] and that JAK3 needs to be present in the complex, as a scaffold, but its catalytic activity is not as crucial as that of JAK1.[77 78] Indeed, absence of JAK3 has a blocking effect while inhibiting the kinase domain only exerts a minor effect.[77 79]

More recently, increased interest in JAKi strategies arose for the need of new treatments for pathological inflammatory conditions like hemophagocytic lymphohistiocytosis (HLH) where overactive T cells and macrophages secrete numerous proinflammatory cytokines, including IFNγ, IL-1β, IL-2, IL-6, IL-10, IL-18 and TNF-α, involving the JAK-STAT pathway.[80] Samewise in COVID-19, the JAK-STAT pathway is implicated in complement hyperactivation in SARS-CoV-2-infected respiratory epithelial cells[81] and in the activation of $CD4^+$ and $CD8^+$ positive T cells, NK cells and monocytes which cooperate with elevated levels of IL-6, IL-9, IL-13, GM-CSF, IFNγ, IL-1β, IL-8, and IL-17. Indeed, very high levels of IL-2, IL-7, IL-10, TNF-α and G-CSF were described in COVID-19 patients requiring intensive care.[82]

Furthermore, JAK-STAT activation promotes senescence of SARS-CoV-2 infected cells, which amplifies inflammation.[83]

Therefore, JAKi, and especially JAK1/JAK2 inhibitors, were suggested as a potential therapy against systemic inflammation in COVID-19[84] because treatment with a JAK1/JAK2 inhibitor to prevent lung injury in severe COVID-19 would be able to reduce cytokine action more effectively than blocking one cytokine at a time (like IL-6.[85]

Inhibitors

The major strategy used for obtaining kinase inhibitors is to obtain compounds that prevent ATP entrance in the ATP binding pocket. ATP is obligatory as a source of phosphate for catalysis. The small molecule inhibitor may bind and compete with ATP, or may bind to close—by sites or distant sites that allosterically prevent ATP binding to the ATP pocket. The kinase inhibitors may bind to active kinases and inhibit them (type I) or may bind to the inactive kinase, and block the transition to active kinase (type II).[86] The very well-known kinase inhibitor imatinib inhibits the Abelson kinase (ABL), PDGF receptor and KIT receptor tyrosine kinases. Imatinib binds to kinases when they are inactive (type II), thus preventing activation. All current JAKi used in the clinics so far are type I inhibitors, and they only recognize and bind kinase domain of JAKs in their active conformation. This is on one hand a limitation, as prebinding activation is a prerequisite for binding, hence pathological signaling occurs at a certain point, but on the other hand the toxic effects due to blocking JAK2 for red blood cell and platelet formation will be less severe.

The industry identified several small molecule ATP competitors with nM affinity to each of the JAKs, or to several JAKs. Two molecules are mainly under focus of this review, baricitinib and ruxolitinib. Baricitinib exhibits an IC50 under 10 nM JAK1 and JAK2 (4.0–5.9 nM for JAK1 and 6.6–8.8 nM for JAK2), 787 nM for JAK3 and 61 nM TYK2, all determined at 1 mM ATP.[87] These values are different as a function of the amount of ATP used and may also depend on the technique to assay inhibition of kinase activity.[87–89] Ruxolitinib exhibits also IC50s of less than 10 nM for JAK1 and JAK2, specifically IC50 3.3–6.4 nM for JAK1, 2.8–8.8 nM for JAK2, 428–487 nM for JAK3 and approximately 10-fold selectivity for TYK2 (IC50 19–30 nM).[87–89]

They are both type I inhibitors with rather low half-life, 4 hours for ruxolitinib and 12.5 hours for baricitinib. In addition, we will discuss tofacitinib (JAK1 and JAK3 inhibitor), upadacitinib (JAK1 inhibitor) and nezulcitinib (pan JAKi, inhaled). We will not discuss the strictly JAK1 specific filgotinib, as its clinical development is most recent. Also, a JAK2 inhibitor, fedratinib which was only recently approved in the clinics after a pause in development, will not be discussed. However, it appears that although all their differences a JAKi class effect is emerging, with potential relevance in COVID-19.

Structural bases for inhibitor action. The first crystal structure of ruxolitinib was solved in complex with the kinase domain of c-Src,[90] while the structure of the JAK2

kinase domain was solved in complex with a pan JAKi.[91] The X-ray crystal structure of baricitinib was initially solved in complex with a member of the Numb-associated kinases, namely BMP2-inducible kinase.[92] Only in 2021, in a very recent study which is being published in *Blood* the structures of ruxolitinib and of baricitinib were reported with the JAK2 kinase domain.[93] Of great interest, these structures allowed the first examination of ruxolitinib and baricitinib engaged with their main target (figure 2B). It also afforded design of ruxolitinib and baricitinib modified molecules where a linker was introduced to a solvent exposed carbon (C2 of the pyrimidine ring) of each inhibitor to which pomalidomide or thalidomide were linked. Proteolysis-targeting compounds were created and showed that they hold the potential to degrade JAK2 in target cells.[93]

JAKi has known a broadening use in the clinics over the past decade and new molecules keep being designed.[94] Ruxolitinib, one of the oldest JAKi, is the agent most used in hematology patients whereas other JAKi such as baricitinib and tofacitinib are used more commonly in systemic rheumatic diseases (tofacitinib for patients with rheumatoid arthritis, psoriatic arthritis or ulcerative colitis and baricitinib for patients with rheumatoid arthritis).

