Bruton’s Tyrosine Kinase (BTK) Inhibitors: New Possible Good Candidates Against COVID-19

Claudia Baratè*, Fabrizio Mavilia1, Edoardo Benedetti2, Gabriele Buda2, Fortunato Morabito3,4, Chiara Baldini2, Francesco Ferro2, Ilaria Puxeddu3, Antonello di Paolo2, Federico Ricci2, Mario Petrini2, Sara Galimberti2

1. Hematology, AOUP, Pisa, Italy
2. Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
3. Biotechnology Research Unit, AO of Cosenza, Cosenza, Italy
4. Hematology and Bone Marrow Transplant Unit, Hemato-Oncology Department, Augusta Victoria Hospital, East Jerusalem, Israel

* Corresponding author: Dr. Claudia Baratè, UO Hematology, AOUP, Pisa, Italy

Received:Received: March 25, 2020; Accepted: April 23, 2020; Published: April 28, 2020

Abstract

COVID-19 is the current severe systemic disease that follows the infection by the new Coronavirus, SARS-CoV-2. It is characterized by a “cytokine” storm, innate immune system failure and by a hypercoagulation status that is responsible for ischemic damage of several organs. The infection starts with the attack of SARS-CoV-2 to ACE2 and CD26 receptors on the human cells, with consequent block of autophagy and increased cell senescence, responsible for hyperinflammation and further overspread of new virions. In the present article we revised the role of the Bruton’s Tyrosine Kinase (BTK) in this scenario and how the BTK inhibitors (BTKIs), already available for therapy of lymphoproliferative diseases and autoimmune disorders, might represent a valid therapeutic option in COVID-19.

Indeed, BTK is actively involved in inflammation; consequently, its inhibition might be advantageous in reducing the hyper-inflammation that characterizes COVID-19, as demonstrated in rheumatological disorders and graft-versus-host disease. Moreover, BTK inhibition might restore autophagy and reduce senescence, so avoiding the overspread of viral infection and sustaining the host antiviral response. Finally, BTKIs might also reduce the thrombotic risk without a significant pro-hemorrhagic effect by blocking CLEC2. The ongoing clinical trials involving ibrutinib, acalabrutinib and zanubrutinib will help to support or to refute our hypotheses.

Keywords: Covid-19, SARS, inflammation, BTK, ibrutinib, acalabrutinib

Coronavirus Disease 19 (Covid-19): Pathogenesis

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (also known as “new Coronavirus”) is the strain of coronavirus responsible for the COVID-19 pandemic affecting worldwide 8,634,575 people and causing, up to June 2020, 460,081 deaths (119,112 in USA, 187,231 in Europe and 4,645 in China; https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases). This virus shows RNA homology higher than 80% with previous Coronaviruses, responsible for the SARS outbreak in China of 2002 and for MERS that occurred in Middle East in 2012 [1]. Two receptors for SARS-CoV-2 have been identified on human cells: the Angiotensin Converting Enzyme 2 (ACE2) [2], and the Dipeptidyl-Peptidase 4 (DPP4), also known as CD26 [3]. These receptors are constitutively expressed in kidney, liver, epithelial cells, exocrine glands, pancreas, lung and gut, so explaining some symptoms and signs typical of COVID-19, such as nausea, vomit, diarrhea, pneumonia and insulin-resistance [4]. CD26 is particularly expressed on pneumocytes, especially in subjects with a history of smoking and chronic lung disease, so justifying the high number of COVID-19 pneumonia that frequently required ventilation and intubation [5].

Several different viral proteins have been identified to be fundamental for virus attack and replication: spike (S) and envelope (E) proteins allow virus to attack host cells, membrane (M) protein is necessary for its interaction with RNA, hemagglutinin esterase (HE) is important for virus release and nucleocapsid (N) protein increases the stability of the new virions [6].

After passage through endoplasmic reticulum and Golgi apparatus, viral RNA, N protein and E glycoproteins assemble to form the new virions that are subsequently released to spread infection [7]. Chloroquine and hydroxychloroquine modify the pH-dependent early phase of virus replication and reduce the production of TNF alpha and IL-6, resulting efficacious in the Coronavirus pandemic [8], although its cost-benefit ratio has been recently debated due to the increased risk of QTc prolongation and possible onset of arrhythmias that these compounds
might induce, especially in combination with antibiotics [9,10].

