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Review Article

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

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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-product 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 to give activated 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 (diarrhea, capillary leak syndrome, and myocardial

dysfunction), has been reported during the recent pandemic [35].

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 must 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 inflammasome, 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], Waldenstrom'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 subjects 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 trigging 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 remibrutinib, 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 Illuminate 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 cGVHD pathogenesis. On the other hand, ibrutinib fails to inhibit Th1 T cells which conversely are key actors in both pathogen and tumor response. Over all these data explain the beneficial effect of ibrutinib in controlling cGVHD without a significant increased number of infections [85]. Indeed, ibrutinib offered 67% of overall responses, with 21% of resolution of organ damage and a significant improvement of patients' quality of life, in a series of 42 patients with cGVHD [86].Similar response rates were observed in skin (88%), mouth (88%), and gut (91%); furthermore, 80% of patients showed response in at least 2 organs. Among responders, 71% sustained response for at least 5 months and significantly reduced the median corticosteroid dose. Plasma levels of soluble factors associated with inflammation, fibrosis, and cGVHD significantly decreased during treatment with ibrutinib; in particular, reduced levels of interferon gamma, IL1, IL8, monocyte chemotactic protein 1, macrophage-derived chemokines, macrophage-inflammatory proteins 1, soluble CD25 and TNF alpha were detected [87].

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-

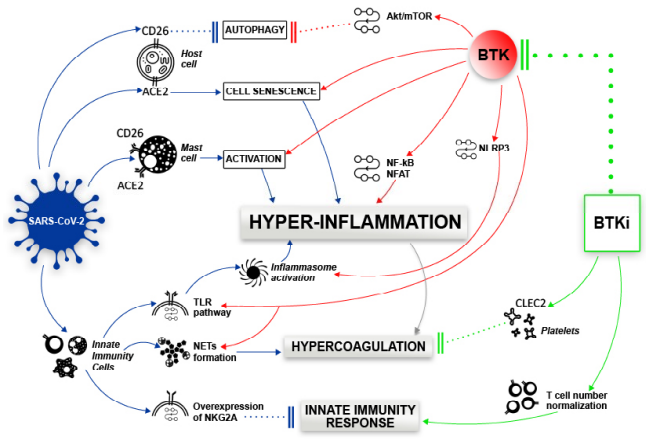

Figure 1. BTK drives hyper-inflammation, immune response failure and hyper-coagulation via different pathways, that might explain its involvement in the COVID-19 pathogenesis. The red lines describe how BTK interferes with different cellular processes (senescence; autophagy, mast cells activation), how it interacts with surface receptors (TLRs family) and how it activates different pro-inflammatory pathways (NFKB; NFAT; NLRP3). All these BTK-driven activities support the hyper-inflammation, coagulation hyper-activation, reduced autophagy and host immune response and increased senescence that characterize COVID-19 (blue lines). The green dotted lines show the effects of potential BTK inhibition. The continuous green line describes how BTK inhibitors work in the contest of the immune response and coagulation that are dysregulated and hyper activated by BTK.

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

1. Sen S, Anand KB, Karade S, et al. Coronaviruses: origin and evolution [published online ahead of print, 2020 Apr 27]. Med J Armed Forces India. 2020;76(2):136-141.
2. Yuan M, Wu NC, Zhu X, et al. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science. 2020;368(6491):630-633.
3. Qi F, Qian S, Zhang S, Zhang Z. Single cell RNA sequencing of 13 human tissues identify cell types and receptors of human coronaviruses. Biochem Biophys Res Commun. 2020;526(1):135-140.
4. Deacon CF. Physiology and Pharmacology of DPP-4 in Glucose Homeostasis and the Treatment of Type 2 Diabetes [published correction appears in Front Endocrinol (Lausanne). 2019 May 03;10:275]. Front Endocrinol (Lausanne). 2019;10:80.
5. Seys LJM, Widagdo W, Verhamme FM, et al. DPP4, the Middle East Respiratory Syndrome Coronavirus Receptor, is Upregulated in Lungs of Smokers and Chronic Obstructive Pulmonary Disease Patients. Clin Infect Dis. 2018;66(1):45-53.
6. Neuman BW, Kiss G, Kunding AH, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol. 2011;174(1):11-22.
7. Longhitano L, Tibullo D, Giallongo C, et al. Proteasome Inhibitors as a Possible Therapy for SARS-CoV-2. Int J Mol Sci. 2020;21(10):3622.
8. Meo SA, Klonoff DC, Akram J. Efficacy of chloroquine and hy-