There is extensive literature on the effects of JAKi in MPNs (in MF and complicated PV). In these conditions, the treatment is effective for alleviating symptoms, improving quality of life, overall survival, decreasing spleen size, but is not curative, as allele burdens do not significantly decrease. Most importantly, resistance to treatment exists, that is not linked to further mutations in the driver *JAK2* gene. This is very different from the situation with imatinib and *BCR/ABL1* driven chronic myeloid leukemia where mutations in ABL kinase domain, the main target, are explaining resistance.[95] These results have been interpreted to suggest that the targets of JAK2 inhibitors such as ruxolitinib are non-mutated JAK1 and JAK2 in inflammatory cells, suggesting that JAKi may act as effective anti-inflammatory agents in MPNs,[96] which is indeed the goal of their use in systemic rheumatic diseases or in newer indications such as or HLH[80] or graft-versus-host disease (GVHd).[97–102]

That ruxolitinib is able to be effective in cases of GVHd resistant to corticotherapy clearly indicates its immunosuppressive effects are not identical to those of corticotherapy.[97 98] The mechanisms assessed in mouse models of GVHd involve JAKi-mediated suppression of pro-inflammatory cytokines and of the proliferation of effector T cells, with ruxolitinib impairing differentiation of $CD4^+$ T cells into IFNγ- and IL17A-producing cells.[103]

Experience with JAKi in COVID-19

Significant clinical experience has been accumulated on the use of JAKi for treating COVID-19 and associated inflammatory status (table 1).

JAKi in use in systemic rheumatic diseases

The effect of the COVID-19 pandemic on people with inflammatory or autoimmune rheumatic diseases remains unclear and, as immune-compromised patients, people with systemic rheumatic diseases are at increased risk of infection, including by SARS-CoV-2.[104] This is due to their underlying immune conditions and to immune-modulating therapies such as biologics. Whether background immunosuppressive medications put individuals with rheumatic disease at an increased or decreased risk for severe SARS-CoV-2 infection is unknown, and evidence is lacking to guide treatment decisions.[105] This population may, however, represent an interesting group to study as some disease-modifying drugs commonly used to treat rheumatic diseases, such as hydroxychloroquine, or biologics targeting interleukin IL-6 (as tocilizumab)[51 52 106 107] or sarilumab,[108] IL-1 (as anakinra)[56] or JAKi are being or have been assessed in patients with severe COVID-19.

It should be added that in times of broad vaccination against COVID-19 in Western countries, one study demonstrated that the overall response rate to COVID-19 vaccine in patients suffering from systemic rheumatic diseases treated with JAKi remained high,[109] in line with rates reported with other immunosuppressants.[110]

In this review, we will develop on the use of JAKi for the treatment of severe form of COVID-19.

Baricitinib

Baricitinib, a JAK1/JAK2 inhibitor, was suggested as soon as February 2020 as a potential treatment for COVID-19 acute respiratory disease[111] using BenevolentAI's knowledge graph. BenevolentAI is a large repository of structured medical information that include different and numerous connections extracted by machine learning. Adaptation/customization to COVID-19 was applied to this resource and approved drugs that may inhibit the viral infection were searched. Baricitinib was identified as a potential molecule that could inhibit infection of lung cells by SARS-CoV-2. One of the key cell subtypes expressing the ACE2 receptor are lung AT2 alveolar cells. Viruses enter by endocytosis and many small molecule kinase inhibitors were shown to prevent entry into cells of different virus types.[112 113] One regulator of endocytosis is AP2-associated protein kinase 1 (AAK1), which belongs to the group of Numb-Associated Kinases. A prediction was formulated that inhibition of AAK1 could prevent SARS-CoV-2 entry and then also intracellular virion assembly. One of the molecules predicted to inhibit AAK1 was baricitinib which also binds to another endocytosis regulator, cyclin G-associated kinase. The prediction was that baricitinib would inhibit both virus entry (via AAK1 targeting) and inflammation by targeting JAK1 and JAK2 downstream many cytokine receptors during cytokine storm.[111]

This work was extended and the affinity and selectivity of the identified drugs were examined in order to point to anti-inflammatory and antiviral drugs. Baricitinib,