We previously reported that the interaction between the new Coronavirus and CD26 might be central since SARS-CoV-2 uses this structure for blocking autophagy, a well-known host first line antiviral defense, and for further sustaining the inflammation loop, that in COVID-19 rapidly becomes excessive and uncontrolled [11]. This hyper-inflammatory status might be also the consequence of increased formation of the “neutrophil extracellular traps” (NETs) that actively sustain inflammation and possibly even thrombotic events that have been frequently reported in COVID-19 [12].

At the same time, the over-activation of the renin angiotensin axis, through the cross-linking of Coronavirus to ACE2 receptors, induces brain, kidney and skeletal muscle dysfunction and cellular senescence [13]. Indeed, SARS-CoV-2 uses its open reading frame (ORF) 9b protein to block the host antiviral action by the proteasome-dependent degradation of the mitochondrial Dynamin 1-like (DRP1) protein. This activity causes mitochondrial abnormalities and dysfunctions, such as hyper fusion, with the final acquisition of senescent phenotype [14]. Once cells have become senescent, they start to over-produce pro-inflammatory cytokines, chemokines and growth factors that sustain COVID-19 onset. In addition, human cells, becoming senescent, spread new virions by producing high amounts of extracellular vesicles that can reach different and far sites where infection disseminates [15]. Moreover, senescence seems to directly favor the viral particles anchorage on the host cell surface by inducing the expression of higher amounts of vimentin [16], an intermediate filament protein implicated in the dynamic organization of the cytoskeleton already described as a key element of virus entry into host cells in previous Coronavirus [17], and HIV-1 infections [18].

Meanwhile, SARS-CoV-2 destabilizes the host antiviral proteins and up-regulates some deubiquitinases by dysregulating ubiquitination processes, allowing virus to express some proteins necessary for its replication [19].

Previous in vitro studies on SARS-CoV-2 showed that the synthesis of the viral RNA and related proteins were strongly reduced by proteasome inhibitors [20]. Consequently, these drugs have been proposed similarly against COVID-19. In particular, the most recently licensed proteasome inhibitor Carfilzomib, was indicated by two different groups the best candidate to interact with SARS-CoV-2 glycoproteins [21,22].

In addition to hyperinflammation, dysregulation of host innate immunity plays a fundamental role in the COVID-19 pathogenesis. Indeed, virus-infected lung cells induce the recruitment of macrophages, monocytes and lymphocytes [23], while neutrophils, together with boosted pro-inflammatory cytokines, such as IL-6 and IL-17, promote the pro-thrombotic state [24].

In addition to the DPP4/CD26 axis, the new Coronavirus impairs host immune response by inducing over-expression of the inhibitory receptor NKG2A expressed on cytotoxic lymphocytes and NK cells, which, in turn, reduces the ability of lymphocytes to produce CD107a, IFN-γ, IL-2, granzyme B, and TNF alpha[25]. At the same time, viral components are recognized by toll-like-receptors (TLRs) that trigger the activation of inflammasome [26].

All these factors converge in a “cytokine storm”, making impossible for the host to proceed to the efficient immune response and to the control of virus-induced inflammation.

In this complex scenario, Bruton’s Tyrosine Kinase (BTK) could play a relevant role.

**BTK as Crossroads between Inflammation and Host Immune Response**

BTK is a 659-amino acid prevalently cytoplasmic protein that belongs to the conserved family of “non-receptors” tyrosine kinases, known as “TEC (Tyrosine Kinase Expressed in hepatocellular Carcinoma) family”. BTK is encoded by a gene located on chromosome X, structurally including: 1) a Src homology 2 (SH2) domain, which is involved in the interaction with phosphorylated tyrosines; 2) a SH3 domain, by which BTK interacts with proline-rich domains of different proteins; 3) a catalytic site, and 4) the N-terminal (PH) domain, necessary for interacting with plasma membrane via phosphatidylinositol triphosphate (PIP3). This latest domain is essential for BTK translocation from the cytoplasm to the membranes and for starting its phosphorylating activity [27].