droxychloroquine in the treatment of COVID-19. Eur Rev Med Pharmacol Sci. 2020;24(8):4539-4547.
9. Gopinathannair R, Merchant FM, Lakkireddy DR, et al. COVID-19 and cardiac arrhythmias: a global perspective on arrhythmia characteristics and management strategies [published online ahead of print, 2020 Jun 3]. J Interv Card Electrophysiol. 2020;1-8.
10. Mehra MR, Desai SS, Ruschitzka F, et al. RETRACTED: Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis [published online ahead of print, 2020 May 22] [retracted in: Lancet. 2020 Jun 5;null]. Lancet. 2020;S0140-6736(20)31180-6.
11. Galimberti S, Morabito F, Gentile M, et al. Dipeptidyl-Peptidase 4 (Cd26): a Possible Therapeutic Target in Covid-19. LOJ Phar & Cli Res 2(1)- 2020. LOJPCR.MS.ID.000128.
12. Ferro F, Elefante E, Puxeddu I, et al. COVID-19: the new challenge for rheumatologists. First update. Clin Exp Rheumatol. 2020;38(3):373-382.
13. Mogi M. Effect of renin-angiotensin system on senescence. Geriatr Gerontol Int. 2020;20(6):520-525.
14. Mogi M. Effect of renin-angiotensin system on senescence. Geriatr Gerontol Int. 2020;20(6):520-525.
15. Malavolta M, Giacconi R, Brunetti D, et al. Exploring the Relevance of Senotherapeutics for the Current SARS-CoV-2 Emergency and Similar Future Global Health Threats. Cells. 2020;9(4):909.
16. Frescas D, Roux CM, Aygun-Sunar S, et al. Senescent cells expose and secrete an oxidized form of membrane-bound vimentin as revealed by a natural polyreactive antibody. Proc Natl Acad Sci U S A. 2017;114(9):E1668-E1677.
17. Yu YT, Chien SC, Chen IY, et al. Surface vimentin is critical for the cell entry of SARS-CoV. J Biomed Sci. 2016;23:14.
18. Fernández-Ortega C, Ramírez A, Casillas D, et al. Identification of Vimentin as a Potential Therapeutic Target against HIV Infection. Viruses. 2016;8(6):98. Published 2016 Jun 15. doi:10.3390/v8060098
19. Gassen NC, Niemeyer D, Muth D, et al. SKP2 attenuates autophagy through Beclin1-ubiquitination and its inhibition reduces MERS-Coronavirus infection. Nat Commun. 2019;10(1):5770.
20. Raaben M, Posthuma CC, Verheije MH, et al. The ubiquitin-proteasome system plays an important role during various stages of the coronavirus infection cycle. J Virol. 2010;84(15):7869-7879. doi:10.1128/JVI.00485-10
21. Wang J. Fast Identification of Possible Drug Treatment of Coronavirus Disease-19 (COVID-19) through Computational Drug Repurposing Study. J Chem Inf Model. 2020;60(6):3277-3286.
22. Hijikata A, Shionyu-Mitsuyama C, Nakae S, et al. Knowledge-based structural models of SARS-CoV-2 proteins and their complexes with potential drugs [published online ahead of print, 2020 May 7]. FEBS Lett. 2020;10.1002/1873-3468.13806.
23. Pedersen SF, Ho YC. SARS-CoV-2: a storm is raging. J Clin Invest. 2020;130(5):2202-2205.
24. Raucci F, Mansour AA, Casillo GM, et al. Interleukin-17A (IL-17A), a key molecule of innate and adaptive immunity, and its potential involvement in COVID-19-related thrombotic and vascular mechanisms. Autoimmun Rev. 2020;19(7):102572.
25. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol. 2020;17(5):533-535.
26. Wang Y, Liu L. The Membrane Protein of Severe Acute Respiratory Syndrome Coronavirus Functions as a Novel Cytosolic Pathogen-Associated Molecular Pattern to Promote Beta Interferon Induction via a Toll-Like-Receptor-Related TRAF3-Independent Mechanism. mBio. 2016;7(1):e01872-15.
27. Corneth, O.B.J, Klein Wolterink, R.G.J, Hendriks, R.W. BTK signaling in B cell differentiation and autoimmunity.
28. Rawlings DJ, Scharenberg AM, Park H, et al. Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. Science. 1996;271(5250):822-825.
29. Rip J, Van Der Ploeg EK, Hendriks RW, et al. The Role of Bruton's Tyrosine Kinase in Immune Cell Signaling and Systemic Autoimmunity. Crit Rev Immunol. 2018;38(1):17-62.
30. Wang Y, Hu J, Liu J, et al. The role of Ca2+/NFAT in Dysfunction and Inflammation of Human Coronary Endothelial Cells induced by Sera from patients with Kawasaki disease. Sci Rep. 2020;10(1):4706. Published 2020 Mar 13.
31. Yang Y, Wang H, Kouadir M, et al. Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors. Cell Death Dis. 2019;10(2):128.
32. Benner B, Scarberry L, Stiff A, et al. Evidence for interaction of the NLRP3 inflammasome and Bruton's tyrosine kinase in tumor-associated macrophages: implications for myeloid cell production of interleukin-1beta. Oncoimmunology. 2019;8(11):1659704.
33. Ekpenyong-Akiba AE, Poblocka M, Althubiti M, et al. Amelioration of age-related brain function decline by Bruton's tyrosine kinase inhibition. Aging Cell. 2020;19(1):e13079.
34. Martínez-Sánchez G, Schwartz A, Donna VD. Potential Cytoprotective Activity of Ozone Therapy in SARS-CoV-2/COVID-19. Antioxidants (Basel). 2020;9(5):389.
35. Licciardi F, Pruccoli G, Denina M, et al. SARS-CoV-2-Induced Kawasaki-Like Hyperinflammatory Syndrome: A Novel COVID Phenotype in Children [published online ahead of print, 2020 May 21]. Pediatrics. 2020;e20201711.
36. Tsukada S, Saffran DC, Rawlings DJ, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. Cell. 1993;72(2):279-290.
37. Lin SC, Chiang BL, Lee YJ, et al. Pseudomonas aeruginosa sepsis presenting as oral ecthyma gangrenosum in identical twins with Bruton tyrosine kinase gene mutation: Two case reports and review of the literature [published online ahead of print, 2020 Apr 17]. J Microbiol Immunol Infect. 2020; S1684-1182(20)30103-1.
38. Jones TPW, Buckland M, Breuer J, et al. Viral infection in primary antibody deficiency syndromes. Rev Med Virol. 2019;29(4):e2049.
39. Herbst S, Shah A, Mazon Moya M, et al. Phagocytosis-dependent activation of a TLR9-BTK-calcineurin-NFAT pathway co-ordinates innate immunity to Aspergillus fumigatus. EMBO Mol Med. 2015;7(3):240-258.
40. Ye B, Zhou C, Guo H, et al. Effects of BTK signalling in pathogenic microorganism infections. J Cell Mol Med. 2019;23(10):6522-6529.
41. Iyer AS, Morales JL, Huang W, et al. Absence of Tec family kinases interleukin-2 inducible T cell kinase (Itk) and Bruton's tyrosine kinase (Btk) severely impairs Fc epsilon RI-dependent mast cell responses. J Biol Chem. 2011;286(11):9503-9513.
42. Theoharides TC. COVID-19, pulmonary mast cells, cyto-