Table 1 Clinical experience with JAK2 inhibitors in COVID-19

Study ID no	Brief title of the study	Promoter/ Sponsor	Status (first posted)	Patient's condition/ inclusion criteria	No of patients	Study type	Study phase	Centers	Intervention/drugs	Primary outcome measures	Reference
			June 2020 (published)	COVID-19-induced (mild to severe) pneumonia	4	Cases report	Pilot study	Monocentric	Baricitinib	▶ Clinical variables and evolution of the COVID-19 disease ▶ Laboratory analysis (blood counts, inflammatory markers/ IL-6 serum levels, viral load)	Stebbing *et al*[117]
NCT04438629	Evaluation of Immune Response in COVID-19 Patients (IMMUNOVID)	Azienda Ospedaliera Universitaria Integrata Verona (sponsor) and Pederzoli Hospital of Peschiera, Italy	March–June 2020 (published), recruiting	COVID-19-induced pneumonia	88 (20 on baricitinib)	Observational, longitudinal, prospective		Bicentric	Baricitinib, 4 mg two times per day 2 days, 4 mg/day 7 days	▶ Evolution of the COVID-19 disease ▶ Laboratory analysis COVID-19 associated immune disorder (time frame: 24 hours) : circulating cell subsets by flow cytometry COVID-19 associated inflammation (time frame: 48 hours): plasma levels of different solubles factors Oxygenation (time frame: 24 hours)	Bronte *et al*[118]
NCT04358614	Baricitinib Therapy in COVID-19: A Pilot Study on Safety and Clinical Impact	Hospital of Prato, Italy	completed (published October 2020)	COVID-19 moderate pneumonia	12 patients in the pilot study 191 (113 on baricitinib) in the second phase	Observational, longitudinal, retrospective	Phase 2 Phase 3	Multicentric (seven in Italy)	Baricitinib 4 mg/d and antiviral therapy (lopinavir/ritonavir) for 2 weeks vs SOC (hydroxychloroquine and lopinavir/ritonavir) for 2 weeks	Mortality rate	Cantini *et al*[120]
NCT04401579	Adaptive COVID-19 Treatment Trial 2	US National Institute of Allergy and Infectious Diseases	Completed (published March 2021)	COVID-19 hospitalized patients	1033 (515 on baricitinib)	Interventional, randomized, double-blind, placebo-controlled	Phase 3	Multicentric (67 international centers)	Remdesivir plus baricitinib 4 mg/d, up to 2 weeks vs Remdesivir plus placebo	Time to recovery	Kalil *et al*[121]
NCT04421027	A Study of Baricitinib (LY3009104) in Participants With COVID-19 (COV-BARRIER)	Eli Lilly and Company	Completed (published August 2021)	O2-dependent COVID-19 patients with risk indicators of aggravation	1585	Interventional, randomized, double-blind, placebo-controlled	Phase 3	Multicentric	Baricitinib 4 mg/d and SOC for 2 weeks vs Placebo and SOC for 2 weeks	▶ Death rate or ▶ Requirement of Non-Invasive Ventilation/ High-Flow Oxygen or Invasive Mechanical Ventilation including ECMO (Time frame: 28 days)	Marconi *et al*[124]
NCT04469114	Tofacitinib in Hospitalized Patients With COVID-19 Pneumonia (STOP-COVID)	Hospital Israelita Albert Einstein, Brazil (sponsor)	Completed (published)	COVID-19 hospitalized patients with pneumonia	289	Interventional, randomized, double-blind, parallel-design, placebo-controlled	Phase 3	Multicentric (15 in Brazil)	Tofacitinib, 10 mg Twice daily up to 14 days vs Placebo	Death or respiratory failure until Day 28	Guimaraes *et al*[131]

Continued

Table 1 Continued

Study ID no	Brief title of the study	Promoter/ Sponsor	Status (first posted)	Patient's condition/ inclusion criteria	No of patients	Study type	Study phase	Centers	Intervention/drugs	Primary outcome measures	Reference
		Hammersmith Hospital, London, UK	June 2020 (published)	HSCT for CML Diabetes Male	1	Case report		Monocentric	Tocilizumab 8 mg/kg (two doses, no effect) Ruxolitinib 5 mg Twice daily 3 days, 10 mg Twice daily 8 days, 5 mg Twice daily 10days	Case report (severe disease)	Innes *et al*[142]
		Aachen University Hospital, Aachen, Germany	May 2020 (published)	MF high-risk for COVID-19 (arterial hypertension, obesity, hyperuricemia, chronic kidney disease) Male	1	Case report		Monocentric	Ruxolitinib, 10 mg BD (patient on drug before COVID-19)	Case report (mild disease)	Koschmieder *et al*[143]
No 47 (Italian Agency for Drug (AIFA) and Istituto Spallanzani) No 17104 (RUXO-COVID (institutional ethic committee in Florence)	RUXO-COVID	Azienda Ospedaliera-Universitaria Careggi, Florence, Italy	April-May 2020 (inclusions) August 2020 (published)	Severe COVID-19 (no mechanical ventilation at diagnosis)	34	Observational, prospective		Monocentric	Ruxolitinib (compassionate use), 5 mg Twice daily 1–2 days, 10 mg Twice daily 1–2 days,	▶ Clinical variables and evolution of the COVID-19 disease ▶ Laboratory analysis (immunophenotyping of peripheral blood cells, intra-cellular and serum cytokines levels)	Vannucchi *et al*[144]
		Schwarzwald–Baar–Klinikum Villingen-Schwenningen, Germany	March-April 2020 (inclusions), June 2020 (published)	Severe COVID-19	105 consecutive patients, 14 patients on ruxolitinib	Retrospective, observational (multidisciplinary board decision on specific medical treatment)	pilote case series	Monocentric	Ruxolitinib, 7.5 mg Twice daily 1–7 days to 15 mg Twice daily	CIS	La Rosee *et al*[145]
NCT04338958	Ruxolitinib in COVID-19 Patients With Defined Hyperinflammation (RuxCoFlam)	University of Jena, Germany (Sponsor)	Completed (published)	COVID-19 patients with hyperinflammation (COVID-19 Inflammation Score (CIS) ≥10/16)	193	Interventional, single arm (open), non-randomized	Phase 2	Multicentric	Ruxolitinib, 10 mg Twice daily to 20 mg Twice daily	Improvement in CIS	La Rosee *et al*[145]
NCT04337359 (expanded access for ruxolitinib)	Ruxolitinib Managed Access Program for Patients Diagnosed With Severe/Very Severe COVID-19 Illness	Policlinico S.Marco Gruppo San Donato University and Research Hospital, Zingonia, Bergamo, Italy	March–April 2020 (inclusions), November 2020 (published)	Severe COVID-19 (no mechanical ventilation at diagnosis)	75 (32 on ruxolitinib, 43 as control)	Interventional, non-randomized		Monocentric	Ruxolitinib, 5 mg Twice daily 7 days, 5 mg/d 3 days + Methyl-prednisolone 1 mg/kg/day IV 3 days, 0.5 mg/kg/day 5 days, then oral prednisone tapered over 2 weeks	Evolution of the COVID-19 disease	D'Alessio *et al*[146]
			February 2020 (included), July 2020 (published)	Severe COVID-19	41 (20 on ruxolitinib, 21 as control)	Interventional, prospective, single-blind, randomized and controlled	Phase 2	Multicentric (three in China)	Ruxolitinib, 5 mg Twice daily and standard-of-care (SOC) vs Placebo (C vitamin, 100 mg Twice daily) and SOC	Time to clinical improvement	Cao *et al*[147]