Once activated, BTK induces phosphorylation of the downstream Phospholipase C gamma 2 (PLC gamma 2) protein, activates calcium channels in endoplasmic reticulum, and recruits the Tumor necrosis factor Receptor-Associated Factor 6 (TRAF6), which in turn activates the IKK complex. This complex induces the ubiquitination-mediated degradation of IkB, that allows NF-kB to translocate into the nucleus, resulting in the final increased B cell survival and inflammation [28,29]. In addition, BTK is able also to trigger the Nuclear Factor of Activated T-cells (NFAT) pathway, notably over-activated in patients with inflammatory conditions, such as the Kawasaki’s disease[30].

BTK is involved in the inflammatory process as active part of the NLRP3 inflammasome, a multimeric protein complex that triggers the release of proinflammatory cytokines, such as IL-1 beta and IL-18, in many inflammatory conditions, including Alzheimer’s disease, diabetes, and infections [31].

It has been found that a variety of stimuli, including the danger-associated molecular patterns (DAMPs) and the pathogen-associated molecular patterns (PAMPs), can activate the inflammasome, either after the interaction of NF-kB with Toll-like receptor 4 (TLR4), by mitochondrial dysfunction, indirectly triggered by calcium efflux, or lysosomal rupture. All these conditions are controlled by BTK. Once activated, the inflammasome, including BTK, cleaves the pro-caspase-1 that in turn cleaves pro-IL-1 beta to its active form that further sustains the inflammatory process [32].

In addition to sustaining inflammation, BTK is also involved in the senescence, that, as above reported, is essential for infection overspread and virus-related organ damage. Indeed, in a murine model, BTK suppression significantly correlated with a decreased accumulation of senescent cells in the brain and with a less anxious behavior of animals [33]. That these BTK-related aspects might be relevant in COVID-19 scenario is well proven by two observations: 1) the clinical outcome of COVID-19 patients occurred when NF-kB was blocked, for example with systemic ozone therapy [34], 2) an increased number of children who, after exposure to SARS-CoV-2, developed the Kawasaki’s syndrome (diabetes, capillary leak syndrome, and myocardial
The fundamental role of BTK in adaptive immunity and infection control has been well understood since 1993, when the X-linked agammaglobulinemia (Bruton’s agammaglobulinemia) has been described for the first time [36]. In this genetic disease, different BTK mutations induce the lack of circulating B cells, the arrest of neutrophil maturation at myelocyte and promyelocyte stages, the defect of dendritic cell maturation and antigen presentation, with the consequent increased of bacterial infection rate [37]. On the contrary, the viral infections in these subjects are rare, because T and NK cells functions are preserved due to the cellular lack of BTK [38]. Nevertheless, in several murine models, BTK appeared to favor infections sustained by X-31 influenza virus39, EBV and HIV-1 [40].

In addition to myeloid and dendritic cells, BTK is also expressed in mast cells, where it is involved in their TLR-mediated activation. It has been reported that BTK positively regulates production of cytokines by mast cells, such as IL-2, IL-4, TNF alpha, and GM-CSF [41]. The relationship between SARS-CoV-2 infection and the activation of mast cells with subsequent “cytokine storm” is undoubtedly supported by the high expression levels of ACE2 on mast cells, especially in lung, where, after virus triggering, they release pro-inflammatory cytokines and chemokines, including leukotrienes that cause bronchoconstriction [42].

Then, a possible further reason for using BTKIs during Coronavirus pandemic is the ability to control mast cells activation. In conclusion, BTK is the actor of many scenes that characterize Coronavirus infection and its related disease. Firstly, it activates NF-kB and NFAT, sustaining the inflammation. Secondly, it is part itself of inflamasome, so sustaining the production of IL1 and other pro-inflammatory cytokines [43]. Thirdly, it is deeply involved in the senescence process, that contributes to damage many organs (especially lung) and to overspread new virions.

Thus, its pharmacological inhibition might represent a possible effective therapeutic option against COVID-19.

