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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 worldwide[1] 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 care[14] 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 described[21] 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 activation[22] 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 PLCγ2 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 effects[41] 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-κB signalling in dendritic cells and macrophages. The effects of Btk inhibitors on platelet PI3K/Akt or NF-κB 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-T[45] 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.

References

1. WHO Situation Report 117. 2020. WHO. [internet]. [accessed May 17 2020]. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

2. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. Arch Intern Med. 2020 Mar 13. [accessed 2020 May 13]:[10p]. doi: 10.1001/jamainternmed.2020.0994

3. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, Liu L, Shan H, Lei C-L, Hui DSC, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med. 2020 Feb 28. [accessed 2020 Mar 31]:[13p]. doi: 10.1056/NEJMoa2002032

4. Zhang C, Wu Z, Li J-W, Zhao H, Wang G-Q. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. International Journal of Antimicrobial Agents 2020 Apr 23.

[accessed 2020 May 13]:[27p]. doi: 10.1016/j.ijantimicag.2020.105954

5. Yakoub-Agha I, Chabannon C, Bader P, Basak GW, Bonig H, Ciceri F, Corbacioglu S, Duarte RF, Einsele H, Hudecek M, et al. Management of adults and children undergoing chimeric antigen receptor T-cell therapy: best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE). Haematologica 2019;105:297–316.

6. Luo P, Liu Y, Qiu L, Liu X, Liu D, Li J. Tocilizumab treatment in COVID-19: A single center experience. J Med Virol. 2020;8:420–5.

7. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmunity Reviews. 2020 Mar 30. [accessed 2020 May 13]:[7p]. doi: 10.1016/j.autrev.2020.102537

8. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, Clark C, Iba T. ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost. 2020;18:1023–6.

9. Tang N, Bai H, Chen X, Gong J, Li D, Sun Z. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy. J Thromb Haemost. 2020;18:1094–9.

10. Fox SE, Akmatbekov A, Harbert JL, Li G, Brown JQ, Vander Heide RS. Pulmonary and Cardiac Pathology in Covid-19: The First Autopsy Series from New Orleans. medRxiv. 2020 Apr 10. [accessed 2020 May 13]:[8p]. doi: 10.1101/2020.04.06.20050575

11. Menter T, Haslbauer JD, Nienhold R, Savic S, Hopfer H, Deigendesch N, Frank S, Turek D, Willi N, Pargger H, et al. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. Histopathology. 2020 May 4. [accessed 2020 May 13]:[27p]. doi: 10.1111/his.14134

12. Lillicrap D. Disseminated intravascular coagulation in patients with 2019-nCoV pneumonia. J Thromb Haemost 2020;18:786–7.

13. Zhang Y, Xiao M, Shang S, Xia P, Cao W, Jiang W, Chen H, Ding X, Shang S. Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. N Engl J Med 2020;382:e38(1)–e38(3).

14. Paranjpe I, Fuster V, Lala A, Russak A, Glicksberg BS, Levin MA, Charney AW, Narula J, Fayad ZA, Bagiella E, et al. Association of Treatment Dose Anticoagulation with In-Hospital Survival Among Hospitalized Patients with COVID-19. J Am Col Cardiol. 2020 May 5. [accessed 2020 May 13]:[9p]. doi: 10.1016/j.jacc.2020.05.001

15. Connors JM, Levy JH. Thromboinflammation and the hypercoagulability of COVID-19. J Thromb Haemost. 2020 Apr 27. [accessed 2020 May 22]:[10p]. doi: 10.1111/jth.14849

16. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nature Reviews Immunology. 2012;13:34–45.

17. Ciceri F, Beretta L, Scandroglio AM, Colombo S, Landoni G, Ruggeri A, Peccatori J, DAngelo A, De Cobelli F, Rovere-Querini P, et al. Microvascular COVID-19 lung vessels obstructive thromboinflammatory syndrome (MicroCLOTS): an atypical acute respiratory distress syndrome working hypothesis. Crit Carae. 2020 Apr 16. [accessed 2020 May 22]:[3p]

18. Hitchcock JR, Cook CN, Bobat S, Ross EA, Flores-Langarica A, Lowe KL, Khan M, Dominguez-Medina CC, Lax S, Carvalho-Gaspar M, et al. Inflammation drives thrombosis after Salmonella infection via CLEC-2 on platelets. J Clin Invest. 2015;125:4429–46.