Continued

Table 1 Continued

Study ID no	Brief title of the study	Promoter/ Sponsor	Status (first posted)	Patient's condition/ inclusion criteria	No of patients	Study type	Study phase	Centers	Intervention/drugs	Primary outcome measures	Reference
trial register no. 81 April 2020 (Italian COVID-19 Ethical Committee) NCT04361903	Ruxolitinib for the Treatment of ARDS in Patients With COVID-19 Infection (COVID-19: Ruxolitinib for the Treatment of cytokinE Storm resPiratory dIstREss Syndrome. RESPIRE Study)		August 2020 (published)	COVID-19-related ARDS	18	Observational, retrospective		Multicentric (three in Italy)	Ruxolitinib, 20 mg Twice daily 2 days and de-escalation to 5 mg Twice daily	Degree of respiratory impairement	Capochiani *et al*[148]
	MPN-COVID	European Leukemia Network		MPNs	175	Observational, retrospective		Multicentric, in 38 European centers (France, Germany, Italy, Poland, Spain and the UK)		▶ Mortality ▶ Sub-analysis of the effect of therapy	Barbui *et al*[151]
		Royal Berkshire Hospital NHS Foundation Trust, Reading, UK		COVID-19 with secondary haemophagocytic lymphohistiocytosis (sHLH) Patients with medical history of lymphoma	2	Cases report		Monocentric	Ruxolitinib + Tocilizumab	Case report (severe diseases)	Portsmore *et al*[153]
NCT04362137	Phase 3 Randomized, Double-blind, Placebo-controlled Multi-center Study to Assess the Efficacy and Safety of Ruxolitinib in Patients With COVID-19 Associated Cytokine Storm (RUXCOVID)	Novartis Pharmaceuticals	Completed	COVID-19-related cytokine storm	432	Interventional, randomized, double-blind, placebo-controlled	Phase 3	Multicentric	Ruxolitinib, 5 mg Twice daily vs Placebo	▶ Death rate ▶ Development of respiratory failure [requiring mechanical ventilation) ▶ Requirement for ICU	
NCT04377620	Assessment of Efficacy and Safety of Ruxolitinib in Participants With COVID-19-Associated ARDS Who Require Mechanical Ventilation (RUXCOVID-DEVENT)	Incyte Corporation	Terminated	COVID-19-associated ARDS AND intubation and mechanical ventilation	211	Interventional, randomized, double-blind, placebo-controlled	Phase 3	Multicentric	Ruxolitinib, 5 mg or 15 mg, Twice daily and SOC vs Placebo and SOC	Death rate	

Continued

Table 1 Continued

Study ID no	Brief title of the study	Promoter/ Sponsor	Status (first posted)	Patient's condition/ inclusion criteria	No of patients	Study type	Study phase	Centers	Intervention/drugs	Primary outcome measures	Reference
NCT04402866	TD-0903 for ALI Associated With COVID-19	Theravance Biopharma (sponsor)	completed (published)	Oxygen-requiring patients with COVID-19-associated: ▲▲ ALI Lung inflammation	235	Randomized, double-blind, parallel-group trial, placebo-controlled	Phase 2	Multicentric (the UK, Moldova and Ukrain)	Nezulcitinib (ascending-dose cohorts from 1 to 10 mg/day) for up to 7 days vs Placebo	No of respiratory failure-free days (Baseline through Day 28)	Singh *et al*[157]

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; ECMO, extracorporeal membrane oxygenation; JAK, Janus kinases; MF, myelofibrosis.

fedratinib and ruxolitinib were highlighted as having similar JAK-STAT inhibitory potency, baricitinib stood out due to its AAK1 inhibition capacity. In a EMEA trial with baricitinib for autoimmune diseases in 4214 patients, a small increase in upper respiratory infection has been seen, but the incidence of severe infections was similar to placebo.[114] That baricitinib holds the potential to inhibit SARS-CoV-2 infection was also reviewed in reference 115.

Experimental evidence showed that indeed baricitinib exerts an antiviral effect separated from its anti-inflammatory effects.[116 117] In a case series of patients with bilateral COVID-19 pneumonia, baricitinib treatment was associated with clinical and radiologic recovery, a rapid decline in SARS-CoV-2 viral load, inflammatory markers and IL6 levels. Collectively, these data supported further evaluation of the anti-cytokine and anti-viral activity of baricitinib and suggested its assessment in randomized trials in hospitalized COVID-19 patients.[117]

Indeed, baricitinib reduced the immune dysregulation in severe COVID-19 patients. A group of 20 patients was treated in an observational, longitudinal trial (IMMUNOVID) with baricitinib at 4 mg two times per day for 2 days, followed by 4 mg per day for another 7 days (NCT04438629).[118] Treated patients exhibited markedly reduced levels of IL6, IL1β and TNFα, recovery of circulating T and B cells and increased antibody production against the Spike protein. This was associated with a reduction in the need for oxygen therapy. This study suggested that baricitinib can prevent progression to severe disease.

Baricitinib reduced lung inflammation in a rhesus macaque model of SARS-CoV-2 infection.[119] While viral shedding was not reduced by baricitinib and T cell responses were similar, animals treated with baricitinib showed reduced inflammation, decreased lung infiltration and exhibited a reduction in lung pathological changes. Lung macrophages production of cytokines and chemokines responsible for inflammation and neutrophil recruitment was reduced by baricitinib.[119]

A beneficial effect of baricitinib was reported in COVID-19 moderate pneumonia in a retrospective multicenter study (NCT04358614).[120] In this study baricitinib reduced COVID-19 mortality rate, intensive care unit admissions of COVID-19 pneumonia and SARS-CoV-2 viral burden in nasopharyngeal swabs and when used during 14 days, did not induce adverse effects.