BTKIs: Who are They? Pros and Cons of a Possible Their Use in COVID-19

BTKIs had been shown to be very effective in several hematological neoplasms, such as chronic lymphocytic leukemia (CLL) [44], Waldenström’s Macroglobulinemia (WM) [45], and mantle cell lymphoma (MCL) [46]. Ibrutinib, the first licensed compound, when compared with ofatumumab, offered a longer survival to 90% of CLL relapsed patients, including those carrying deletions of chromosome 17 or/and TP53 mutations [47,48]. In first line, ibrutinib induces 90% of overall responses, with 83% and 73% of patients who are respectively alive and disease-free after 5-years of treatment [49].

Acalabrutinib, a novel irreversible BTKI with higher potency and selectivity than ibrutinib, seems to be also effective, with a lower probability of cardiac adverse events in respect of ibrutinib [50], 95% of overall responses and 24-months overall survival and progression-free survival of 91.5% and 87.2%, respectively [51]. Finally, zanubrutinib, one of the newer drugs, in a small series of relapsed/refractory CLL cases, elicited 84.6% of responses, with a 12-months event-free survival of 92.9% [52].

From experience in the oncologic context, we can now derive solid information about the most frequent toxicities of BTKIs, which is a major point to be considered when we hypothesize their use in COVID-19. Firstly, we have to keep in consideration BTKIs-induced platelets dysfunction. In CLL, ibrutinib was associated with low-grade ecchymosis and petechiae in 50% of cases, with major hemorrhages ranging from 1% to 9% [53]. Indeed, ibrutinib inhibits the collagen-induced platelet aggregation by interfering with the glycoprotein VI-mediated pathway [54]. This activity might be useful in ischemic conditions, such as after myocardial infarction, when ibrutinib and tirabrutinib, another novel BTKI, have been successfully employed to inhibit platelet aggregation [55].

During COVID-19, the number of platelets is often reduced, either because infection impairs their bone marrow production or because of their reduced half-life due to their peripheral destruction, with a pathogenetic mechanism similar to that observed in the macrophage activation syndrome [56]. Nevertheless, at least 25% of patients show elevated D-dimer levels, with a situation mimicking disseminated intravascular coagulation (DIC) [57]. It has been reported that BTKIs can block the platelet tyrosine kinase-linked receptor CLEC-2, implicated in a hypercoagulation state. Notably, CLEC-2 has only a minimal role in the classical hemostatic function of platelets; therefore, it is unlikely that its inhibition may cause bleeding [58]. Accordingly, it has been suggested that BTKIs in COVID-19 might reduce the microvascular and venous thrombosis without increasing the bleeding risk [59].

Another side effect which should not underestimate arising from the treatment of hematological neoplasms with BTKIs is represented by the infections. Pneumonia have been reported in 12% of patients, and average infection rate was estimated to be 7.1/100 patient-months during the first 6 months of treatment with ibrutinib and 2.6/100 during the following phases of treatment [60]. A pooled analysis of 4 randomized controlled studies where ibrutinib has been used in CLL or MCL patients found 8% of grade ≥3 pneumonia [61], while another meta-analysis found that 1 of every 5 patients developed any grade of lung infection [62]. Noticeably, the infection rate observed in hematological patients treated with continuous ibrutinib is unlikely to overlap that of COVID-19 in which the treatment length should be very short, thus reducing the risk of infection. Moreover, it is well known that the population receiving BTKIs because of CLL or lymphoma is basically characterized by an impaired immune response.

Interestingly, some data from literature might support the idea that BTKIs might be also useful during the early phase of SARS-CoV-2 infection. In a murine model of pneumococcal pneumonia, ibrutinib reduced the lung recruitment of monocytes and neutrophils and TNF alpha secretion by macrophages [63]. Analogously, knockout BTK mice experienced longer survival compared with those with wild type gene after Listeria monocytogenes infection [64]. Similarly, BTK-deficient mice showed a lower number of colon infiltrating macrophages during intestinal colonization by Candida albicans, showing again its protective role even against fungal infection [65]. Finally, BTK inhibition caused the death of HIV1-infect-
ed cells [66].

Furthermore, ibrutinib could induce autophagy through inhibition of Akt/mTOR pathway. Indeed, it has been recently reported that ibrutinib significantly reduced the Mycobacterium Tuberculosis load in mediastinal lymph nodes and spleen of infected mice through inhibition of this pathway [67]. A phenomenon of tumor shrinkage has been previously reported in glioblastoma by a similar mechanism of autophagy induction [68].

