Novel treatments for immune thrombocytopenia

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Available online: Immune thrombocytopenia (ITP) is an autoimmune disease characterized by reduced numbers of platelets which can cause an increased risk of bleeding. In most adults, ITP is typically a chronic condition and often requires treatment. Major bleeding including intracerebral hemorrhage (ICH) is rare and occurs predominantly in patients with platelet counts below $10^9/L$ [1]; however, the bleeding risk rises with increasing age and other comorbidities [2]. Patients with ITP have a 4–5-fold increased risk of death from bleeding or infection [3,4], and quality of life is often reduced.

Summary

Primary immune thrombocytopenia (ITP) is caused by platelet autoantibodies and T-cell dysregulation. Both platelets and their precursor megakaryocytes may be targeted leading to platelet destruction and underproduction. Current treatments for ITP are inadequate since they do not reverse the disease process and generally do not result in durable remissions. In addition, many treatments are limited by side effects including infection and potentially thrombosis. Novel agents that are currently in development target certain key steps in the disease process, including: (1) the interaction between T-cell and antigen presenting cells (CD40–CD154 interaction); (2) the binding of the Fc portion of platelet autoantibodies to Fc-receptors on macrophages (soluble Fc-RIIb); and (3) the signaling pathways leading to platelet phagocytosis by macrophages (Syk inhibition). Other strategies have been to augment platelet production by simulating thrombopoiesis or by neutralizing physiological inhibitors of megakaryopoiesis. Targeted therapies in ITP have the potential to improve disease morbidity and mortality while limiting systemic side effects. Before these agents can be used in practice, additional clinical studies are needed with rational study outcomes including platelet count, bleeding and quality of life. An individualized treatment strategy is needed for patients since ITP is a distinctly heterogeneous disease.
Given the morbidity and mortality associated with ITP, better treatments are needed to achieve and maintain disease control. In this article, we discuss the limitations of current therapies and highlight new treatments for ITP that are currently in development. We begin with a summary of the immune pathways that are disrupted in ITP and how these pathways may be targets for novel therapies (figure 1).

**Overview of pathophysiology of ITP**

Primary ITP represents a spectrum of pathophysiological events, which together result in a reduction in the number of platelets.
of circulating platelets. Additionally, increasing clinical and experimental evidence suggests that ITP results from the loss of self-tolerance for platelet proteins. As a result, platelets and possibly their precursor megakaryocytes are rapidly destroyed by autoantibodies and by cell-mediated toxicity. Experiments from the 1950s implicated a circulating plasma factor as the cause of platelet destruction in ITP and further studies demonstrated a platelet-specific immunoglobulin G (IgG) in many patients [7,8]. More recently, the cellular mechanisms underlying the immune dysregulation in ITP patients have been better defined, including defects in regulatory [9,10] and cytotoxic T-cells [11,12].

**Loss of tolerance to platelet autoantigens**

The normal response to antigens is mediated through helper (CD4+) and cytotoxic (CD8+) T-cells. During maturation in the thymus, T-cells that react strongly with self-antigens are deleted [13] to ensure that immune cells can differentiate between self and non-self. In addition, safeguards are in place in the peripheral circulation to avoid T-cell autoreactivity. For example, for T-cells to become activated, they must be properly stimulated by binding to major histocompatibility complex (MHC) molecules and CD40 on antigen presenting cells [14] via the T-cell receptor (TcR) and CD154 (CD40 ligand) on T-cells [15]. This activation process initiates the humoral immune response. If platelet-autoreactive T-cells are stimulated by this process [16], autoantibodies are produced that react with platelets and/or megakaryocytes. Thus, interruption of the CD40–CD154 interaction is a potential target for therapy in ITP.

**Autoantibodies against platelets and megakaryocytes**

IgG autoantibodies have been identified in many ITP patients, with the most frequent target being platelet glycoproteins (GP) IbβIIa and IbβIIx [17]. Autoantibody-coated platelets bind to Fc-receptors (FcR) on macrophages in reticuloendothelial tissues which leads to phagocytosis and platelet destruction [18,19]. Blocking FcR binding or interrupting the signaling pathways that ultimately lead to phagocytosis are also being explored as potential targets for ITP treatment [20]. Bone marrow megakaryocytes also express platelet proteins; thus, these cells may also be affected by platelet autoantibodies. In vitro experiments using plasma or isolated IgG from ITP patients have demonstrated that some IgG antibodies can bind megakaryocytes and impair their growth and proliferation [17]. Consistent with these observations, in vivo platelet survival studies using autologous radiolabelled platelets have shown that platelet production is often impaired [21]. In recent years, the use of thrombopoietin (TPO) receptor agonists to augment platelet production has been shown to be effective in up to 75% of ITP patients [22] by correcting the relative impairment in platelet production.