Results from the trial of the ACTT-2 trial assessing baricitinib plus remdesivir for hospitalized adults with COVID-19 (NCT04401579)[121] give support to the approach of inhibiting JAK1/JAK2 in severe COVID-19 disease. This adaptive, double-blind, randomized and placebo-controlled phase 3 trial on 1033 patients (515 receiving both drugs and 518 receiving just remdesivir) evaluated baricitinib plus remdesivir in hospitalized adults with COVID-19. Remdesivir was administered for ≤10 days, and either baricitinib or placebo (control) for ≤14 days. The primary outcome was time to recovery and a secondary outcome was clinical status at day 15. The

combination of baricitinib plus remdesivir was superior to remdesivir alone in both counts with a 1-day shortening of recovery time in the baricitinib arm (median 7 days, 95% CI (6 to 8 days) vs 8 days in the control group, 95% CI (7 to 9 days)) and 30% higher odds of improvement in clinical status at day 15 (OR, 1.3; 95% CI (1.0 to 1.6)). Patients receiving high-flow oxygen or noninvasive ventilation at enrollment had a time to recovery of 10 days with combination treatment and 18 days with control (rate ratio for recovery, 1.51; 95% CI (1.10 to 2.08)). The 28-day mortality rate was 5.1% in the combination group and 7.8% in the control group (HR for death, 0.65; 95% CI (0.39 to 1.09)). Thus, baricitinib plus remdesivir was superior to remdesivir alone in reducing recovery time and accelerating improvement in clinical status among patients with COVID-19, notably among those receiving high-flow oxygen or non-invasive ventilation. Following the result of this trial, the FDA issued on November 19, 2020 an emergency use authorization (EUA) for baricitinib in combination with remdesivir, for the treatment of suspected or laboratory confirmed COVID-19 in hospitalized adults and pediatric patients 2 years of age or older requiring supplemental oxygen, invasive mechanical ventilation, or extracorporeal membrane oxygenation (ECMO).[122]

The role of addition of glucocorticoids was not evaluated in the ACTT-2 trial, but dexamethasone stayed permitted in standard indications as ARDS. As a matter of fact, the adapative, randomized, blinded controlled phase 3 ACTT-4 trial, which primary objective was to evaluate the clinical efficacy of baricitinib in combination with remdesivir vs dexamethasone and remdesivir as assessed by the mechanical ventilation-free survival by day 29, was closed to enrolement in April 2021, prior to full enrolment, after intermediate efficacy analysis found that neither treatment regimen was significantly better than the other.[123]

Then, results were announced from the phase 3 COV-BARRIER (NCT04421027) trial of baricitinib in hospitalized COVID-19 patients.[124] The international, double-blinded, placebo-controlled study randomized 1525 patients to baricitinib or standard of care alone. Although the study did not meet its primary endpoint for improvement in progression to non-invasive ventilation or invasive mechanical ventilation, or death, there was a statistically significant improvement in mortality for patients treated with baricitinib in addition to standard of care, including corticosteroids and remdesivir.[124] Importantly, the COV-BARRIER study showed that the survival benefits provided by baricitinib were independent of the presence or absence of concomitant use of steroids (mostly dexamethasone). Following these results, the FDA revised the EUA for baricitinib on July 28, 2021, to an EUA authorizing the drug alone for the treatment of COVID-19 in hospitalized adults and pediatric patients 2 years of age or older requiring supplemental oxygen, non-invasive or invasive mechanical ventilation, or ECMO. It has to be mentioned that baricitinib was approved expressely mentioning the fact that it was not 'FDA-approved as a treatment for COVID-19'.[125]

In conclusion, in the USA[126] and Japan[127] (in consideration by the EMA) baricitinib in combination with low-dose dexamethasone is currently recommended for the treatment of COVID-19 patients requiring high-flow oxygen or non-invasive ventilation or low-flow oxygen but with significantly elevated inflammatory markers. The same conclusions were reached by the WHO from the eighth version of its 'A living WHO guideline on drugs for COVID-19' from January 2022, which strongly recommended baricitinib for patients with severe or critical COVID-19 in combination with corticosteroids.[128]

In all those indications, baricitinb was indicated as an alternative treatment to the IL-6 inhibitor tocilizumab. To date in an extremely quickly evolving field, use of baricitinib is not recommended in patients requiring mechanical ventilation or ECMO, as it was judged that more data were needed in this population. Baricitinib seemed nevertheless a reasonable alternative to tocilizumab if the latter is not available.

As for patients with systemic rheumatic diseases, the indication to discontinue treatment in patients with known exposure to SARS-CoV-2 seems prevalent but is still debated and should be discussed with specialist physicians keeping in mind the risk of disease flare.[129]

