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Less Can Be More When Targeting Interleukin-6-Mediated Cytokine Release Syndrome in Coronavirus Disease 2019

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Abstract: Coronavirus disease 2019 pandemic caused by severe acute respiratory syndrome-coronavirus-2 is a worldwide public health emergency that will have a lasting generational impact in terms of mortality and economic devastation. Social distancing to prevent viral transmission and supportive care of infected patients are the main interventions now available. This global health crisis therefore merits innovative therapies. Cytokine release syndrome mediated by interleukin-6 is a critical driver of coronavirus disease 2019 mortality. Herein, we review and discuss key immunologic effects of direct interleukin-6 blockade, downstream nonselective Janus kinase inhibition, and selective Janus kinase 2 suppression to treat coronavirus disease 2019–related cytokine release syndrome. We provide evidence that selective targeting of interleukin-6 or Janus kinase 2 is well informed by existing data. This contrasts with broad, nonselective blockade of Janus kinase-mediated signaling, which would inhibit both deleterious and beneficial cytokines, as well as critical host antiviral immunity.

Key Words: coronavirus disease 2019; severe acute respiratory syndrome-coronavirus-2, cytokine release syndrome, interleukin-6, JAK

Coronavirus disease 2019 (COVID-19), the severe infection caused by the virus severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), has affected almost 5 million individuals and killed over 300,000 worldwide by mid-May 2020 (1). These statistics continue to climb and more universal testing will identify increased numbers of people with minimal or no symptoms. Public health officials have employed social distancing and regional stay-at-home directives as the only available interventions to flatten the curve of newly diagnosed cases and hospitalizations. Healthcare providers can only provide aggressive supportive care and repurpose existing therapeutic agents against this novel virus, without the benefit of time to conduct controlled clinical trials (2, 3).

INTERLEUKIN-6 AS A DRIVER OF COVID-19 CYTOKINE RELEASE SYNDROME

Early in the characterization of COVID-19 disease, physicians in Wuhan, China, recognized that patients exhibited a second wave of symptoms consistent with cytokine release syndrome (CRS), characterized by high levels of interleukin-6 (IL-6), high fevers, and hypoxic pneumonitis often requiring mechanical ventilation (4, 5). As seen in other clinical settings prone to CRS (6), clinical investigators have identified this as a manifestation of an overly robust immune response to the SARS-CoV-2 (4, 5, 7). Mechanistically, pathogenic Th1 T cells fuel CRS by producing GM-CSF, which induces $CD14^{+}CD16^{+}$ monocytes to release IL-6, causing the resultant CRS (5, 7). Investigators in China thus made the rational choice to use the anti-IL-6 receptor monoclonal antibody (Mab), tocilizumab, to treat CRS and reduce the sequelae of IL-6-mediated inflammation (8–11) (**Fig. 1**). Emerging data show that early administration of tocilizumab can reverse the inflammatory pneumonitis associated with COVID-19, which in the best-case scenario results in radiographic improvement within 3 weeks of treatment (8). Investigators in the United States are similarly studying the efficacy of tocilizumab in COVID-19 pneumonia in a multicenter, randomized, placebo-controlled phase III clinical trial (ClinicalTrials.gov: NCT04320615, **Table 1**). Tocilizumab is FDA and EMA approved for the treatment of CRS after chimeric antigen receptor (CAR) T-cell therapy in the United States and Europe (6), and tocilizumab is now approved in China for the treatment of COVID-19-induced CRS. In the context of treating

