




# Vaccine-Induced Immune Thrombotic Thrombocytopenia (VITT): Targeting Pathomechanisms with Bruton Tyrosine Kinase Inhibitors

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## Abstract

A series of cases with rare thromboembolic incidents including cerebral sinus vein thrombosis (some of them fatal) and concomitant thrombocytopenia occurring shortly after vaccination with the coronavirus disease 2019 (COVID-19) vaccine AZD1222 (Vaxzevria) have caused significant concern and led to its temporary suspension in many countries. Immediate laboratory efforts in four of these patients have identified a tentative pathomechanism underlying this syndrome termed initially vaccine-induced prothrombotic immune thrombocytopenia (VIPIT) and renamed recently vaccine-induced immune thrombotic thrombocytopenia (VITT). It encompasses the presence of platelet-activating antibodies to platelet factor-4/heparin complexes, possibly emulated by polyanionic constituents of AZD1222, and thus resembles heparin-induced thrombocytopenia (HIT). Because these immune complexes bind and activate platelets via Fcγ receptor IIA (FcγRIIA), high-dose intravenous immunoglobulin G has been suggested for treatment of VITT in addition to non-heparin anticoagulants. Here we propose inhibitors of Bruton tyrosine kinase (Btk) approved for B cell malignancies (e.g., ibrutinib) as another therapeutic option in VITT, as they are expected to pleiotropically target multiple pathways downstream of FcγRIIA-mediated Btk activation, for example, as demonstrated for the effective inhibition of platelet aggregation, dense granule secretion, P-selectin expression and platelet-neutrophil aggregate formation stimulated by FcγRIIA cross-linking. Moreover, C-type lectin-like receptor CLEC-2- and GPIb-mediated platelet activation, the interactions and activation of monocytes and the release of neutrophil extracellular traps, as encountered in HIT, could be attenuated by Btk inhibitors. As a paradigm for emergency repurposing of approved drugs in COVID-19, off-label use of Btk inhibitors in a low-dose range not affecting haemostatic functions could thus be considered a sufficiently safe option to treat VITT.

## Keywords

- ▶ COVID-19 vaccine
- ▶ thrombocytopenia
- ▶ platelet
- ▶ Btk
- ▶ FcγRIIA

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Vaccines are critical to effectively contain the coronavirus disease 2019 (COVID-19) pandemic. Four vaccines have been approved by the European Medicines Agency (EMA) as of 30 March 2021: two mRNA-based vaccines encoding the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from Pfizer/BioNTech and Moderna, and two recombinant adenoviral vector-based vaccines encoding the spike protein from AstraZeneca/Oxford University and Janssen/Johnson&Johnson. More than 120 million doses of vaccine had been administered in Europe (<https://ourworldindata.org/covid-vaccinations>) by 31 March 2021. Despite unprecedented scientific and industrial efforts, supply of vaccines still falls short of urgent demand to reach protective immunity in the general population, increasing the pressure to use all approved vaccines.