kine storms, and beneficial actions of luteolin. Biofactors. 2020;46(3):306-308.
43. Galimberti S, Baldini C, Baratè C, et al. The CoV-2 outbreak: how hematologists could help to fight Covid-19. Pharmacol Res. 2020;157:104866.
44. Bond DA, Woyach JA. Targeting BTK in CLL: Beyond Ibrutinib. Curr Hematol Malig Rep. 2019;14(3):197-205.
45. Steven Peter Treon, Christina K Tripsas, Guang Yang, et al. A Prospective Multicenter Study Of The Bruton's Tyrosine Kinase Inhibitor Ibrutinib In Patients With Relapsed Or Refractory Waldenstrom's Macroglobulinemia. Blood 2013; 122 (21): 251.
46. Owen C, Berinstein NL, Christofides A, et al. Review of Bruton tyrosine kinase inhibitors for the treatment of relapsed or refractory mantle cell lymphoma. Curr Oncol. 2019;26(2):e233-e240.
47. Byrd JC, Brown JR, O'Brien S, et al. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med. 2014;371(3):213-223.
48. Morabito F, Gentile M, Monti P, et al. TP53 dysfunction in chronic lymphocytic leukemia: clinical relevance in the era of B-cell receptors and BCL-2 inhibitors [published online ahead of print, 2020 Jun 27]. Expert Opin Investig Drugs. 2020;1-12.
49. Burger JA, Barr PM, Robak T, et al. Long-term efficacy and safety of first-line ibrutinib treatment for patients with CLL/SLL: 5 years of follow-up from the phase 3 RESONATE-2 study. Leukemia. 2020;34(3):787-798.
50. Morabito F, Recchia AG, Vigna E, et al. An in-depth evaluation of acalabrutinib for the treatment of mantle-cell lymphoma. Expert Opin Pharmacother. 2020;21(1):29-38.
51. Sun CCL, Nierman PK, Kendall EK, et al. Clinical and biological implications of target occupancy in CLL treated with the BTK inhibitor acalabrutinib [published online ahead of print, 2020 Mar 20]. Blood. 2020;blood.2019003715.
52. Xu W, Yang S, Zhou K, et al. Treatment of relapsed/refractory chronic lymphocytic leukemia/small lymphocytic lymphoma with the BTK inhibitor zanubrutinib: phase 2, single-arm, multicenter study. J Hematol Oncol. 2020;13(1):48.
53. Brown JR. How I treat CLL patients with ibrutinib. Blood. 2018;131(4):379-386.
54. Kamel S, Horton L, Ysebaert L, et al. Ibrutinib inhibits collagen-mediated but not ADP-mediated platelet aggregation. Leukemia. 2015;29(4):783-787.
55. Bhatti M, Ayton S, Michail O, et al. Effect of Bruton's tyrosine kinase inhibitors on platelet aggregation in patients with acute myocardial infarction. Thromb Res. 2019;179:64-68.
56. Griffin G, Shenoi S, Hughes GC. Hemophagocytic lymphohistiocytosis: An update on pathogenesis, diagnosis, and therapy. Best Pract Res Clin Rheumatol. 2020;101515.
57. Xu P, Zhou Q, Xu J. Mechanism of thrombocytopenia in COVID-19 patients. Ann Hematol. 2020;99(6):1205-1208.
58. Nicolson PLR, Nock SH, Hinds J, et al. Low dose Btk inhibitors selectively block platelet activation by CLEC-2 [published online ahead of print, 2020 Jan 16]. Haematologica. 2020; haematol.2019.218545.
59. Nicolson PL, Welsh JD, Chauhan A, et al. A rationale for blocking thromboinflammation in COVID-19 with Btk inhibitors [published online ahead of print, 2020 Jun 19]. Platelets. 2020;1-6.