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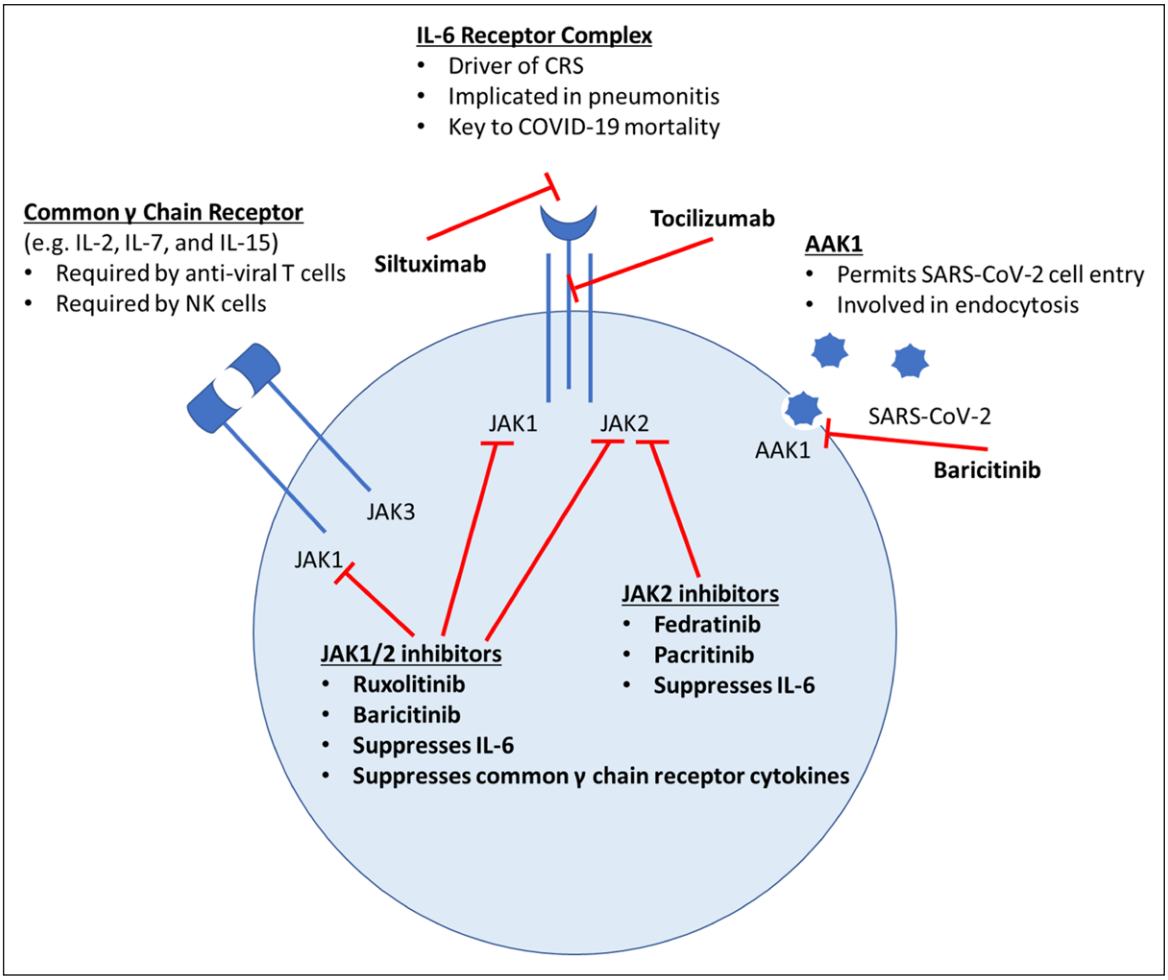

Figure 1. The differential effects of anti-interleukin (IL)-6 monoclonal antibody (Mab) and Janus kinase (JAK) inhibitors as coronavirus disease 2019 (COVID-19) therapy. The IL-6 receptor complex uses JAK1 and JAK2 to mediate signal transduction, while JAK1 and JAK3 are required by the common γ-chain receptors. The broad suppressive effects of JAK1/2 inhibitors, like ruxolitinib and baricitinib, concurrently diminish the activity of IL-6 and the common γ chain receptors. Conversely, IL-6 cytokine or receptor blockade with monoclonal antibodies or selective JAK2 inhibitors, fedratinib and pacritinib, spare common γ-chain receptor activity. While the JAK1/2 and JAK2 inhibitors reduce IL-6 activity and likely COVID-19 cytokine release syndrome (CRS), the broader suppressive effects of ruxolitinib and baricitinib limit overall antiviral immunity by targeting JAK1 required by T and NK cells alike. Baricitinib is unique, however, in that it has direct antiviral effects on severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) by binding AAK1 and limiting host cell endocytosis of viral particles.

