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A rationale for blocking thromboinflammation in COVID-19 with Btk inhibitors

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A novel Coronavirus infection (COVID-19) leading to pneumonia and severe acute respiratory distress syndrome (ARDS) was first reported in Wuhan, Hubei Province, China in December 2019 and has subsequently spread to almost all other countries in the world. On 11th March 2020, the WHO declared the COVID-19 outbreak a global pandemic. As of 16th of May 2020, there are 4,425,485 confirmed cases with 302,059 deaths worldwide¹ Patients with severe illness may develop dyspnoea and hypoxaemia within one week of onset of symptoms, which may quickly progress to ARDS or end-organ failure.² In the order of 20% of infected patients are admitted to hospital and of these, approximately 25% will die.³ Only supportive treatments are available such as oxygen via a face mask or mechanical ventilation as required. Thus there is an urgent and unmet clinical need in these patients for specific therapies that prevent or treat the pathophysiology of the disease.

The sudden deterioration seen during the illness course in patients with COVID-19 has been attributed to inappropriate immune activation, mediated through excessive macrophage activation and release of cytokines, including interleukin (IL)-6.⁴ This is similar to that seen with the Cytokine Release Syndrome (CRS) of Chimeric Antigen Receptor-T cell (CAR-T) therapy.⁴ Indeed, the anti-IL-6 monoclonal antibody tocilizumab is used to treat CRS in CAR-T therapy and is being trialed in patients with COVID-19.^{5,6} The very high ferritin levels seen in the most severely affected patients is indicative of macrophage activation and is at similar levels to those seen in conditions driven by overactive macrophages such as Haemophagocytic Lymphohistiocytosis (HLH).⁷

Abnormal coagulation parameters have been observed in patients with COVID-19 (thrombocytopenia, raised D-dimer and prolonged prothrombin time [PT]) ⁸ and these are reported to predict a poor prognosis.⁹ Multiple studies in the last few weeks have shown increases in microvascular thrombosis post-mortem in patients who have died from COVID-19.^{10,11} Moreover, recent reports have noted increased thromboembolic events^{12,13} and there is some evidence of improved outcomes with prophylactic- and therapeutic-doses of low molecular weight heparin (LMWH). The evidence of benefit of both of these treatments is stronger in those patients admitted to intensive care¹⁴ or in those with more severe coagulopathy.⁹

It is not known exactly what triggers the coagulopathy in patients with COVID-19 but it does not seem to resemble the pathophysiology of disseminated intravascular coagulation (DIC) where there is widespread activation of platelets and procoagulant proteins initially causing microvascular thrombosis followed by consumption of these elements and resultant bleeding. In COVID-19 bleeding is not as prominent a feature as the thrombosis in the microvasculature of the lungs and other organs.¹⁵

It is well known that inflammatory states increase the risk of thrombosis and it is increasingly apparent that platelets form a vital link between the immune system and coagulation. Immunothrombosis is the process by which platelets contribute to the function of the innate immune system by forming thrombi in vessels which catch pathogens. Thromboinflammation is the pathological formation of thrombi in response to inflammatory stimuli. It is suspected to play a critical role in the development of DIC as well as in arterial and venous thrombosis.¹⁶ Two recent papers have speculated that thromboinflammation resulting from pathological immune activation by monocytes and other immune cells could be the mechanism by which COVID-19 related hypercoagulability occurs^{15,17} but none have proposed specific, targetable pathways involved in this process in COVID-19.

In a mouse model of thromboinflammation triggered by attenuated salmonella infection, our group has shown that tissue macrophages showing up-regulation of podoplanin in response to inflammatory challenge activate the platelet tyrosine kinase-linked receptor CLEC-2 which leads to hepatic thrombus formation.¹⁸ In this model, depletion of macrophages, or genetic deletion of podoplanin or CLEC-2 blocks thrombus formation. Platelet CLEC-2 is also a pre-requisite for thrombus formation in a venous thrombosis mouse model.¹⁹ In these mice, podoplanin is upregulated in the subendothelium on stromal cells surrounding the thrombus. Despite this strong evidence for CLEC-2's role in mouse thromboinflammation, no studies exist showing similar findings in humans. In previously unpublished work however, we have also found increased expression of podoplanin in the venous valves adjacent to a femoral vein thrombus in a patient who died with deep vein thrombosis

(DVT) but not in the unaffected valves of the same vein or the equivalent valves in the contralateral leg (see Figure 1).