60. Byrd JC, Furman RR, Coutre SE, et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia [published correction appears in N Engl J Med. 2014 Feb 20;370(8):786]. N Engl J Med. 2013;369(1):32-42.
61. O'Brien S, Hillmen P, Coutre S, et al. Safety Analysis of Four Randomized Controlled Studies of Ibrutinib in Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma or Mantle Cell Lymphoma. Clin Lymphoma Myeloma Leuk. 2018;18(10):648-657.e15.
62. Tillman BF, Pauff JM, Satyanarayana G, et al. Systematic review of infectious events with the Bruton tyrosine kinase inhibitor ibrutinib in the treatment of hematologic malignancies. Eur J Haematol. 2018;100(4):325-334.
63. de Porto AP, Liu Z, de Beer R, et al. Btk inhibitor ibrutinib reduces inflammatory myeloid cell responses in the lung during murine pneumococcal pneumonia. Mol Med. 2019;25(1):3.
64. Chadwick RW, George SE, Chang J, et al. Comparative gastrointestinal enzyme activity and activation of the promutagen 2,6-dinitrotoluene in male CD-1 mice and male Fischer 344 rats. Cancer Lett. 1990;52(1):13-19.
65. Strijbis K, Yilmaz OH, Dougan SK, et al. Intestinal colonization by Candida albicans alters inflammatory responses in Bruton's tyrosine kinase-deficient mice. PLoS One. 2014;9(11):e112472.
66. Guendel I, Iordanskiy S, Sampey GC, et al. Role of Bruton's tyrosine kinase inhibitors in HIV-1-infected cells. J Neurovirol. 2015;21(3):257-275.
67. Hu Y, Wen Z, Liu S, et al. Ibrutinib suppresses intracellular mycobacterium tuberculosis growth by inducing macrophage autophagy. J Infect. 2020;80(6):e19-e26.
68. Wang J, Liu X, Hong Y, et al. Ibrutinib, a Bruton's tyrosine kinase inhibitor, exhibits antitumoral activity and induces autophagy in glioblastoma. J Exp Clin Cancer Res. 2017;36(1):96.
69. Weber ANR, Bittner Z, Liu X, et al. Bruton's Tyrosine Kinase: An Emerging Key Player in Innate Immunity. Front Immunol. 2017;8:1454.
70. Volmering S, Block H, Boras M, et al. The Neutrophil Btk Signalosome Regulates Integrin Activation during Sterile Inflammation. Immunity. 2016;44(1):73-87.
71. Cheung TT, McInnes IB. Future therapeutic targets in rheumatoid arthritis? Semin Immunopathol. 2017;39(4):487-500.
72. Hutcheson J, Vanarsa K, Bashmakov A, et al. Modulating proximal cell signaling by targeting Btk ameliorates humoral autoimmunity and end-organ disease in murine lupus. Arthritis Res Ther. 2012;14(6):R243. Published 2012 Nov 8.
73. Haselmayer P, Camps M, Liu-Bujalski L, et al. Efficacy and Pharmacodynamic Modeling of the BTK Inhibitor Evobrutinib in Autoimmune Disease Models. J Immunol. 2019;202(10):2888-2906.
74. Theoharides TC. COVID-19, pulmonary mast cells, cytokine storms, and beneficial actions of luteolin. Biofactors. 2020;46(3):306-308.
75. Gschwandtner M, Paulitschke V, Mildner M, et al. Proteome analysis identifies L1CAM/CD171 and DPP4/CD26 as novel markers of human skin mast cells. Allergy. 2017;72(1):85-97.
76. Dispenza MC, Krier-Burris RA, Chhiba KD, et al. Bruton's tyrosine kinase inhibition effectively protects against human IgE-mediated anaphylaxis [published online ahead of print, 2020 Jun 2]. J Clin Invest. 2020;138448.
77. Gabizon R, London N. A Fast and Clean BTK Inhibitor. J Med