CRS after CAR T-cell therapy, tocilizumab has not caused severe adverse reactions, secondary infections, or deaths (6). Moreover, while tocilizumab blockade of the IL-6 receptor reduces the rampant systemic inflammation observed with CRS, there is no collateral damage to dendritic cell or T-cell function (12). Clinical investigators are also comparing siltuximab, a direct anti-IL-6 chimeric Mab (13), with glucocorticoids in treating COVID-19 pneumonia (ClinicalTrials.gov: NCT 04329650; Table 1).

TARGETING JANUS KINASE1/2 TO SUPPRESS IL-6 RECEPTOR ACTIVITY

In contrast to blockade of the IL-6 cytokine or its receptor, others have sought to blunt the severity of COVID-19 by therapeutically targeting signal transduction mediated by the IL-6 receptor, using the Janus kinase (JAK) 1/2 inhibitor, ruxolitinib (14, 15) (Table 1). Ruxolitinib is FDA-approved for the treatment of myelofibrosis (16), and more recently, steroid-refractory graft-versus-host disease (17). While ruxolitinib suppresses IL-6 receptor activity (18, 19), its very broad effects on JAK1/2 signaling also eradicate the functions of other important common gamma chain cytokines, e.g., IL-2, IL-7, and IL-15 (20, 21) (Fig. 1). Others and we have also demonstrated that ruxolitinib profoundly impairs key cell mediators of host antiviral immunity, chiefly beneficial cytotoxic T lymphocytes, natural killer cells, and dendritic cells (20–25). While ruxolitinib diminishes the systemic response to IL-6, as well as impairing Th1 cells implicated in the initiation of CRS, its overall suppression of cellular and innate immunity can also disable the clearance of SARS-CoV-2. Similar to a SCID-like immune phenotype, broad JAK1/2 inhibition by ruxolitinib is complicated by serious infections like cryptococcal pneumonia, tuberculosis, hepatitis B, and cytomegalovirus (24, 26, 27). Given that ruxolitinib clearly reduces antiviral immunity, the rationale to test its use in treating patients with severe or very severe COVID-19 illness merits at least equipoise or serious reconsideration. Caution is especially warranted in the context of an expanded access program for ruxolitinib, managed by Novartis in the United States (ClinicalTrials.gov: NCT 04337359), and a phase 3 clinical trial of

TABLE 1. Inhibitors of Interleukin-6 Receptor Signal Transduction

Drug	Mechanism	Immune Profile	Antiviral Activity	Coronavirus Disease 2019 Trial NCT #
Tocilizumab (7–10)	Anti-IL-6R Mab	≠ DCs ≠ T cells	Not reported	NCT 04320615
Siltuximab (13)	Anti-IL-6 Mab	Not reported	Not reported	NCT 04329650
Ruxolitinib (17–21)	JAK1/2 inhibitor	↓↓ DCs ↓↓ T cells ↓↓ NK cells	Weak AAK1	NCT 04337359 NCT 04331665 NCT 04334044
Baricitinib (28, 29)	JAK1/2 inhibitor	↓↓ T cells	Strong AAK1	NCT 04358614 NCT 04340232 NCT 04346147 NCT 04320277 NCT 04321993 NCT 04345289
Fedratinib (21, 31, 32)	JAK2 inhibitor	↓↓ DCs ≠ T cells ↓ NK cells	Weak AAK1	Not reported
Pacritinib (20)	JAK2 inhibitor	≠ T cells ↓↓↓ NK cells	Not reported	Not reported

COVID-19 = coronavirus disease 2019, DC = dendritic cells, JAK = Janus kinase, NK = natural killer.
≠ = no change; ↓ = decrease in cell number and/or function.

ruxolitinib, sponsored by Incyte in the United States and Novartis outside the United States, both of which are enrolling patients with CRS due to COVID-19 (ClinicalTrials.gov: NCT 04331665 and NCT 04334044).