**Cytotoxic T-cells against platelets and megakaryocytes**

Although platelet autoantibodies are thought to play an important role in ITP, only about 50–65% of patients have detectable anti-GP IbβIIa or anti-GP IbβIIx autoantibodies on their platelets, and far fewer have autoantibodies detectable in the circulation [23,24]. Other potential mechanisms of thrombocytopenia in ITP include a direct lytic effect of cytotoxic T-lymphocytes on platelets [11] and/or megakaryocytes [25]; and an abnormal effector T-cell response [25,26]. In addition, T-regulatory cells are dysfunctional or reduced in some ITP patients [27], a defect that may be corrected after successful treatment with TPO receptor agonists, rituximab or dexamethasone [9,10,28].

**Current treatment of primary ITP**

ITP represents a heterogeneous spectrum of disease in terms of its presentation and pathophysiology; therefore, patient management must be individualized. A small proportion of patients with stable thrombocytopenia and platelet counts above 50 × 10⁹/L may undergo spontaneous remissions [29]; but for those who require treatment, corticosteroids, intravenous immune globulin (IVIG) and Rh-immune globulin (RhIg) are accepted first-line treatments [30]. Corticosteroids often produce responses that last weeks or months but typically require ongoing drug exposure. Clinical data suggest that 3 to 6 cycles of high-dose dexamethasone administered at 2- to 4-week intervals may be associated with durable responses in some patients [31] and may be more effective than daily prednisone. RhIg is currently used less often due to a black box warning from national health authorities about the possibility of fatal reactions related to intravascular hemolysis.

Second-line treatments include rituximab, splenectomy, TPO receptor agonists [30] and immunosuppressant medications [32]. Of all available medical treatments, only rituximab can achieve durable responses in a significant proportion of patients [33]. In an observational study (n = 77 adults and n = 66 children), 21% of adults and 26% of children with chronic ITP treated with rituximab maintained a response for at least 5 years [34]. In contrast, splenectomy is associated with a higher rate of durable responses (60–70%) that are generally longer-lasting (continuing after up to 12-year follow-up) [35]. TPO receptor agonists are a novel class of medications that increase platelet production through activation of the TPO receptor on megakaryocytes and hematopoietic progenitor cells. TPO is endogenously produced in the liver, but plasma levels are normal or decreased in patients with ITP [36]. The two TPO receptor agonists that are licensed for ITP are romiplostim and eltrombopag. Both agents have been shown to be effective in phase III trials [37,38] and in long-term follow-up studies [39,40]. The risk of thromboembolism with these agents remains uncertain [41].
The limitations of current medical treatments for ITP are their inability to induce sustained platelet count responses, and their associated risks of infection and thrombosis. Novel treatments that are safe and that target specific pathways in the disease process may provide better alternatives.

**Novel agents for the treatment of ITP**

**Therapies targeting the CD40–CD154 interaction**

The initiation of the T-cell dependent B-cell response depends on the intersection between CD40 (on antigen presenting cells) and CD154 (on T-cells). Activated platelets also express CD154 which can directly stimulate B-cells [42] but the function of CD154 on platelet remains uncertain. Blocking the CD40–CD154 interaction on T-cells can interrupt the stimulation of autoreactive T-cells in patients with ITP [43] (figure 1). Blocking antibodies against CD154 have been developed. In a phase I, multicenter, dose-escalating study, 20 patients with ITP with a platelet count less than 50 × 10⁹/L (mean 29 × 10⁹/L) were treated with toralizumab (IDEC-131), a humanized monoclonal anti-CD154 antibody. Of 5 patients who received the highest dose (10 mg/kg), 3 patients achieved a platelet count above 50 × 10⁹/L lasting at least 6 weeks. Side effects included hot flashes, fatigue, dizziness, and gastrointestinal symptoms. There was a significant decrease in anti-GPIIbIIIa antibodies and antibody-producing B-cells and a trend towards decreased GPIIbIIIa-induced T-cell proliferation after treatment [44]. Subsequent open-label studies with toralizumab (n = 31) and ruplizumab (hu5c8; n = 15), another humanized anti-CD154 monoclonal, were done over a decade ago. All patients had ITP for at least 2 months with platelet counts at or below 30 × 10⁹/L and had failed multiple therapies. Of the 31 patients initially treated with toralizumab, one achieved a platelet count above 150 × 10⁹/L and 4 achieved a platelet count above 50 × 10⁹/L. Of 15 patients treated with ruplizumab, 4 achieved a platelet count above 150 × 10⁹/L lasting at least one week and 2 patients achieved a platelet count above 50 × 10⁹/L. Three patients who relapsed were salvaged successfully with toralizumab. The overall response with anti-CD154 mAb was 24% [45].