Chem. 2020;63(10):5100-5101.

78. Angst D, Gessier F, Janser P, et al. Discovery of LOU064 (Remibrutinib), a Potent and Highly Selective Covalent Inhibitor of Bruton's Tyrosine Kinase. J Med Chem. 2020;63(10):5102-5118.
79. Gao S, Yuan L, Li C, et al. A novel small molecule compound possesses immunomodulatory properties on bone marrow-derived dendritic cells via TLR7 signaling pathway and alleviates the development of SLE. Int Immunopharmacol. 2017;47:47-52.
80. Clark A, Jit M, Warren-Gash C, et al. Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study [published online ahead of print, 2020 Jun 15]. Lancet Glob Health. 2020;S2214-109X(20)30264-3.
81. Conti P, Younes A. Coronavirus COV-19/SARS-CoV-2 affects women less than men: clinical response to viral infection. J Biol Regul Homeost Agents. 2020;34(2):10.23812/Editorial-Conti-3.
82. Greil R, Tedeschi A, Moreno C, et al. Ibrutinib decreases obinotuzumab induced secretion of cytokines associated with infusion-related reactions in patients with CLL: Analysis from the ILLUMINATE study. Hematol Oncol. 2019;37: 210-212.
83. Fiorcari S, Maffei R, Audrito V, et al. Ibrutinib modifies the function of monocyte/macrophage population in chronic lymphocytic leukemia. Oncotarget. 2016;7(40):65968-65981.
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.
85. Jaglowski SM, Blazar BR. How ibrutinib, a B-cell malignancy drug, became an FDA-approved second-line therapy for steroid-resistant chronic GVHD. Blood Adv. 2018;2(15):2012-2019.
86. King-Kallimanis BL, Wroblewski T, Kwitkowski V, et al. FDA review summary of patient-reported outcome results for ibrutinib in the treatment of chronic graft versus host disease. Qual Life Res. 2020;29(7):1903-1911.
87. Miklos D, Cutler CS, Arora M, et al. Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. Blood. 2017;130(21):2243-2250.
88. Treon SP, Castillo JJ, Skarbnik AP, et al. The BTK inhibitor ibrutinib may protect against pulmonary injury in COVID-19-infected patients. Blood. 2020;135(21):1912-1915.
89. Thibaud S, Tremblay D, Bhalla S, et al. Protective role of Bruton tyrosine kinase inhibitors in patients with chronic lymphocytic leukaemia and COVID-19 [published online ahead of print, 2020 May 20]. Br J Haematol. 2020;10.1111/bjh.16863.
90. Roschewski M, Lionakis MS, Sharman JP, et al. Inhibition of Bruton tyrosine kinase in patients with severe COVID-19. Sci Immunol. 2020;5(48):eabd0110.

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