More than 20 million doses of the COVID-19 vaccine AstraZeneca (AZD1222, ChAdOx1 nCoV-19) have been administered in European Union (EU) countries and the United Kingdom as of 25 March 2021.<sup>1</sup> However, recent reports of rare severe cerebral venous sinus thrombosis (CVST) shortly after vaccination have prompted the temporary suspension of the vaccine in 16 continental European countries in mid-March. On 26 March 2021, EMA authorized the further use of the COVID-19 vaccine AstraZeneca, which was renamed Vaxzevria.<sup>2</sup> According to the Robert Koch Institute, 2.7 million first doses and 767 second doses of the vaccine were given in Germany as of 29 March 2021, and a total of 31 cases of CVST in Germany after vaccination with Vaxzevria had been reported to the Paul-Ehrlich Institute as of 29 March 2021. Coincident thrombocytopenia was documented in 19 cases. In nine cases, the outcome was fatal. With the exception of two men, 36 and 57 years old, all reports concerned women aged 20 to 63 years.<sup>3</sup> German authorities decided therefore on 30 March 2021 to suspend Vaxzevria for regular vaccinations of persons younger than 60 years. However, in the United Kingdom where 13.7 million doses of this vaccine have been applied, only 5 comparable cases have been reported so far in the media,<sup>4</sup> initially hinting at batch-specific effects related to adenoviral vaccine constituents. More recently, a United Kingdom government update through 21 March 2021 documented 22 reports of CVST and 8 reports of other thrombosis events with low platelet counts.<sup>5</sup> As of 4 April 2021, a total of 169 cases of CVST and 53 cases of splanchnic vein thrombosis were reported to Eudra-Vigilance.<sup>6</sup> Around 34 million people had been vaccinated with Vaxzevria in the European Economic Area (EEA) and United Kingdom by this date. On April 7, EMA's safety committee (Pharmacovigilance Risk Assessment Committee [PRAC]) had concluded that unusual blood clots with low blood platelet counts should be listed as very rare side effects of Vaxzevria.<sup>6</sup>

A cooperative effort led by Andreas Greinacher at Greifswald University rapidly unravelled a tentative pathogenic mechanism underlying these rare incidents of mostly intracranial venous thromboses associated with thrombocytopenia, which they named vaccine-induced prothrombotic immune thrombocytopenia (VIPIT) and that was renamed recently vaccine-induced immune thrombotic thrombocy-

topenia (VITT) (see section "Note").<sup>7</sup> They were alarmed by an index patient (female) with a splanchnic, followed by a cerebral venous and aortal thrombosis shortly after the first vaccination, and documented a series of eight similar cases (seven females and one male), seven presenting cerebral vein thrombosis and one with pulmonary embolism. All had concomitant thrombocytopenia (13,000–100,000/ $\mu$ L). First symptoms had been observed from 4 to 16 days after vaccination, and four patients died. Sera of four patients were available for further investigations. In all four patients, antibodies directed against platelet factor-4 (PF4)/heparin complexes were found and these sera activated washed test platelets from normal donors weakly in the absence and strongly in the presence of added PF4. Thus, laboratory findings in these rare incidents after Vaxzevria vaccination resembled heparin-induced thrombocytopenia (HIT), a thrombocytopenic prothrombotic disorder caused by the formation of immunoglobulin G (IgG) antibodies against new epitopes exposed after association of heparin or other polyanions with PF4 (CXCL4) secreted from platelets.<sup>8</sup> By their Fc domains, these immune complexes bind to Fc $\gamma$ RIIA on the surface of platelets and thus cross-link these receptors and induce platelet activation.<sup>8,9</sup> Indeed platelet activation by the VITT sera was inhibited by high concentrations of either heparin or IgG shielding Fc $\gamma$ RIIA.<sup>7</sup> Interestingly, direct addition of the AZD1222 vaccine to washed platelets or first pre-incubating the platelets with diluted vaccine and subsequently washing them enhanced platelet reactivity to VITT sera in the presence of PF4. In analogy to heparin, polyanionic deoxyribonucleic acid (DNA) or after cleavage by deoxyribonuclease (DNase), polyanionic DNA fragments or nucleoprotein of AZD1222 might pre-activate platelets, as well as spike protein, if transcribed in excess and binding to angiotensin-converting enzyme 2 (ACE2) on platelets as has been suggested for Sars-Cov-2.<sup>10</sup> Until vaccine virus polymerase chain reaction (PCR) data might become available, it can only be speculated whether this can occur in vivo under rare circumstances, for example, during coincident infection with a wild-type virus substituting cross-functional *E1* gene that is deleted in the vaccinia adenovirus to abrogate its replication.<sup>11</sup> A sensitization of platelets may likewise occur during other forms of coincidental infection or superinfection. Taken together, this possibility would support a concept implicating polyanionic components of AZD1222 as binding partners for PF4 causing the prothrombotic disorder. Based on these striking analogies with HIT, the authors suggested non-heparin anticoagulants and high-dose intravenous immunoglobulin G (IVIG) to treat VITT.<sup>7</sup>