19. Payne H, Ponomaryov T, Watson SP, Brill A. Mice with a deficiency in CLEC-2 are protected against deep vein thrombosis. Blood. 2017;129:2013–20.

20. Rayes J, Bourne JH, Brill A, Watson SP. The dual role of platelet-innate immune cell interactions in thrombo-inflammation. Res Pract Thromb Haemost. 2019;4:23–35.

21. Rolfes V, Ribeiro LS, Hawwari I, Böttcher L, Rosero N, Maasewerd S, Santos MLS, Próchnicki T, de Souza Silva CM, de Souza Wanderley CW, et al. Platelets Fuel the Inflammasome Activation of Innate Immune Cells. CellReports. 2020;31:107615. doi: 10.1016/j.celrep.2020.107615

22. Lin L, Xu L, Lv W, Han L, Xiang Y, Fu L, Jin M, Zhou R, Chen H, Zhang A. An NLRP3 inflammasome-triggered cytokine storm contributes to Streptococcal toxic shock-like syndrome (STSLS). PLoS Pathog. 2019;15:e1007795–24. doi: 10.1371/journal.ppat.1007795

23. Liu Q, Zhou Y-H, Yang Z-Q. The cytokine storm of severe influenza and development of immunomodulatory therapy. Cell Mol Immunol. 2015;13:3–10.

24. Lorenz V, Stegner D, Stritt S, Vogtle T, Kiefer F, Witke W, Schymeinsky J, Watson SP, Walzog B, Nieswandt B. Targeted downregulation of platelet CLEC-2 occurs through Syk-independent internalization. Blood. 2015;125:4069–77.

25. Gitz E, Pollitt AY, Gitz-Francois JJ, Alshehri OM, Mori J, Montague S, Nash GB, Douglas MR, Gardiner E, Andrews RK, et al. CLEC-2 expression is maintained on activated platelets and on platelet microparticles. Blood. 2014;124:2262–70.

26. Spalton JC, Mori J, Pollitt AY, Hughes CE, Eble JA, Watson SP. The novel Syk inhibitor R406 reveals mechanistic differences in the initiation of GPVI and CLEC-2 signaling in platelets. J Thromb Haemost. 2009;7:1192–9.

27. Nicolson PLR, Nock SH, Hinds J, Garcia-Quintanilla L, Smith CW, Campos J, Brill A, Pike JA, Khan AO, Poulter NS, et al. Low dose Btk inhibitors selectively block platelet activation by CLEC-2. Haematologica. 2020 Jan 16. [accessed 2020 Jan 16];[13p]. doi: 10.3324/haematol.2019.218545

28. Nicolson PLR, Hughes CE, Watson S, Nock SH, Hardy AT, Watson CN, Montague SJ, Malcor J-D, Thomas MR, Pollitt AY, et al. Inhibition of Btk by Btk-specific concentrations of ibrutinib and acalabrutinib delays but does not block platelet aggregation to GPVI. Haematologica. 2018;103:2097-108.

29. Busygina K, Jamasbi J, Seiler T, Deckmyn H, Weber C, Brandl R, Lorenz R, Siess W. Oral Bruton tyrosine kinase inhibitors selectively block atherosclerotic plaque–triggered thrombus formation in humans. Blood. 2018;131:2605–16.

30. Quek LS, Bolen J, Watson SP. A role for Bruton's tyrosine kinase (Btk) in platelet activation by collagen. Curr Biol. 1998;8:1137–40.

31. Akinleye A, Chen Y, Mukhi N, Song Y, Liu D. Ibrutinib and novel BTK inhibitors in clinical development. J Hematol Oncol. 2013 Aug 19. [accessed 2018 Jun 26]:[9p]. doi: 10.1186/1756-8722-6-59

32. Weers M de, Verschuren M, Kraakman M, Mensink R, Schuurman R, van Dongen J, Hendriks RW. The Bruton's tyrosine kinase gene is expressed throughout B cell differentiation, from early precursor B cell stages preceding immunoglobulin gene rearrangement up to mature B cell stages. Eur J Immunol. 1993;23:3109–14.

33. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Kilsak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, et al. Deficient

Expression of a B Cell Cytoplasmic Tyrosine Kinases in Human X-Linked Agammaglobulinemia. Cell. 1993;72:279–90.

34. Liang C, Tian D, Ren X, Ding S, Jia M, Xin M, Thareja S. The development of Bruton's tyrosine kinase (BTK) inhibitors from 2012 to 2017: A mini-review. Eur J Med Chem. 2018;151:315–26.

35. Kander EM, Zhao Q, Bhat SA, Hirsch J, Byrd JC, Ooka L, Wiczer T, Woyach JA, Awan FT, Rogers KA, et al. Venous and arterial thrombosis in patients with haematological malignancy during treatment with ibrutinib. Br J Haematol. 2019;187:399–402.

36. Hughes CE, Navarro-Núñez L, Finney BA, Mourão-Sá D, Pollitt AY, Watson SP. CLEC-2 is not required for platelet aggregation at arteriolar shear. J Thromb Haemost. 2010;8:2328–32.

37. Bender M, May F, Lorenz V, Thielmann I, Hagedorn I, Finney BA, Vogtle T, Remer K, Braun A, Bosl M, et al. Combined In Vivo Depletion of Glycoprotein VI and C-Type Lectin-Like Receptor 2 Severely Compromises Hemostasis and Abrogates Arterial Thrombosis in Mice. Arterioscler Thromb Vasc Biol. 2013;33:926–34.

38. McMullen JR, Boey E, Ooi J, Seymour JF, Keating MJ, Tam CS. Ibrutinib increases the risk of atrial fibrillation, potentially through inhibition of cardiac PI3K-Akt signaling. Blood. 2014;124:3829–30.

39. Bye AP, Unsworth AJ, Desborough MJ, Hildyard CA, Appleby N, Bruce D, Kriek N, Nock SH, Sage T, Hughes CE, et al. Severe platelet dysfunction in NHL patients receiving ibrutinib is absent in patients receiving acalabrutinib. Blood Adv. 2017;1:2610–23.

40. Chen LS, Bose P, Cruz ND, Feng S, Jiang Y, Wu Q, Thompson PA, Kroll MH, Qiao W, Huang X, et al. A pilot study of lower doses of ibrutinib in patients with chronic lymphocytic leukemia. Blood. 2018;132:2249-59

41. Cohen S, Tuckwell K, Katsumoto TR, Zhao R, Galanter J, Lee C, Rae J, Toth B, Ramamoorthi N, Hackney JA, et al. Fenebrutinib versus Placebo or Adalimumab in Rheumatoid Arthritis: A Randomized, Double-Blind, Phase II Trial (ANDES Study). Arthritis Rheumatol. 2020 Apr 9.[accessed 2020 May 22]:[34p]. doi: 10.1002/art.41275

42. NCT03395210 A Study of PRN1008 in Adult Patients With Immune Thrombocytopenic Purpura (ITP). 2018. ClinicalTrials.gov. [internet]. [accessed March 31 2020]. https://www.clinicaltrials.gov/ct2/show/NCT03395210

43. Btk inhibitor calms pemphigus vulgaris with low-dose steroids. 2019. MDedge Dermatology [internet]. [accessed May 14 2020]. https://www.mdedge.com/dermatology/article/195941/autoimmune-diseases/btk-inhibitor-calms-pemphigus-vulgaris-low-dose

44. Ito M, Shichita T, Okada M, Komine R, Noguchi Y, Yoshimura A, Morita R. Bruton's tyrosine kinase is essential for NLRP3 inflammasome activation and contributes to ischaemic brain injury. Nat Commun. 2015;6:1–11.

45. Ruella M, Kenderian SS, Shestova O, Klinchisky M, Melenhorst JJ, Wasik MA, Lacey SF, June CH, Gill S. Kinase inhibitor ibrutinib to prevent cytokine-release syndrome after and-CD19 chimeric antigen receptor T cells for B-cell neoplasma. Leukemia. 2017;31:246-8.