In addition to their role in driving inflammatory thrombosis platelets are key regulators of the innate immune response in both sterile and infectious conditions.²⁰ The platelet-macrophage interaction has been well described²¹ and remains especially relevant to the pathogenesis of the CRS observed in COVID-19 and this is perhaps unsurprising with recent evidence highlighting a clear role for platelets triggering inflammasome activation²² and then proinflammatory cytokine production that characterises the CRS.²³

We speculate that blockade of CLEC-2 might prevent immune-mediated activation of platelets and thus block thromboinflammation during COVID-19. Inhibitors of non-receptor signalling kinases downstream of CLEC-2 are effective in completely blocking platelet CLEC-2 function. The Src kinase inhibitor dasatinib and Syk kinase inhibitors R406 (the active metabolite of fostamatinib) and PRT-06018 have been shown to block CLEC-2 mediated platelet aggregation.²⁴⁻²⁶ Recently we and the Siess group have shown that targeting the kinase Btk is very effective at blocking downstream PLCy2 phosphorylation, all forms of platelet CLEC-2 function and thrombus formation under flow at drug concentrations that leave other platelet receptor signalling pathways intact.²⁷⁻²⁹ Btk is a non-receptor signalling kinase downstream of CLEC-2 in platelets and the B-cell receptor as well as at low levels in myeloid and erythroid cells.³⁰⁻³² It was first discovered as the causative mutated protein in the congenital immunodeficiency X-linked agammaglobulinaemia (XLA).³³ Btk inhibitors are currently licensed for the treatment of B-cell malignancies but are also being trialed for the treatment of autoimmune disease. They are broadly separated into two types; those that bind covalently to Cysteine-481 in the ATP binding site in the kinase domain and thus prevent kinase activity and those that reversibly associate to the Src Homology (SH) 3 domain when Btk is in its inactive conformation and prevent change to the active conformation.³⁴

Both ibrutinib and dasatinib have completed randomised clinical trials (RCTs) in B-cell malignancies and chronic myeloid leukaemia/prostate cancer respectively. There is evidence from post-hoc analysis of these trials that both ibrutinib and dasatinib significantly reduce venous thrombosis (see Table 1) and that ibrutinib also reduces arterial thrombosis.³⁵ There may be an added benefit to using Btk inhibitors rather than anticoagulants to reduce thrombosis in COVID-19; namely the lack of bleeding side effects. We and other groups have shown that CLEC-2 has only a minimal role in the classical haemostatic function of platelets^{36,37} and thus CLEC-2 inhibition itself is unlikely to cause bleeding. It is important to note that patients who lack Btk have no demonstrable CLEC-2 mediated platelet function and do not bleed excessively.^{27,30} Increased bleeding and atrial fibrillation are the main clinically significant side effects associated with the first generation Btk inhibitor

ibrutinib, and to a lesser extent the second generation drug acalabrutinib. They have been shown by us and other groups to be mediated by off-target effects on other kinases due to the high drug doses used in patients with haematological malignancy.^{28,38,39} Chen *et al.* showed that patients with chronic lymphocytic leukaemia could have their ibrutinib doses reduced from 480 mg to 140 mg once daily and still achieve a trough Btk occupancy of 97%.⁴⁰ Indeed the highly Btk selective fenebrutinib reduced joint inflammation in rheumatoid arthritis without any reported bleeding side effects⁴¹ and there is so little bleeding associated with the novel Btk inhibitor Rilzabrutinib that it is currently in phase II trials for immune thrombocytopenia (ITP).⁴² Other potential effects could be mediated through Btk's known role in PI3K/Akt and NF-kB signalling in dendritic cells and macrophages. The effects of Btk inhibitors on platelet PI3K/Akt or NF-kB have not been directly studied. The most frequently experienced adverse events in studies of the latest-generation and more targeted Btk inhibitors are self-limiting gastrointestinal side effects and headaches.^{41,43} Given the severity of COVID-19 we feel that these side effects would be well tolerated for the (likely) short duration of treatment required.