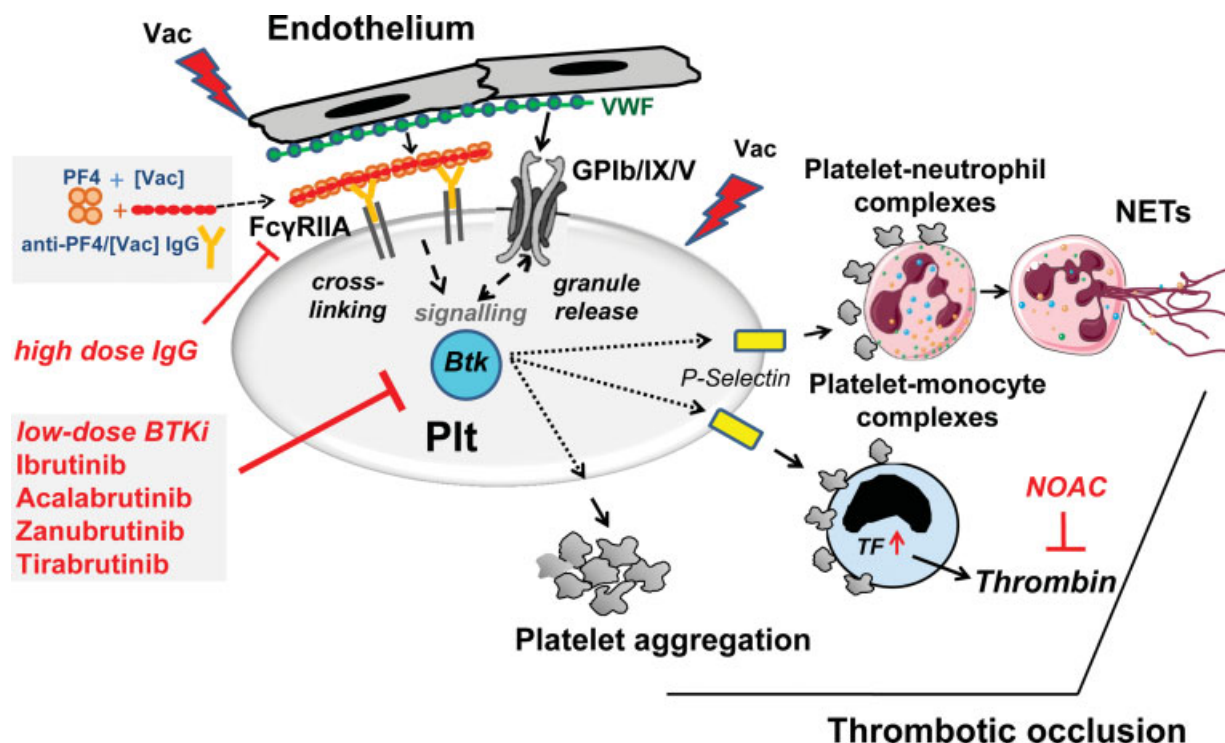
As IVIG might not be available globally for pathogenesis-guided experimental therapy of VITT, we want to draw attention to an additional pharmacologic option that could block fatal platelet activation by the Fc $\gamma$ RIIA pathway apparently operative in VITT: platelet Fc $\gamma$ RIIA stimulation leads to downstream activation of Bruton tyrosine kinase (Btk)<sup>12</sup> as a decisive signalling pathway for subsequent steps of platelet activation.<sup>13</sup> We have recently shown that platelet activation (including aggregation, dense granule secretion and P-selectin expression) and formation of platelet-neutrophil