46. Gauthier J, Hirayama AV, Purushe J, Hay KA, Lymp J, Li DH, Yeung CCS, Sheih A, Pender BS, Hawkins RM, et al. Feasibility and efficacy of CD19-targeted CAR T cells with concurrent ibrutinib for CLL after ibrutinib failure. Blood. 2020;135:1650–60.

47. Treon SP, Castillo J, Skarbnik AP, Soumerai JD, Ghobrial IM, Guerrera ML, Meid KE, Yang G. The BTK-inhibitor ibrutinib may protect against pulmonary injury in COVID-19 infected patients. Blood. 2020 Apr 17. [accessed 2020 Apr 25]:[9p}. doi: 10.1182/blood.2020006288

48. Montillo M, Brien SOA, Tedeschi A, Byrd JC, Dearden C, Gill D, Brown JR, Barrientos JC, Mulligan SP, Furman RR, et al. Ibrutinib in previously treated chronic lymphocytic leukemia patients with autoimmune cytopenias in the RESONATE study. Blood Cancer J. 2017;7:e524. doi: 10.1038/bcj.2017.5

49. Barr PM, Robak T, Owen C, Tedeschi A, Bairey O, Bartlett NL, Burger JA, Hillmen P, Coutre S, Devereux S, et al. Sustained efficacy and detailed clinical follow-up of first-line ibrutinib treatment in older patients with chronic lymphocytic leukemia: extended phase 3 results from RESONATE-2. Haematologica. 2018;103:1502–10.

50. Dreyling M, Jurczak W, Jerkeman M, Santucci Silva R, Rusconi C, Trneny M, Offner F, Caballero D, Joao C, Witzens-Harig M, et al. Ibrutinib versus temsirolimus in patients with relapsed or refractory mantle-cell lymphoma: an international, randomised, open-label, phase 3 study. Lancet. 2016;387:770–8.

51. Huang X, Qiu L, Jin J, Zhou D, Chen X, Hou M, Hu J, Hu Y, Ke X, Li J, et al. Ibrutinib versus rituximab in relapsed or refractory chronic lymphocytic leukemia or small lymphocytic lymphoma: a

randomized, open-label phase 3 study. Cancer Med. 2018;7:1043–55.

52. Cortes JE, Saglio G, Kantarjian HM, Baccarani M, Mayer J, Boqué C, Shah NP, Chuah C, Casanova L, Bradley-Garelik B, et al. Final 5-Year Study Results of DASISION: The Dasatinib Versus Imatinib Study in Treatment-Naïve Chronic Myeloid Leukemia Patients Trial. J Clin Oncol. 2016;34:2333–40.

53. Araujo JC, Trudel GC, Saad F, Armstrong AJ, Yu EY, Bellmunt J, Wilding G, McCaffrey J. Docetaxel and dasatinib or placebo in men with metastatic castration-resistant prostate cancer (READY): a randomised, double-blind phase 3 trial. Lancet Oncol. 2017;14:1307–16.

54. OpenEpi: Open Source Epidemiologic Statistics for Public Health version 3.01. 2006. Dean AG, Sullivan KM, Soe MM. [internet]. [accessed Sept 05 2018]. https://www.OpenEpi.com

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. 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 *et al.*[48]	715	0	Ofatumumab	132.3	2	0	0.024[a]
NCT01722487	Barr *et al.*[49]	323	0	Chlorambucil	209.5	0		
NCT01646021	Dreyling *et al.*[50]	180.7	1	Temsirolimus	72.9	2	0.202	0.224[a]
NCT01973387	Huang *et al.*[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 *et al.*[52]	1295	2	Imatinib	1300	1	2.008	0.623[a]
NCT0074449	Araujo *et al.*[53]	850.9	8	Placebo	703	28	0.236	<0.001[a]
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 www.clinicaltrials.gov 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. [a]Statistical analysis performed using a Mid-P exact test. [b]Statistical analysis performed using a Mid-P exact test with stratification by trial.[54]