In addition, BTKIs allow a partial reconstitution of normal B cells and help to repair the T-cell defects in CLL patients. Indeed, multiple studies reported that ibrutinib decreases Th2 cytokines, normalizes total T-cell number, and decreases T-regulatory cells [69], so exerting an “immunomodulating” activity potentially useful action that could be useful also during COVID-19. Patients with severe COVID-19 share symptoms with those with “rheumatological” diseases, often showing cardiovascular, central nervous system, gastrointestinal and kidney damage. Thus, clinical trials conducted in the “rheumatological” setting might support the use of BTKIs as “anti-inflammatory” drugs in SARS-CoV-2. Indeed, BTK is required for the activation of neutrophils recruited in the sites of inflammation [70], thus supporting the concept that BTKIs might be beneficial in settings with amplified inflammation, such as rheumatoid arthritis (RA) and lupus erythematosus systemic (SLE) [71]. Mouse models of these diseases clearly demonstrated that BTKIs were able to inhibit B cells triggering these autoimmune disorders: mice treated with the BTK inhibitor PCI-32765 displayed a significant decrease in spleen size compared to the vehicle-treated mice, associated with a significant reduced number of activated T and B cells and plasmablasts [72]. Evobrutinib is a novel, highly selective, irreversible BTK inhibitor that in RA and SLE preclinical models resulted very effective, with reduction of disease severity and histological damage consequent to the decrease of B cell activation and autoantibodies production [73].

Another interesting possible positive effect of BTKIs in COVID-19 is based on their ability of blocking the BTK-dependent mast cell activation. Mast cells, physiologically involved in the development of inflammation via release of multiple pro-inflammatory cytokines and chemokines, contain both ACE2 [74], and CD26 [75]. As above reported, these are the two receptors for SARS-CoV-2, and their presence on mast cells might explain at least in part some symptoms resembling the macrophage activation syndrome or graft-versus-host disease (GVHD) [43]. Some already published data support the use of BTKIs against mast cells: in a murine model, pretreatment with two doses of acalabrutinib prevented IgE-mediated anaphylaxis [76], and relbrutinib, a novel, potent, highly selective BTK inhibitor [77], seems to be promising in treatment of chronic spontaneous urticaria and Sjogren’s Syndrome [78].

Moreover, BTKIs seem to be effective in reducing inflammation also by interfering with the TLR pathway. Murine models of SLE clearly demonstrated that TLR7 protected animals at the beginning of viral infection, sustaining subsequently, when virus is cleared, excessive inflammation [79]. The role of TLR7 has been recently discussed in relationship with the lower incidence of COVID-19 in women: 6% of males were at high risk of COVID-19 compared with 3% of females [80]. Women are naturally less susceptible to viral infections based on a different innate immunity since they have higher levels of CD4+ T cells, more antibodies which remain in the circulation longer and lower levels of IL-6. Interestingly, X chromosome encodes for TLR7 as well as many other proteins, including TLR8, CD40L and CXCR3, which influence the response to viral infections and vaccinations [81]. These findings might be relevant to explain the different rate of infection of new Coronavirus between males and females.

A clear clinical demonstration of anti-inflammatory action of BTKIs comes from the “hematological” experience. Baseline cytokine levels were similar in the two arms of Iluminate trial, comparing ibrutinib plus obinutuzumab versus chlorambucil plus obinutuzumab in CLL patients. As expected, all cytokine levels (IL6, IL8, IL18, MCP1, MIP1α, and TNFα) increased after infusion of obinutuzumab, but the median increase in cytokines was lower in the ibrutinib arm [82]. These data well correlated with the recent demonstration that ibrutinib itself exerts an additional “anti-inflammatory” effect, trough the reduction of the phagocytic ability and the increase of the immunosuppressive profile of fibroblast-shaped adherent cells differentiated from peripheral blood-derived monocytes or nurse-like cells (NLCs) in CLL patients [83].