Tofacitinib

Tofacitinib is a JAK1 (IC50 15 nM) and JAK3 (IC50 45–55 nM) inhibitor known to be effective against cytokines signaling via JAK1 and JAK3.[77 87] It also inhibits JAK2 with less potency (IC50 71–77 nM), while the IC50 for TYK2 is 472–489 nM.[87 130] The efficacy and safety of tofacitinib in patients hospitalized with COVID-19 pneumonia has been recently reported in the STOP-COVID trial, a randomized, double-blind, interventional phase 3 trial (NCT04469114) of 289 patients randomized 1:1 between placebo and tofacitinib 10 mg two times per day; most patients received also glucocorticoids.[131] Tofacitinib led in hospitalized patients with COVID-19 pneumonia to a lower risk of death or respiratory failure through day 28 when compared with placebo. Specifically, death from any cause through day 28 occurred in 2.8% of the patients in the tofacitinib group vs 5.5% in the placebo group (HR, 0.49; 95% CI 0.15 to 1.63), with a cumulative incidence of death or respiratory failure through day 28 of 18.1% vs 29.0% in the tofacitinib vs placebo group (risk ratio, 0.63; 95% CI 0.41 to 0.97; p=0.04).[131] These results let the US National Institute of Health (NIH) to recommend tofacitinib as an alternative to baricitinib when unavailable.[126] Of note, the WHO recommended against the use of tofacitinib in January 2022, considering lack of evidence from small trials and possible increase in serious side effects with tofacitinib.[128 132]

Upadacitinib

Upadacitinib was reported to be a JAK1 specific inhibitor.[133] It exhibits sub nM IC50 for JAK1 (0.76 nM), 2 nM

for JAK2 and >100 nM for JAK3 and TYK2.[130] Its in vitro pharmacology was determined in primary peripheral mononuclear cells from healthy subjects in comparison with baricitinib and tofacitinib.[134] In primary cells upadacitinib also inhibited JAK2 downstream IL3 type cytokines, not only JAK1 downstream inflammatory cytokines like IL6.[134] Given this profile of inhibition, this inhibitor may be useful in counteracting the broad signaling via JAK1 and JAK2 in cytokine storm. Of interest an in-silico docking study found significant binding of kinase inhibitors like upadacitinib to the RNA dependent RNA polymerase kinase-like folded NiRAN domain.[135]

JAKi most in use in hematology patients

Ruxolitinib

Ruxolitinib is a JAK1 and JAK2 inhibitor used for around 10 years in the clinics for the treatment of MPNs MF and PV.

In general, hematological cancer patients exhibit enhanced mortality on SARS-CoV-2 infection when compared with healthy individuals.[136 137] A cohort study in Wuhan on hospitalized hematological cancer patients showed an incidence of 10% that developed COVID-19 infection. While the incidence was relatively similar to that in healthcare providers (7%), the mortality rate of hematological patients (no MPN in the study) was increased to 62% vs 0% of healthcare providers,[138] estimated 41 times higher than hematological patients without SARS-CoV-2 infection.[139] Interestingly, a lower rate of SARS-CoV-2 infection was recorded both in China and elsewhere for *BCR/ABL1* positive chronic myeloid leukemia.[137 140] However, no significant attenuation of T cell response was detected in MPN patients compared with healthy subjects.[141]

Ruxolitinib was tested in several clinical settings, from one patient studies to phase 3 clinical trials (table 1, ClinicalTrials.gov). However, although a positive impression first came from case reports or small, uncontrolled or non-randomized cases series[142–148] that ruxolitinib might be helpful to fight COVID-19, these results were not confirmed in larger randomized trials. Press releases have been issued after the results of two phase 3, randomized, placebo-controlled clinical trials with the use of ruxolitinib in hospitalized non-ventilated patients (RUXCOVID, NCT04362137) and in ventilated patients (RUXCOVID-DEVENT, NCT04377620). Neither study met its primary endpoint. In the RUXCOVID study, 432 patients were enrolled. The results showed no improvement in the proportion of patients who experienced death, respiratory failure requiring mechanical ventilation or admission to the intensive care unit, by day 29 for patients receiving ruxolitinib 5 mg bidaily compared with those receiving standard of care alone (12.0% vs 11.8%; p=0.769). There was also no clinically relevant benefit in mortality rate by day 29 or in time to recovery.

The phase 3 RUXCOVID-DEVENT study evaluated ruxolitinib 5 mg bidaily or 15 mg bidaily as a treatment for patients with COVID-19 associated ARDS on mechanical ventilation. There was no statistically significant improvement in mortality through day 29 compared with placebo. However, when US study participants were analyzed separately there was significance, as it also could be detected for the overall population when data from both treatment arms were pooled. The safety findings in both RUXCOVID and in RUXCOVID-DEVENT were consistent with those expected for ruxolitinib and for patients with COVID-19 infection.

The development of ruxolitinib in the COVID-19 setting has not proceeded after these results[149] and ruxolitinib has not be retained by the WHO[128] or other drug agencies as a drug to be used in the setting of COVID-19.

Many reasons could explain the fact that ruxolitinib, which has the same spectrum of JAKs inhibition as baricitinib, did not improve patients with COVID-19. One would be that BenevolentAI did not predict ruxolitinib as having a possible impact on reducing SARS-CoV-2 pathogenicity. Furthermore, it is not clear if patients in the RUXCOVID and RUXCOVID-DEVENT trials were constantly on antiviral therapy. Nevertheless, coadministration of remdesivir with baricitinib in the COV-BARRIER trial concerned less than 20% of the patients and the placebo and test arms were appropriately balanced.[124] Hence, it is unlikely that it influenced the results of the study. Doses of ruxolitinib in the RUXCOVID trial were also lower than in most publications, but higher doses did not improve outcome in the RUXCOVID-DEVENT trial. As for the best time of intervention, no phase 3 study evaluated early treatment with ruxolitinib but both RUXCOVID and RUXCOVID-DEVENT studied different time of evolution of the disease and neither met its endpoint. Finally, best efficacy of baricitinib or tofacitinib is achieved in combination with corticosteroids, and such combination was not studied in a dedicated trial in the case of ruxolitinib. Once again however, publications mainly focus on trials with positive ending. Details of failed ones are much more difficult to get access to and the decision behind the arrest in the development of ruxolitinib can only be interfered from press releases by Novartis/Incyte.

