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Janus Family Kinase (JAK) Inhibitors in HLH and Severe COVID-19

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After its emergence in Wuhan, China, the rapid transmission of a novel betacoronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), led to a worldwide pandemic, coronavirus disease 2019 (COVID-19). Thankfully, the overwhelming majority of COVID-19 patients have mild respiratory symptoms or remain entirely asymptomatic (1). SARS-CoV-2 is highly immunogenic, and pre-existing immunity to SARS-CoV-2, possibly due to its homology with endemic coronaviruses causing the "common cold", is observed to varying degrees in many uninfected individuals (2-4), and may thus explain the prevalence of asymptomatic carriers (5-7). However, a hyperinflammatory response to SARC-CoV-2, pathogenically analogous to that observed in hemophagocytic lymphohistiocytosis (HLH) promotes the development and progression of both acute respiratory distress syndrome (ARDS) and systemic manifestations of severe COVID-19, and is thus a dominant driver of mortality. The underlying immunologic mechanisms that promote severe COVID-19, while increasingly appreciated (8, 9), also have significant therapeutic implications.

The pathogenic role of monocytes/macrophages

High viral titers and the inflammatory response leads to a dramatic increase in the accumulation of monocytes/macrophages in the lungs of SARS, including COVID-19, patients (10, 11). Monocyte-derived macrophages are abundant in the bronchoalveolar lavage fluid of COVID-19 patients, whereas tissue-resident (alveolar) macrophages are relatively less abundant, and gene set enrichment analysis of scRNA-seq data

suggests, perhaps not surprisingly, that they are “classically” (M1) polarized (11). Macrophage hemophagocytosis, associated with classically polarized macrophages, is a pathologic hallmark of HLH, and is similarly observed in the lungs of patients with SARS (10). Similarly, scRNA-seq performed in PBMC obtained from COVID-19 patients and healthy controls demonstrates a significant expansion of classical monocytes and enrichment for TNF and IL-1β-responsive genes (12, 13).

A strain of SARS-CoV has been adapted for mouse studies and provides compelling evidence for the pathogenic role of monocytes/macrophages in SARS. C57BL/6 mice infected with mouse adapted SARS-CoV rapidly clear the virus and develop only mild symptoms, whereas infected Balb/c mice develop severe symptoms that are associated with a significant expansion of pulmonary monocytes/macrophages. As type 1 interferons contribute to the inflammatory response in severe COVID-19 (13), this model was utilized to further dissect their role in disease pathogenesis. In contrast to Balb/c mice which succumb to SARS-CoV, those lacking expression of the IFNαβ receptor (Ifnar-/-) developed only mild symptoms and survived (14). Interestingly, Ifnar-/- mice cleared SARS-CoV with similar kinetics to those observed in control Balb/c, but succumbed to infection with alternative RNA viruses (including influenza A). Therefore, the pathogenic role of type 1 interferons is apparently unique to SARS-CoV, and is not explained by impaired viral clearance. Instead, the loss of type 1 interferon signaling significantly impaired the recruitment and activation of inflammatory monocytes while

increasing the total number of viral-specific T cells. Antibody-mediated CCR2 blockade dramatically reduced monocyte infiltration in Balb/c mice and led to complete protection from lethal SARC-CoV challenge. SARS-CoV-2 has been detected in lymph node macrophages (15), and ACE2 (the SARS-CoV-2 receptor utilized for viral entry) is a type 1 interferon target gene (16). While the possibility of macrophage-mediated uptake of viral-containing immune complexes or phagocytosis of infected cells cannot be excluded, these observations raise the intriguing possibility that macrophages are a viral reservoir. Collectively, the available data suggests that monocytes/macrophages play a pathologic role in severe COVID-19 (8), analogous to that observed in HLH, and are attractive therapeutic targets.

The rationale for cytokine (and JAK/STAT) blockade in COVID-19: Lessons learned in HLH

While cytokine blockade is a rational therapeutic strategy, and despite initially promising outcomes, a phase III trial (COVACTA trial) investigating tocilizumab in severe COVID-19 failed to meet its primary or key secondary endpoints. However, the pleiotropic and partially redundant functions associated with most cytokines may pose a challenge for therapeutic strategies targeting a single cytokine. As most cytokines implicated in severe COVID-19 converge on the JAK/STAT pathway, JAK inhibition is a rational alternative strategy, and one that is further supported by the experience with this strategy in secondary HLH.

As interferons are pathogenic in secondary HLH, ruxolitinib was investigated in pre-clinical HLH models and was shown to significantly reduce HLH-associated laboratory and clinical abnormalities (17), and was superior to more targeted, cytokine (IFNγ)-specific blockade (18). The first prospective clinical trial investigating ruxolitinib in adults with secondary HLH further supports this strategy (19). In this small study, seven patients with secondary HLH received ruxolitinib, all of whom experienced the rapid resolution of HLH-associated symptoms and laboratory abnormalities following treatment. Cytopenias significantly improved within the first week of treatment and transfusion independence was rapidly achieved. Therefore, treatment was associated with relatively rapid hospital discharge and superior survival when compared with historic controls. In a similar population of historic controls, the 120-day mortality was 49%. By comparison, no deaths were observed in patients treated with ruxolitinib. The macrophage-specific hemoglobin-haptoglobin scavenger receptor (CD163), while not specific for HLH-associated macrophage activation, was examined as a pharmacodynamics biomarker for macrophage activation, and a significant reduction in plasma soluble CD163 was observed following treatment. Therefore, the immunologic similarities between severe COVID-19 and secondary HLH, combined with ruxolitinib's clinical activity in HLH, should bolster enthusiasm for the JAK inhibitors being investigated in a number of COVID-19 clinical trials (Table 1).

