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Relevance of the Bruton Tyrosine Kinase as a Target for COVID-19 Therapy

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ABSTRACT

The outbreak of the novel coronavirus disease 2019 (COVID-19) has emerged as one of the biggest global health threats worldwide. As of October 2020, more than 44 million confirmed cases and more than 1,160,000 deaths have been reported globally, and the toll is likely to be much higher before the pandemic is over. There are currently little therapeutic options available and new potential targets are intensively investigated. Recently, Bruton tyrosine kinase (BTK) has emerged as an interesting candidate. Elevated levels of BTK activity have been reported in blood monocytes from patients with severe COVID-19, compared with those from healthy volunteers. Importantly, various studies confirmed empirically that administration of BTK inhibitors (acalabrutinib and ibrutinib) decreased the duration of mechanical ventilation and mortality rate for hospitalized patients with severe COVID-19. Herein, we review the current information regarding the role of BTK in severe acute respiratory syndrome coronavirus 2 infections and the suitability of its inhibitors as drugs to treat COVID-19. The use of BTK inhibitors in the management of COVID-19 shows promise in reducing the severity of the immune response to the infection and thus mortality. However, BTK inhibition may be contributing in other ways to inhibit the effects of the virus and this will need to be carefully studied.

Introduction

In December 2019, an outbreak of pneumonia cases of unknown origin was reported in Wuhan, the capital of the Hubei Province in P.R. China (1). Soon afterwards, the microbe responsible for this disease was identified as a novel coronavirus by various independent investigators (2–4). The causative agent has since been described as a single-stranded RNA zoonotic virus and named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with the disease resulting of the infections being finally called by the World Health Organization as coronavirus disease 2019 (COVID-19; ref. 5). COVID-19 is a highly contagious disease, due to a basic reproduction number (R0) that has initially been calculated to be close to 2–3 (likely between 1.4 and 6.5), and can be spread by any activity that results in the release of droplets of saliva (such as coughs and sneezes, singing, shouting, or talking), as well as touching a mucosa (such as nose, eyes, or mouth) after being in contact with a contaminated surface (6). As of October 2020, more than 44 million confirmed cases and more than 1,160,000 deaths have been reported globally (https://coronavirus.jhu.edu/map.html). The actual numbers of affected individuals may be much higher, given the fact that many cases go undiagnosed because of lack of testing or being asymptomatic.

Various studies demonstrated that patients with cancer are at high risk of COVID-19 complication and mortality compared with the general population (7–9). Importantly, patients with hematologic malignancies have shown highest levels of mortality from COVID-19 comparing with the rest of cancers (10). Bruton tyrosine kinase (BTK), a key enzyme that has been implicated in various hematologic malignancies (11), is a nonreceptor tyrosine kinase and a member of the Tec family (12). BTK is mutated in the inherited immunodeficiency disease, X-linked agammaglobulinemia (13), a disorder that results in low peripheral blood B cells, low levels of immunoglobulins, and recurring infections (14). BTK has been characterized as a unique therapeutic target in B-cell malignancies, due to its involvement in B-cell maturation (15, 16). BTK inhibition has been a successful field in drug discovery and various small-molecule inhibitors have been developed, including acalabrutinib (17), LFM-A13, dasatinib, ONO-4059, CC-292, zanubrutinib, and ibrutinib (18). Ibrutinib is the most advanced in clinical development and has allowed much needed new treatment options for patients with chronic lymphocytic leukemia (CLL) and other hematologic malignancies (15, 18). Of note, the kinase function of BTK has recently been found to be also important for stability and activity of proteins involved in essential tumor suppressor pathways in epithelial cells, such as p53 (16, 19, 20), p73 (20, 21), and MDM2 (20, 22). This indicates that BTK is a pleiotropic kinase with a more complex activity than suspected previously, which performs very different functions depending on the context (23).