aggregates stimulated by FcγRIIA cross-linking or sera from HIT patients were completely suppressed by incubating blood with low concentrations of several Btk inhibitors (BTKi) *in vitro*.<sup>13</sup> Approved BTKi are now widely used as standard drugs for the long-term oral therapy of several B cell malignancies with a remarkable safety profile<sup>14</sup> and exerted an apparently protective effect in case of coincident symptomatic COVID-19.<sup>15,16</sup> Of note, the platelet-inhibitory concentrations of the approved BTKi ibrutinib, acalabrutinib, zanubrutinib and tirabrutinib in blood were much lower than the drug levels reached in patients treated with oral standard doses for B cell disorders.<sup>13,17</sup> Furthermore, intake of a single dose of ibrutinib (280 mg) by human healthy volunteers rapidly blocked (3 hours after intake) platelet aggregation and secretion on maximal stimulation of FcγRIIA on platelets in blood *ex vivo*.<sup>13</sup> Stimulus/receptor-selective platelet inhibition was sustained for up to 2 days, which is explained by covalent binding of ibrutinib to Btk and the lack of *de novo* protein synthesis in platelets. Suppression of Btk-mediated platelet activation has previously been shown to be maintained by low ibrutinib dosage (140 mg per day or on alternate days).<sup>18,19</sup>

The pathogenesis of HIT and obviously likewise VITT involves other cells in addition to platelets including monocytes, neutrophils and endothelial cells. BTKi suppressed P-selectin expression on platelets,<sup>13</sup> which is crucially involved in their interaction with monocytes to promote tissue factor expression amplifying thrombin formation.<sup>20,21</sup> BTKi also inhibit FcγRIIA-mediated stimulation of monocytes and

neutrophils.<sup>22</sup> Neutrophil accumulation and neutrophil extracellular trap (NET) release contribute to thrombosis in HIT,<sup>23</sup> and HIT immune complexes induce NET release via interaction with FcγRIIA on neutrophils and through neutrophil-platelet association<sup>24</sup> inhibited by BTKi.<sup>13</sup> Moreover, inhibition of autoreactive B-lymphocytes by BTKi is expected to reduce the production of pathogenic anti-PF4 antibodies.<sup>25</sup> Overall, these findings establish a plethora of deleterious mechanisms beyond platelet activation that could be pleiotropically targeted by BTKi in the pathogenesis of VITT (→ Fig. 1).

Whereas in COVID-19 signs of a general prothrombotic disposition are well documented,<sup>26,27</sup> the uncommon predominant localization of thrombosis in cerebral sinus veins in VITT is puzzling. An additional localizing factor appears to be operative. In SARS-CoV-2 infection, neuro-invasion may occur and spike protein is involved,<sup>28</sup> but CVST has not emerged as a prominent subset in the heterogeneous neurologic manifestations.<sup>29</sup> From fatal cases of VITT, autopsy-based evidence might become available to clarify if and by which mechanisms localized damage to the endothelium of cerebral sinus and splanchnic veins has occurred. Damaged endothelium could then bind circulating free and/or platelet-bound PF4-IgG complexes in VITT-prone patients. PF4 has recently been shown to bind at multiple sites along the surface of extended strings of von Willebrand factor (VWF) released from the endothelium following photochemical injury.<sup>30</sup> The PF4/VWF complexes were recognized by antibodies from HIT patients and promoted platelet adhesion



**Fig. 1** Model for the multiple roles of Bruton tyrosine kinase in the pathomechanisms of vaccine-induced immune thrombotic thrombocytopenia (VITT) and proposed therapeutic interventions. Btk, Bruton tyrosine kinase; BTKi, Bruton tyrosine kinase inhibitors; FcγRIIA, Fcγ fragment of immunoglobulin (IgG) low-affinity IIA receptor; GPIb-V-IX, glycoproteins Ib, V, IX; NETs, neutrophil extracellular traps; NOAC, new oral anticoagulants; PF4, platelet factor-4; Plt, platelet; TF, tissue factor; VWF, von Willebrand factor; Vac, Vaxzevria; [Vac], polyanionic constituents of Vaxzevria.