In addition to the potential antithrombotic benefit of Btk inhibition in COVID-19, a direct reduction in pathologic inflammation may be observed. Inhibition of Btk has been shown to reduce myeloid cell cytokine release, and subsequent ischaemic injury in a mouse brain ischaemia/reperfusion injury model.⁴⁴ Btk inhibitors are also being trialled as an adjunct to CAR-T treatment and have been shown to lower cytokine release in CRS in mouse CAR-T⁴⁵ as well as reduce CRS severity and CRS associated cytokines in patients undergoing CAR-T treatment.⁴⁶ Specifically in COVID-19, there is a small case series showing unexpectedly mild disease in patients with B-cell malignancy treated with ibrutinib.⁴⁷

To conclude, anticoagulants such as LMWH and immunosuppressants such as tocilizumab may have a role separately in the treatment of the thrombosis and inflammation associated with COVID-19, but Btk inhibitors may well perform both of these roles simultaneously; not only are Btk inhibitors likely to reduce the microvascular and venous thrombosis in COVID-19 by blocking platelet CLEC-2, but they may also diminish pathological excessive inflammation by blocking cytokine release. Moreover, unlike anticoagulants, they may well reduce thrombosis without an associated increase in bleeding. They may even be able to be given alongside anticoagulants and IL-6 inhibitors in order to further reduce thrombosis and inflammation. The evaluation of Btk inhibitors in COVID-19 is certainly worthy of consideration in prospective clinical trials.

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Ethical Considerations

The University of Pennsylvania Institutional Review Board (IRB) approved all human protocols, and all procedures were performed in accordance with these protocols.

Conflict of interest

PLRN, MRT and SPW have received research funding from Novartis. PLRN, AC, RT and SPW have received research funding from Principia Biopharma. MRT, PLRN and SPW have received research funding from Rigel Pharmaceuticals.

Author Contributions

PLRN wrote the manuscript and performed the data collection from the ibrutinib and dasatinib trials. JW and MK performed the experiments on human tissue from the patient with DVT. All authors critically appraised the manuscript.

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Figure legend and table

Figure.1: Podoplanin is upregulated on venous valve surrounding thrombus in human veins in vivo.

Femoral vein from a patient with DVT identified at post mortem was sectioned, fixed and stained with the anti-human podoplanin (hPDPN) antibody NZ-1 at a dilution of 1:200. (A) Femoral vein valve from unaffected leg with no surrounding DVT stained with hPDPN (red) and 4,6- diamidino-2-phenylindole (DAPI, blue). Arrows show locations of lymphatic vessels in the vein wall. (B). Femoral vein valve from the affected leg with no surrounding DVT stained with hPDPN. (C) Femoral vein valve from the affected leg with surrounding DVT stained with hPDPN. (C) Femoral vein valve from the affected leg with surrounding DVT stained with hPDPN. Arrows show infiltrating cells staining positive for human podoplanin. Vein Lumen (L), Vein Wall (W), Venous Valve (Va).

Trial Number	Reference	Ibrutinib Exposure (person years)	VTEs in ibrutinib group	Control treatment	Control treatment exposure (person years)	VTEs in control group	VTE rate ratio (95% CI)	P-value
NCT01578707	Montillo <i>et al.</i> ⁴⁸	715	0	Ofatumumab	132.3	2	0	0.024 ^a
NCT01722487	Barr <i>et al.</i> ⁴⁹	323	0	Chlorambucil	209.5	0		
NCT01646021	Dreyling <i>et al.</i> ⁵⁰	180.7	1	Temsirolimus	72.9	2	0.202	0.224ª
NCT01973387	Huang <i>et al.</i> ⁵¹	144.9	0	Rituximab	37.8	0		
Total		1363.6	1		452.5	4	0.071 (0.003-0.584)	0.012 ^b
Trial Number	Reference	Dasatinib Exposure (person years)	VTEs in dasatinib group	Control treatment	Control treatment exposure (person years)	VTEs in control group	VTE rate ratio (95% CI)	P-value
NCT0048124	Cortes <i>et al.</i> ⁵²	1295	2	Imatinib	1300	1	2.008	0.623ª
NCT0074449	Araujo <i>et al.⁵³</i>	850.9	8	Placebo	703	28	0.236	<0.001ª
Total		2145.9	10		2003	29	0.289 (0.134-0.582)	<0.001 ^b

Table 1: Ibrutinib and Dasatinib RCTs with VTEs expressed as drug exposure in person years.

www.clinicaltrials.gov was searched using the terms "ibrutinib OR PCI-32675 OR imbruvica" and "dasatinib OR BMS-354825 OR sprycel" on the 20/9/2018 and results were then filtered to only include RCTs with results. Four ibrutinib and two dasatinib RCTs were identified. Results on <u>www.clinicaltrials.gov</u> were scrutinised to identify adverse events and severe adverse events and the number of venous thromboembolic (VTE) episodes were recorded. The relevant publications for each trial were used to identify median treatment duration so that drug treatment could be standardised and expressed as exposure in person years. ^aStatistical analysis performed using a Mid-P exact test. ^bStatistical analysis performed using a Mid-P exact test with stratification by trial.⁵⁴