Recently, various investigations have demonstrated a positive correlation between BTK activity and severity of COVID-19 infection (24–29). In this context, administration of acalabrutinib to 19 hospitalized patients with severe COVID-19 infection resulted in improvement in oxygenation in a majority of them ($n = 18$), often within 1–3 days (24). In addition, a positive correlation between ibrutinib treatment and COVID-19 infection severity was demonstrated previously (25). Another study corroborated that ibrutinib is effective in decreasing the severity of COVID-19, with 6 of the 8 patients having recovered after treatment (26). Importantly, various clinical trials (ClinicalTrials.gov identifier: NCT04382586 and NCT04346199) are underway to fully evaluate the potential benefit

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of BTK inhibitors in COVID-19. In this minireview, we will discuss possible mechanisms by which BTK inhibitors could attenuate the symptoms of COVID-19 and provide a novel therapeutic option for the pandemic.

Factors That May Contribute to The Effects of BTK Inhibitors on Severe COVID-19

Despite the clear effects of BTK inhibitors on the prognosis of patients with COVID-19, as discussed above, there is limited mechanistic data reported so far that could explain these responses. Given the known functions of BTK, it is fair to assume that the main clinical impact of its inhibition could be related to a reduction of the activity of lymphocytes and thus, the exaggerated inflammation state brought upon by the virus, particularly in the lung. This is consistent with the fact that anti-inflammatories, such as dexamethasone, have been shown to improve the health and prognosis of patients with COVID-19 (30). However, we propose that BTK could be involved in the response to SARS-Cov-2 in other ways as well, which would further strengthen its relevance as a therapeutic target.

BTK inhibition blunts the hyperinflammatory response in lung

Virus-induced hyperinflammation is a major cause of disease severity and death in infected patients with COVID-19 (31–33). Indeed, it has been recently proposed that the actual cause of death by COVID-19 could be the organ inflammation and injury due to the immune response itself, instead of the direct effects of the virus on tissue (34). This hyperinflammation is characterized by increased serum levels of several inflammatory cytokines and chemokines, such as G-CSF, GM-CSF, macrophage inflammatory protein–1α, IL1β, IL6, IL7, IL8, IL9, IL10, IFNγ, IFNγ–inducible protein 10, and monocyte chemoattractant protein 1 (35–37), increased neutrophil-to-lymphocyte ratio (38–40), and high macrophage activity (41).

Using mouse models, it has been observed that the pharmacologic blockade of BTK results in impaired immunity because of inhibition of macrophages (42). Importantly, BTK-driven macrophage inhibition is mediated by blocking the activation of both the inflammasome and NF-κB (42). Of note, both inflammasome and NF-κB pathways are known to play an essential role in cytokine storm in severe COVID-19 (43, 44). Therefore, patients with either solid or hematologic malignancies treated with BTK inhibitors could be at an advantage should they be infected by SARS-CoV-2 because their immune system would already be attenuated and this would prevent extreme responses to the virus.

Accumulating evidence has shown that BTK is implicated in the regulation of proinflammatory processes in lung, which promote irreversible tissue destruction (45–47). In this context, it has been shown that ibrutinib treatment of patients with chronic graft-versus-host disease inhibits the IL2 inducible T-cell kinase (ITK), which is involved in the selective activation of T cells that drive immune reactivity toward healthy tissues (48). Moreover, the administration of ibrutinib in mice with overwhelming lung inflammation resulted in reduction of alveolar macrophage activation, neutrophil influx, cytokine release, and plasma leakage into the lung (49). This supports the hypothesis of the potential anti-inflammatory activity of BTK inhibitors in lung tissue. Further studies have confirmed the role of BTK in monocyte/macrophage and neutrophil activity (50, 51), which contributes to the inflammatory response to infections. A study that examined lungs of patients with COVID-19 confirmed that the infiltrate of immune cells in alveoli was majorly macrophages and monocytes, while moderate multinucleated giant cells, minimal lymphocytes, eosinophils, and neutrophils were also observed (52). Moreover, macrophages express Toll-like receptors (TLR), which play an essential role in recognizing single-stranded RNA from viruses such as SARS-CoV-2 (53). BTK plays a key role in the activation of TLRs signaling through NF-κB, which triggers the expression of various inflammatory cytokines and chemokines (IL1β, IL6, TNFα, IL12, IL8, and CCL2) and phagocytosis (24, 54, 55). Consistent with this, it was demonstrated that treating patients with COVID-19 with acalabrutinib for 14 days resulted in the normalization of IL6 and a reduction of lymphopenia in most cases (24). This reduction is possibly associated with a decrease in inflammatory cytokines and chemokines (56). Of note, lymphopenia contributes to the severity of COVID-19 infection (57), and lethal victims of COVID-19 were reported to have a significantly lower lymphocyte count than survivors (39).