and thrombus growth under flow. Notably, platelet adhesion to the PF4-VWF-HIT antibody complexes was inhibited by antibodies that blocked not only FcγRIIA but also the GPIb-IX complex on platelets. Intriguingly, VWF stimulation of GPIb also activates Btk,<sup>31</sup> and BTKi reduce VWF-/GPIb-mediated platelet activation in blood (as measured by ristocetin-induced aggregation) and platelet adhesion to VWF surfaces under flow in vitro and ex vivo.<sup>13,18,19,32–34</sup> Furthermore, Btk is critical in mediating platelet C-type lectin-like receptor 2 (CLEC-2) activation by podoplanin.<sup>35</sup> Podoplanin is highly expressed in human thrombosed veins,<sup>36</sup> mice with a deficiency in CLEC-2 are protected against deep vein thrombosis,<sup>37</sup> and CLEC-2 activation by podoplanin contributes to inflammation-driven murine hepatic thrombosis.<sup>38</sup> Recently, low concentrations of ibrutinib and acalabrutinib have been shown to inhibit CLEC-2-stimulated platelet aggregation.<sup>39</sup> Taken together, this suggests additional possibly favourable effects of BTKi on VITT beyond inhibition of FcγRIIA on platelets.

As an alternative strategy, an inhibitor of spleen tyrosine kinase (Syk), which is also activated downstream of platelet FcγRIIA,<sup>40</sup> could be considered. R406, the active metabolite of the Syk inhibitor fostamatinib has been shown to inhibit FcγRIIA-induced platelet activation triggered by heparin-PF4 autoantibodies in vitro,<sup>41</sup> and fostamatinib has been recently approved for the treatment of therapy-resistant immune thrombocytopenia (ITP), an autoimmune disease characterized by autoantibodies against platelet GPIb/GPIX and αIIbβ3 integrin and classically treated with IVIG.<sup>42,43</sup> By inhibiting Fc receptor signalling on macrophages and neutrophils that recognize platelet-bound antibodies, fostamatinib reduces platelet phagocytosis and removal, mainly in the spleen,<sup>33</sup> and reverses thrombocytopenia. However, the therapeutic levels of R406 are apparently too low to directly inhibit platelets ex vivo.<sup>44</sup> Similar observations have been made recently with the new Syk inhibitor entospletinib,<sup>45</sup> at present in phase 3 clinical trials of acute myeloid leukaemia (AML).

In conclusion, several mechanisms amenable to inhibition by approved BTKi are at the centre of HIT and VITT pathophysiology according to the first key observations by Greinacher et al.<sup>7</sup> In addition to ibrutinib, other irreversible BTKi have meanwhile been approved (acalabrutinib, zanubrutinib, tirabrutinib) for the treatment of B cell malignancies.<sup>46–49</sup> Especially with concomitant thrombocytopenia, bleeding risks must be taken into account that might, however, be rather moderate and tolerable: the reversible covalent BTKi rilzabrutinib is currently being tested in clinical trials of ITP patients at doses, which inhibit Btk-mediated platelet activation in blood in vitro (von Hundelshausen et al, unpublished findings), and no bleeding events were reported after completing phase 2, although at study entry patients had only a median platelet count of ~14,000/μL (for reference, please see von Hundelshausen and Siess<sup>17</sup>). A short-term application of approved BTKi in a low Btk-specific dosage that leaves other pathways of haemostatic platelet functions intact<sup>17–19,32</sup> might be regarded as safe enough for a pathophysiology-guided compassionate off-label use in

selected cases of VITT. Although the database is yet limited, this might represent a reasonable paradigm for emergency repurposing of approved drugs in COVID-19.

#### Note

After acceptance of this review, two articles have been published which describe further cases of vaccine-induced thrombotic thrombocytopenia (VITT). See below:

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2. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and thrombocytopenia after ChadOx1 nCoV-19 vaccination. *N Engl J Med* 2021;384(22):2124–2130

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#### Conflict of Interest

None declared.

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