The COVID-19 pandemia had a major impact on how MPN patients are managed.[150] Studies on the evolution of MPN patients with respect to COVID-19 infection led by default to examination of effects of ruxolitinib because many patients were under treatment and sudden discontinuation of ruxolitinib is generally avoided in MPNs due to potential rebound effects on myeloid proliferation.

In a retrospective study with 175 MPN patients, it was reported that MPN patients' mortality was higher than in the general population and was 48% in MF, while ET, PV and prefibrotic MF appeared more similar to the general population.[151] That ruxolitinib treatment was significantly more frequent in patients who died in comparison with survivors (p=0.006) may be explained because the severity of the MPN was higher and necessitated treatment. Following multivariable analysis, ruxolitinib treatment alone did apparently not impact on mortality in this study. In an independent manner, it was established

that discontinuing ruxolitinib in MPN patients with COVID-19 is associated with increased mortality.[151] Therefore, in MPN patients on treatment with ruxolitinb who would develop COVID-19, its discontinuation should always be considered with care and discussed in concertation with the referent hematologistand guided by the presence of objective clinical reasons for discontinuation like worsening clinical condition, inability to take peroral medication or contraindications for ruxolitnib treatment (thrombocytopenia, anemia, bleeding, and bacterial sepsis), all of whom might be negative prognostic factors per se.[152]

A combination of anti-IL-6 therapy and ruxolitinib was proposed[153] based on the resemblance of inflammation in severe COVID-19 with the inflammatory picture detected in the pathological condition HLH.[154] This laboratory profile predicted severe evolution.[155] Such patients were thought to possibly benefit from combining IL-6 inhibitor tocilizumab and ruxolitinib. In latest guidelines on COVID-19; however, no combination of tocilizumab and any JAKi is recommended.

Nezulcitinib

Nezulcitinib is a panJAKi with IC50s of 10.3 nM, 10.6 nM, 10.2 NM and 9.2 nM for JAK1, JAK2, JAK3 and TYK2, respectively[156] that can be administered by inhalation. A phase 1 study in healthy subjects of inhaled nezulcitinib demonstrated safety and minimal plasma exposure when given by inhalation. This was also supported by no effects on NK activity. Single and multiple doses of inhaled nezulcitinib at 1, 3 and 10 mg had no safety issues.[156] A phase 2 clinical trial with inhaled nezulcitinib in severe COVID-19 is underway and initially reported trends for improved oxygenation and clinical status, shortened hospitalization, and fewer deaths vs placebo (NCT04402866).[157] However, in the long-run the top-line data news reported that inhaled nezulcitinib failed to meet the primary endpoint in hospitalized patients with COVID-19 with acute lung injury and impaired oxygenation.[158]

Meta-analyses and comparative assessment of JAKi

Several meta-analysis studies were reported on safety and efficacy of use of JAKi, especially baricitinib and ruxolitinib in COVID-19.[159–161]

One examined effect of JAKi in patients with COVID-19 published between Jan 1, 2020 and March 6, 2021. Six cohort studies and five clinical trials were assessed with 2367 patients.[159] Precise timing of start of JAKi treatment was not consistently reported. JAKi decreased use of invasive mechanical ventilation, but only had marginal effects on rates of intensive care unit admissions and ARDS. The precise effect of timing of JAKi treatment which could be critical could not be assessed and correlated with effects.[159] The quality assessment of the published studies was performed with the Newcastle-Ottawa and Jadad scales.[159]

In another study[160] several electronic databases (PubMed, EuropePMC, the Cochrane Central Register of Controlled Trials) were searched up to December 11, 2020 for published studies bearing keywords “COVID-19″ along with (“JAK inhibitor” or “Ruxolitinib” or “Tofacitinib” or “Fedratinib” or “Baricitinib”) and (“Severe” or “Mortality”). Five studies with 1190 patients were included. Use of JAKi was significantly associated with a reduced risk of mortality and clinical improvement in COVID-19 hospitalized patients.

The same overall conclusions were reported by a meta-analysis,[161] which examined two databases for RCTs in hospitalized patients, where patients were assigned to JAKi or standard of care. Pooling data from 4 such trials with 1338 subjects and treatment with either baricitinib, ruxolitinib, tofacitinib and nezulcitinib. Treatment with a JAKi led to a significant reduction in the risk of COVID-19 death by 43%.[161]

Although these meta-analyses provide a positive image on the efficacy of JAKi in COVID-19, there are several weaknesses. There is redundancy in the studies interrogated. Head-to-head comparisons of different inhibitors in similar settings was not performed. In addition, lack of systematic information on the timing of start of treatment and the variability of simultaneous glucocorticoid treatment further complicate interpretation.

Potential adverse effects

There are known adverse effects of the JAKi, which have been described in phase 3 clinical trials with ruxolitinib in MF and PV and then observed for other JAK2 inhibitors.[162–164] Given that JAK2 is essential for formation of red blood cells and platelets, anemia and thrombocytopenia can be induced by the use of JAK2 inhibitors.[162–164] In addition, given the involvement of JAKs in the immune response especially via IFNγ, reactivation of latent herpes simplex, zona-zoster, hepatitis B infections and tuberculosis are well known potential adverse effects. Several patients treated for MPNs with ruxolitinib developed aggressive B cell lymphomas.[165] Given that the timeframe of administration of JAKi is rather short (less than 30 days) in COVID-19 the expectation is that the effects reported after long-term use will not be detected.