JAK inhibitor target selectivity: Is a JAK of all trades beneficial in severe COVID-19?

Given the central role of monocyte/macrophages in severe COVID-19, therapeutic strategies to either target their polarization state or deplete them outright are certainly rational. Colony-stimulating factor-1 (CSF-1, or M-CSF) is required for normal macrophage homeostasis and viability, as mice lacking functional CSF-1 or CSF-1 receptor (CSF-1R, c-fms, CD115) have a marked decrease in tissue resident macrophages (20, 21). Therefore, CSF-1R antagonists, including both tyrosine-kinase inhibitors and antagonistic monoclonal antibodies, are being exploited as a strategy to deplete tissue resident macrophages, particularly in many cancers, and in COVID-19 (NCT04415073). However, recent evidence suggests that alternative (and CSF-1 independent) cytokines promote monocyte/macrophage expansion in virally infected mice (22). Therefore, in addition to the well-described role of cytokine- (and JAK-dependent) signaling in regulating macrophage polarization (23), JAK inhibition may also impair their expansion and/or survival, particularly in combination with CSF-1R antagonists. In fact, CSF-1R has been identified as an “off target” for the JAK inhibitors pacritinib and fedratinib, and these agents were shown to deplete monocyte-derived macrophages in *ex vivo* studies (24). Pacritinib is being investigated in a randomized, placebo-controlled, phase III study in patients with severe COVID-19, including those with cancer (PRE-VENT study, NCT04404361).

Cytokine production by monocytes/macrophages upon recognition of viral RNA is mediated by Toll-like receptors (TLRs), and TLR signaling is particularly important for the early production of type 1 interferons and for classical macrophage polarization (25). TLR signaling is dependent upon the formation of the Myddosome, which includes MyD88 in complex with the IRAK family of serine-threonine kinases, IRAK1 and IRAK4. TLR engagement and Myddosome assembly leads to autophosphorylation and activation of IRAK1, eventually culminating in NFκB and MAPK activation. In addition to CSF-1R, IRAK-1 is an additional "off target" for pacritinib (IC_{50} <50nM), both of which may be inhibited at clinically achievable concentrations (26).

In addition to immunologically relevant targets, selected agents may also impair viral entry. Upon binding its cell surface receptor (ACE2), SARS-CoV-2 viral particles gain entry into the cell by clathrin-mediated endocytosis, a process which requires a number of adaptor proteins and kinases, including adaptor-associated protein kinase 1 (AAK1). AAK1-dependent endocytosis is utilized by manner viruses, and targeted agents that impair its activity inhibit viral entry (27). Baricitinib, a JAK1/JAK2/TYK2 inhibitor, was observed to inhibit AAK1 (and related kinases) at clinically achievable concentrations (IC_{50} <50nM)(28). This activity may be relatively specific to baricitinib, as inhibition by alternative JAK inhibitors (ruxolitinib and fedratinib) was less significant, with IC_{50}'s in cell-based assays approaching 1 μM. Additional JAK inhibitors, including pacritinib, were not examined. A pilot study compared outcomes in moderate COVID-19

patients receiving baricitinib (n=12) or hydroxychloroquine (n=12). All patients received lopinavir/ritonavir. More rapid improvements in clinical and laboratory indicators of disease severity were noted in baricitinib treated patients (29). These preliminary findings were subsequently confirmed in a larger, multicenter study that included consecutive patients treated with either baricitinib (n=113) or hydroxychloroquine (n=78). Again, more rapid clinical improvement was noted in patients receiving baricitinib (30).

The variable selectivity of JAK inhibitors for non-JAK ("off") targets may certainly contribute to clinically significant differences in their anti-viral and immunologic effects. Pacritinib, for example, given its ability to inhibit CSF-1R may be particularly promising, as monocytes/macrophages are a therapeutic target, whereas baricitinib, given its ability to impair AAK1-dependent viral entry may have unique anti-viral properties. In addition to their "off targets", the JAK inhibitors also have significant differences in selectivity for each of the four JAK isoforms (JAK1, JAK2, TYK2, JAK3), which is summarized in Table 1. Different cytokine receptors utilize different JAK isoforms, and those receptors utilizing more than a single JAK isoform may be variably dependent upon a given isoform. Therefore, the relative selectivity of currently available JAK inhibitors, and the pleiotropic, yet non-redundant, role of the JAK isoforms, suggest that differences in JAK selectivity among JAK inhibitors may be associated with clinically significant differences in immunologic effects. As these differences in selectivity are not

absolute, the immunologic disparities among clinically available JAK inhibitors are complex, potentially subtle, but likely dose-, time-, and context-dependent, and certainly warrant further study. The immunologic similarities between severe COVID-19 and HLH not only support the many ongoing clinical trials with JAK inhibitors in COVID-19, but also suggest that hope for the future may not be limited to the development of an effective vaccine, but may also be found in the hematologist's medicine cabinet.

Data Availability Statement: Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Table 1. Janus family kinase inhibitors being studied in COVID-19.

	Target Specificity							
Agent	**JAK1**	**JAK2**	**TYK2**	**JAK3**	**CSF1R**	**IRAK1**	**AAK1**	**Clinical Trial(s)**
Baricitinib	++	++	+	-	NR	-	+	ACTT-2 (randomized, phase III)
Pacritinib	-	++	++	++	++	++	NR	PRE-VENT (randomized, phase III)
Ruxolitinib	++	++	++	++	-	-	-	Phase II/III
Tofacitinib	++	++	+	++	NR	-	-	Randomized phase IIb

++IC_{50} <25 nM; **+**IC_{50} 25-100 nM; **-**IC_{50} >100 nM; NR, not reported

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