Another type of immune dysregulation related to COVID-19 severity is the polarization of macrophages to an M1 state (27) that produced proinflammatory-related factors, such as IL6, IL12, and TNF (58). BTK is crucial for M1 macrophage polarization, with BTK-deficient mice having markedly reduced recruitment of M1 macrophages (59). It has been demonstrated that BTK inhibition abrogates M1 polarization through suppression of CSF1 and IL10 *in vivo* (60).

Various investigations have suggested a positive correlation between COVID-19 severity and lymphopenia, a condition defined by abnormally low counts of lymphocytes (36, 57, 61). Currently, it is unclear how lymphopenia enhances SARS-CoV-2 infection. However, it has been suggested that lymphopenia promotes the cytokine storm (62). Importantly, it has been shown that treating patients with CLL with ibrutinib results in elevating the absolute lymphocyte count in the peripheral blood (63, 64).

It is worth to mention that some BTK inhibitors, including ibrutinib, bind covalently and noncovalently to other kinases and inhibit their activity, such as the SRC family kinases (65). Interestingly, some of the SRC family kinases have been implicated in replication of viruses (66). In this context, inhibition of SRC by saracatinib has been reported to block MERS-CoV at early stages of the viral life cycle (67). Thus, we hypothesize that BTK inhibitors may also inhibit SARS-CoV-2 replication through SRC inhibition.

Currently, there is no doubt that anti-inflammatory therapy plays a key role in the management of patients with COVID-19 through preventing further injury and organ damage or failure. So far, various drugs that possess an anti-inflammatory profile have been investigated in respect of their potential use as a therapeutic strategy for treating the consequences of a SAR-COV-2 infection, such as baricitinib (68), tocilizumab (69), and corticosteroids, including dexamethasone (70, 71). These drugs have been found to mitigate cytokine production and consequently abolish the cytokine storm induced by SARS-CoV-2. Baricitinib has been mainly used as a therapeutic strategy to interrupt the entry of SARS-CoV-2 into lung cells through blocking ACE2-mediated endocytosis, apart from its anti-inflammatory properties (72, 73). On the other hand, the effects of tocilizumab in patients with COVID-19 are mainly caused by its inhibition of the IL6 receptor and the cytokine storm (74). The molecular mechanisms underlying the effects of dexamethasone on COVID-19 severity are broad and can be partly explained by its anti-inflammatory properties, as well as being a cytokine suppressor (75).

We postulate that the mechanisms of action of BTK inhibitors as a therapeutic agent for COVID-19 are vastly different from other anti-inflammatory drugs. Importantly, they may prevent the cytokine

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storm through different pathways, such as inhibiting macrophages (42), inducing prolonged lymphocytosis (64), suppressing ITK (48), and blocking TLRs signaling activation (24), which requires signaling cascade for production of cytokines and chemokines. Therefore, BTK inhibitors, unlike other therapeutic strategies, have a wider range of effects on the immune response to SARS-CoV-2. Because of this, it could also be possible to identify the patients with COVID-19 that would more likely respond to therapies with BTK inhibitors by analyzing the degree of the inhibition of these pathways when exposed to these drugs.