Several JAKi are being investigated for potential thrombotic risk.[166] This is apparently the case for tofacitinib and upacitinib (JAK1 inhibitor).[166] This issue is of great relevance given the thrombotic risk of COVID-19. Also, baricitinib should be examined for potential thrombotic risk.[167] In September 2021, FDA issued an updated warning about increased risk of serious heart-related events, cancer, bloot clots and death for JAKi that treat certain chronic inflammatory conditions.[168] This warning is based on a review of a large randomized safety clinical trial involving tofacitinib, but the warning has been extended to other JAKi treating such chronic inflammatory condition, such as baricitinib and upacitinib. This warning may not be relevant for treatment with JAKi in COVID-19 due to the short treatment duration, while the safety trial concerned chronic longer exposure to JAKi.

A couple of potential adverse effects of JAKi including cardiotoxicity and hepatotoxicity, as well as toxicity of combinations including JAKi have been reviewed in.[169] The coadministration, of either baricitinib or ruxolitinib, along with IL-6 inhibitors (eg, tocilizumab, siltuximab) could produce additive immunosuppression with possible severe bacterial or fungal infections. Ruxolitinib inhibits colchicine metabolism and this is relevant in patients with renal or hepatic impairment. Favipiravir may enhance baricitinib exposure. In contrast, no adverse effects have been reported for simultaneous use of JAKi and remdesivir or dexamethasone.

CONCLUSIONS

Although the pandemic situation seems to wear-off in western countries and vaccines against SARS-CoV-2 have led to limiting hospitalisations, predicting the future evolution of SARS-CoV-2 infection in the human population is difficult. Many questions remain concerning new outbreaks, evolution of the virus or how long protection following natural infection or vaccination will last. Therefore, the potential for drugs to treat people suffering from COVID-19 remains of interest.

The major targets for inhibition in COVID-19 are the entry and replication of SARS-CoV-2 and the immunopathological phenomena triggered by the delayed cytokine storm inducing lung pathology and ARDS. Since cytokines act via JAKs, JAK1 and JAK2 inhibitors may emerge as potential useful therapeutic tools in the COVID-19 pathology. A JAK1 and JAK2 inhibitor, baricitinib also inhibits the Numb Associated Kinase AAK1, which is involved in endocytosis of the virus. Hence baricitinib would act both via inhibiting the immunopathology and inflammation due to JAK1/JAK2 inhibition, but also via direct inhibition of virus entry and assembly due to AAK1 inhibition. Positive results from the double-blind, randomized, placebo-controlled ACCT-2 and COV-BARRIER trials led to the FDA recommendation of baricitinib in certain settings of COVID-19 and its recommendation in U.S. NIH practice guideline for treatment of COVID-19. The same conclusison were reached by the expert committee of the WHO that recommends baricitinib in severe and criticial COVID-19 since the eighth version of its 'living guideline on drugs for COVID-19' from January 2022. Second, the JAK2 and JAK3 inhibitor tofacitinib also got recommended by the U.S. NIH in case of baricitinib unavailability. However other alternative exists, such as IL-6 receptor blockers, which should be used in priority according to latest WHO guidelines.

Ruxolitinib is the oldest JAKi on the market and the only one used in the hematological setting. It is often used in the treatment of MF and certain PV patients, and it was shown that on SARS-CoV-2 infection the inhibitor is generally not stopped and should be continued to control the MPN. It has been suggested that ruxolitinib may reduce immunopathological reactions as it does for GVHd. Although small non-controlled studies suggested some benefit for ruxolitinib in the treatment of COVID-19 and meta-analyses suggested a positive effect, the two randomized phase 3 studies failed to meet their primary endpoints, which led to suspension of ruxoltinib development program in the setting of COVID-19. The different outcomes between these RCTs and smaller studies point to the complexity of conducting clinical studies in patients with COVID-19. The outcomes may have been affected by differences in standards of care at different sites and in different regions at different times during the course of the pandemic. These standards of care include varying proportions of patients receiving corticosteroids, which has been shown to affect the outcomes for hospitalized patients. The studies also enrolled patients at different stages of COVID-19 and patients with more advanced disease may respond better to JAKi. Differences in outcomes between ruxolitinib and baricitinib in different studies may also reflect the doses used.

The results on JAKi clinical use analyzed in several meta-analyses and in the studies detailed above suggest but do not prove a role for JAKi in COVID-19, as the only robust data are for an inhibitor that also exerts other effects than JAKi. Better designed trials with precisely controlled time of introduction of JAKi will be critical in the future. A favorable action of JAKi would be predicted by the mechanistic understanding of the COVID-19 immunopathology involving cytokines that require JAK1 for signaling. However, robust clinical confirmation is still needed. In principle, JAKi can be envisaged at times of development of cytokine storm but should not be used too early in COVID-19, as type I IFN signaling requires JAK1. Careful timing is required since pathological secretion of IL-6/IL-1 may occur in the same time-frame with the requirement for type I IFN action to clear the virus, and therefore JAKi may need to be associated with antiviral molecules.

As more molecules keep being approved in emergency for treatment of COVID-19, RCTs are important to compare their definitive effect and determine the best indication(s) for each molecule. Also, RCTs with combination therapies would be all the more important for patient who might be already on treatment with JAKi—which it might be dangerous to stop abruptly in an emergency situation.

More data will emerge on secondary effects of JAKi in the setting of COVID-19. For example, both JAKi and COVID-19 were linked to potential thrombotic risk of their own but no evidence to date exist for an increased thrombotic risk while using JAKi for treating COVID-19. Moreover, adverse effects known from long-term treatment with JAKi, as immunosuppression, are less likely to be relevant in COVID-19, where treatment is of short duration, but should be kept in mind.

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ORCID iDs
Gabriel Levy http://orcid.org/0000-0001-6746-3083
Stefan N Constantinescu http://orcid.org/0000-0002-8599-2699

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Correction: *JAK inhibitors and COVID-19*

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