JAK INHIBITORS AS ANTIVIRAL THERAPY

In contrast to ruxolitinib, another JAK1/2 inhibitor, baricitinib, is another potential therapeutic agent against SARS-CoV-2 (28, 29). An advantage of baricitinib over ruxolitinib, however, is that it can not only target IL-6 signal transduction; but it also exerts antiviral activity by neutralizing AAK1, a protein involved in viral entry by SARS-CoV-2 (28) (**Table 1**, **Fig. 1**). Hence baricitinib is also a candidate antiviral medication. Despite ruxolitinib's capacity to bind AAK1 as well, it is 20-fold less potent in this regard than a comparable dose of baracitinib (29). Standard doses of ruxolitinib would therefore not achieve meaningful antiviral activity in COVID-19 patients. The unique antiviral effect of baricitinib combined with its ability to suppress IL-6 signal transduction have therefore led to its evaluation in a number of clinical trials for the treatment of COVID-19 (ClinicalTrials.gov: NCT 04358614, NCT 04340232, NCT 04346147, NCT 04320277, NCT 04321993, and NCT 04345289).

SELECTIVE JAK2 INHIBITION TO REDUCE IL-6 SIGNAL TRANSDUCTION

Fedratinib and pacritinib are selective JAK2 inhibitors that exhibit negligible effects on JAK1 at standard doses (20, 30, 31) (Table 1). We have shown that fedratinib reduces dendritic cell maturation yet spares the activity of viral-specific T cells (32), and it exhibits intermediate suppression of NK cells compared with ruxolitinib (21). Moreover, the chief toxicities associated with fedratinib include gastrointestinal side effects, anemia, and rare encephalopathy, which thiamine supplementation can prevent (31). We have also shown that unlike broad JAK1/2 inhibition by ruxolitinib, selective JAK2 inhibition by pacritinib spares both nonalloreactive T cells specific for nominal antigens and the induction of beneficial Tregs, while significantly limiting NK cell activity (20). In a large, randomized clinical trial, the most common adverse events attributed to pacritinib were diarrhea and thrombocytopenia (30). To date, serious infectious complications have not been reported with either selective JAK2 inhibitor (30, 31). Additionally, fedratinib and pacritinib efficiently suppress Th1 cells that initiate CRS pathogenesis via GM-CSF (5, 20, 32). Similar to ruxolitinib, fedratinib has weak activity against AAK1 at standard doses and is therefore unable to exert direct antiviral effects against SARS-CoV-2 in the way that baracitinib can (29). The effects of pacritinib on AAK1 are unknown. Given that fedratinib and pacritinib spare antigen-specific T-cell function and have minimal risks for opportunistic infections, selective JAK2 inhibitors warrant preferential testing in treating COVID-19 CRS over broader JAK1/2 inhibitors like ruxolitinib (Figure 1).

CONCLUSIONS

COVID-19 is the most significant infectious global health threat experienced in generations. There is thus an essential need for innovative and novel applications of existing therapeutics to address the morbidity and mortality of this deadly virus. We must nevertheless thoughtfully weigh the risks and benefits of experimental investigations and rely on established data to guide interval decisions before there are clear-cut conclusions. The hunger for game changing therapies should not cloud clinical judgment and practice, diminish the need for disciplined clinical research, or minimize scientific rigor. One must carefully consider the severe immune consequences of ablating common gamma chain cytokines. While CRS has emerged as a critical driver of COVID-19 pathology and death, approaches to target IL-6 selectively are better informed by current data than broad, nonselective blockade of JAK1/2-mediated signaling, which would inhibit both deleterious and beneficial cytokines.

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