Altogether, this shows that BTK could be important for the COVID-19–driven cytokine storm observed in the severe cases. Mechanistically, this could possibly be mediated by an elevation of its kinase activity, which *ex vivo* analysis showed increased BTK autophosphorylation in blood monocytes from patients with severe COVID-19 when compared with blood monocytes from healthy volunteers (24). Despite this evidence, the effect of BTK inhibitors on inflammation warrants further investigations.

BTK inhibition attenuates the COVID-19–associated respiratory distress syndrome

The overexpression of phosphorylated BTK in the lungs is associated with lung injury, and the induction of an acute respiratory distress syndrome has been reported previously (76–78). Accordingly, BTK has been proposed to be involved in sepsis-induced acute lung injury (ALI; ref, 76). Importantly, intratracheal injection of BTK siRNA confers potent protection against sepsis-induced ALI in a mouse model of cecal ligation and puncture-induced sepsis-induced ALI, as demonstrated by a dramatic reduction in epithelial cell apoptosis, pathologic scores, vascular permeability, pulmonary edema, and the expression of inflammatory cytokines and neutrophil infiltration in the lung tissues of septic mice (76). Moreover, inhibiting BTK with ibrutinib rescued mice from lethal influenza-driven ALI (77). The data showed that BTK inhibition reduced alveolar hemorrhage and caused dramatic morphologic alterations to the lungs, with interstitial thickening, and the presence of alveolar exudate (77).

The latest experimental evidence also shows that BTK is involved in trauma hemorrhagic shock-induced lung injury in rats, and blocking BTK activity with the LFM-A13 inhibitor protects lungs from this injury (78). In addition, at least one study has shown that BTK overexpression in lung is associated with collagen deposition around airways and total basal membrane thickness, suggesting a role for BTK in reducing airway stiffness and increasing airway resistance (45). The function of BTK in collagen deposition is mediated by matrix metalloproteinase-9 (45).

All these data together indicate a strong implication of BTK in different models of lung injury and show that its inhibition can ameliorate some of the symptoms involved. Therefore, we postulate that BTK inhibitors could also be decreasing the severity of COVID-19–related lung pathology by a direct effect on the general components of the exacerbated damage response to the infection. This possibility should be carefully explored.

BTK inhibitors may reduce thrombosis in patients with COVID-19

Many patients with COVID-19 are suffering with high risk of thrombotic complications, with 20% of severely ill patients affected by venous thromboembolism (79, 80). This has a critical impact on the prognosis of the disease. BTK inhibitors, specifically ibrutinib, are associated with reduced venous thrombosis and arterial thrombosis (81, 82), through mechanisms that are not fully understood. Thus, this may be an added advantage to using BTK inhibitors in COVID-19. Importantly, these drugs would lack the bleeding side effects of regular anticoagulants (29).

The Role of BTK in Cancer Patients with COVID-19

Patients with cancer have been described as one of the most susceptible groups in the COVID-19 pandemic, having shown so far a high mortality ratio (7–9). Specifically, patients with hematologic cancers (leukemia, myeloma, and lymphoma), lung cancer, or metastatic cancer (stage IV) had the uppermost frequency of severe events, described as a condition requiring admission to an intensive care unit, the use of mechanical ventilation, or death (83). However, the risk factors that lead to high susceptibility in patients with cancer are poorly explained. We will consider these patients separately in this review, in light of the important role of BTK in cancer.