However, the most convincing evidence that BTKIs are able to exert a worthy anti-inflammatory activity comes from the finding that ibrutinib can successfully treat resistant chronic GVHD (cGVHD) after failure of one or more lines of systemic therapy [84]. For this reason, ibrutinib has been licensed also for this indication by U.S. Food and Drug Administration (FDA). Notably, in addition to inhibiting BTK, ibrutinib is an irreversible inhibitor of the Interleukin-2 inducible Tyrosine Kinase (ITK), involved in cytokine release and activation of Th2 lymphocytes, already demonstrated to be involved in peripheral blood-derived monocytes or nurse-like cells (NLCs) in CLL patients [83].

In conclusion, BTK sustains viral infection and inflammation; the efficacy of BTKIs in “rheumatological” diseases and cGVHD support their potential use in COVID-19. Btkis against SARS-Cov-2 and COVID-19: What We Already Learnt from the Experience of the Last Months

As above reported, many in vitro and in vivo studies clear-
ly showed that BTKIs have an anti-inflammatory action and that probably are not detrimental during viral infections. However, the proof of concept arises from recently published clinical observations and experiences in COVID-19.

Indeed, Treon and colleagues reported that few patients with Waldenstrom’s macroglobulinemia presented symptoms of COVID-19. Specifically, only 5 subjects on therapy with ibrutinib 420 mg/day experienced mild Coronavirus-related cough, fever, headache, anorexia, and diarrhea, however none of them required hospitalization. Notably, a patient receiving ibrutinib 140 mg/day required mechanical ventilation after SARS-CoV-2 infection, showing scarce response to Tocilizumab. However, he rapidly recovered with no further need of mechanical ventilation when ibrutinib dose was increased, demonstrating that ibrutinib at therapeutic dose might be effective in COVID-19 [88].

Analogously, 8 CLL patients receiving BTKIs were hospitalized for COVID-19 at the Mount Sinai hospital. BTKI was held in 6 cases, and 2 of them developed severe respiratory failure and expired. On the contrary, two patients who continued ibrutinib had short hospital stays, minimal oxygen requirement, and rapid and full recover [89]. Finally, another study including 19 patients with severe COVID-19 hospitalized at NIH (Bethesda) reported that a short-term course of acalabrutinib (10-14 day) improved oxygenation in the majority of patients and significantly reduced inflammation, as demonstrated by reduction of IL6 plasma levels [90].

In conclusion, even if still on a small number of patients, these pivotal observations seem to encourage employing BTKIs in COVID-19.

Outlook

In the present article we revised the role of BTKIs in the light of COVID-19 pathogenesis (Figure 1).

Overall, we think that the above mentioned in vitro and in vivo data might support the use of BTKIs against the new Coronavirus, based on 3 major likely beneficial effects.

Firstly, BTK is actively involved in inflammation via TLR and ITK inhibition and as constitutive part of the inflammasome. Consequently, its inhibition might be advantageous in reducing the hyper-inflammation that characterizes COVID-19, as clearly proven by the successful use of BTKIs in rheumatological conditions and cGVHD.

Secondly, BTK inhibition might restore autophagy and reduce senescence, so avoiding the overspread of viral infection and sustaining the host antiviral response, as also demonstrated by the “not detrimental” antimicrobial activity of BTKIs in murine models.

Thirdly, BTK inhibition might also reduce the “thromboinflammation” where the block of CLEC2 might reduce the thrombotic risk without a significant pro-hemorrhagic effect.

However, the results of the ongoing clinical trials are mandatory. Indeed, 5 studies have been recently registered in the “clinical trial.gov” website. Ibrutinib will be administered for 2-4 weeks to patients requiring supplemental oxygen for pulmonary distress related to SARS-CoV-2 infection in two trials (NCT04375397 and NCT04439006). Analogously, acalabrutinib will compared with the best supportive care in other 2 studies (NCT04380688, NCT04346199). Finally, zanubrutinib also will be compared with placebo or best supporting care in another ongoing study (NCT04382586).

In conclusion, BTK seems to be a key player in the COVID-19 scenario, and we think that its inhibition may be crucial in the fight against the new Coronavirus.

References

8. Meo SA, Klonoff DC, Akram J. Efficacy of chloroquine and hy-


matol.2019.218545.


84. Rahmat LT, Logan AC. Ibrutinib for the treatment of patients with chronic graft-versus-host disease after failure of one or more lines of systemic therapy. Drugs Today (Barc). 2018;54(5):305-313.