Potential double action of BTK inhibitors in COVID-19 patients with blood malignancies

BTK inhibitors may be particularly helpful in patients with COVID-19 with blood malignancies (24–26, 84), given the oncogenic activity of BTK and the beneficial effects that the drugs already have on these cancers (85–87). The outcomes of administrating ibrutinib to 8 patients with COVID-19 with CLL have been already described previously (26). In this study, BTK inhibitor treatment had to be stopped in 6 of the patients ("BTKi-held") and was allowed to continue in only 2 patients. Two of the total of 8 patients in this cohort developed severe respiratory failure and died of it, while the rest of patients showed mild-to-moderate symptoms. Importantly, the other 2 patients that were able to continue on ibrutinib instead had minimal oxygen requirements, with associated short hospital stays and, eventually a full recovery (26). Another study focused on ibrutinib given to patients with Waldenstrom macroglobulinemia with COVID-19 (25). It showed that 5 of 6 patients had mild symptoms and recovered promptly, while only 1 patient required hospitalization and mechanical ventilation, but eventually recovered fully. Although the numbers of cases in these studies were low, they nevertheless suggest that BTK inhibition may be particularly effective in patients with certain blood malignancies. Further studies will be needed to confirm this and fully understand the mechanisms involved.

The importance of BTK in cell death suggests unwanted side effects of the inhibitors in COVID-19

In solid cancers, BTK has not been shown to have the key oncogenic functions as seen in leukemias. On the contrary, BTK-dependent phosphorylation upregulates the stability and activity of p53 in epithelial cells (16), a critical tumor suppressor in humans (88). Several studies have confirmed that p53 is also involved in the host cell's nonspecific antiviral defense system (89). For example, viral infection results in induction of p53-mediated type I IFN signaling (90). Accordingly, knockout of p53 promotes replication of the SARS-CoV replicons (91), a novel coronavirus that broke out in 2003 and caused SARS at the beginning of the millennium, with a global lethality of approximately 10% (92). Given the similitudes between SARS-CoV and SARS-CoV-2, it should be investigated whether p53 has the same effect on the latter.

Viruses utilize different pathways to attenuate p53 activity and thus, avoid the induction of death in infected cells. For instance, coronavirus produces papain-like protease 2, which directly interacts and

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deubiquitinates cellular oncoprotein and p53 inhibitor, MDM2, and thus, promotes proteasomal degradation of p53 (93). This inhibits the p53-mediated production of type I IFN signaling and apoptosis and ensures viral growth (93). It is worth to mention that we previously identified BTK as an inhibitor of MDM2-mediated p53 degradation (22). Moreover, proteo-transcriptomics analysis of COVID-19–infected cells showed upregulation of various prosurvival pathways, including mTOR, hypoxia-inducible factor-1 alpha, and PI3K/protein kinase B (PI3K/AKT; ref. 94). p53 is known for suppressing all these pathways (94–98). Because of this, whether BTK inhibitors interfere with p53 activity in patients with COVID-19 should be analyzed. Given the fact that the exacerbated immune response seems more determinant in the cause of death than the viral load (34) and that the inhibitors would be given for short periods of time, the negative consequences would likely be negligible.

Summary and Final Conclusions

The urgent need of drugs to control the symptoms of COVID-19 has led to unexpected findings, including the potential importance of BTK inhibitors in increasing the survival of the most serious cases of the disease. This is consistent with the hypothesis that the major factor of poor prognosis is an excessive immune response, which BTK inhibition would reduce by the mechanisms we discussed. Despite many recent studies unanimously supporting the potential interest of BTK inhibitors as novel COVID-19 treatments, it would still be necessary to further study the mechanisms involved in the positive effects and carefully consider any negative side effects. One risk is an impaired humoral immunity, which may increase the susceptibility of the patients to secondary infections, such as pneumonia. Also, BTK inhibition in patients with COVID-19 with solid cancers might cause adverse effects due to suppression of p53 activity, perhaps accelerating tumor growth, favoring metastasis, or even interfering with antineoplastic treatments.

The limited studies published so far have raised the exciting possibility that BTK inhibition may reduce the mortality due to COVID-19. While these findings may have an important clinical impact, at the moment they cannot be considered conclusive, because of the small cohorts analyzed. To clarify the relationship between BTK inhibitors and COVID-19, larger-scale datasets are necessary, as well as more detailed mechanistic studies.

Authors' Disclosures

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