The occurrence of thromboembolic events in both human and animal models halted further trials with the monoclonal anti-CD154 [44,46]. As CD154 is expressed on the surface of activated platelets, thrombotic events were thought to be mediated by FcR binding on platelets and subsequent platelet activation. Newer monoclonal anti-CD154 antibodies with a modified Fc portion are currently being tested in clinical trials. These molecules may potentially be less thrombogenic because the Fc portion cannot bind or activate platelet FcR [47].

**Therapies targeting FcR binding and signaling**

In patients with ITP, IgG-coated platelets are rapidly cleared via FcR on macrophages [20]. Occupancy of FcR is believed to be one of the main mechanisms of action of Rhlg and IVIG, since Fc fragments isolated from IVIG have a similar effect on increasing platelet counts [48,49] and blocking FcγRIII can reverse ITP in a mouse model [50]. Therefore, interference with FcR binding or its downstream effects are promising targets for ITP therapies. Rozrolimupab is a mixture of 25 recombinant human anti-RhD monoclonal antibodies. The combination of multiple recombinant antibodies may produce a more potent biological effect than human-derived Rhlg while avoiding donor exposures and minimizing the risk of infection [51]. Rozrolimupab was evaluated in a multicenter, phase I/II dose-finding trial of 61 patients with ITP. At the highest dose, 62% of patients achieved a platelet count response and only one patient required rescue treatment. However, hemolytic anemia was a limiting side effect. A fall in hemoglobin of 25 g/L by 7 days occurred in 25% of patients, 4 patients required red blood cell transfusion, and 2 patients had a decrease in hemoglobin level of 30 g/L or more [52].

Crosslinking Fc-receptors on the surface of macrophages induces intracellular signaling via phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs). Downstream recruitment and activation of the protein tyrosine kinase Syk results in cytoskeletal changes and ultimately phagocytosis [53]. Inhibition of Syk decreases the efficiency of phagocytosis [54] and in clinical studies has been shown to improve outcomes in patients with rheumatoid arthritis [53] and ITP. In a pilot open-label, single-arm cohort dose-escalation study, 16 adults with chronic ITP with platelet counts below 30 × 10⁹/L were treated with the oral Syk inhibitor R788 [20]. Of 16 patients, 8 had improved platelet counts over 50 × 10⁹/L and 4 had transient responses. Toxicities occurred in some patients including diarrhea, vomiting, and increased liver enzymes.

Inhibitory Fc gamma receptors, such as FcγRIIB, prevent the consumption of platelets by macrophages. These receptors mediate the protective effect of IVIG [49]. FcγRIIB receptors are also expressed on B-cells and control B-cell activation [55]. A soluble FcγRIIB molecule (SM101) has been developed to compete for pathogenic immune complexes and prevent stimulation of memory B-cells that can lead to pathogenic autoantibody production. This compound was recently evaluated in a randomized, double-blinded, placebo-controlled, dose-escalation phase 1 study of 36 ITP patients. Three patients who received the highest dose achieved sustained platelet count responses over 50 × 10⁹/L [56].

**Therapies targeting platelet production**

New TPO receptor agonists are currently under investigation. Avatrombopag, which may be more potent than eltrombopag, has additive effects when combined with recombinant human TPO [57] and clinical data are promising [58]. Other TPO receptor agonists that have undergone clinical...
development include LGA-4665, NIP-004, NIP-002, and butyrazamide [59].

A different approach to augmenting platelet production is to neutralize physiological inhibitors of megakaryopoiesis. Megakaryocytes and platelets express VPAC1, a stimulatory G-protein coupled receptor. In vivo, pituitary adenyl cyclase-activating peptide (PACAP) and vasoactive intestinal peptide (VIP) bind to this receptor to inhibit megakaryocyte growth and differentiation. Blocking this interaction may improve thrombopoiesis. The anti-VPAC1 monoclonal IgG1 antibody has been shown to stimulate thrombopoiesis in murine models of both immune and non-immune thrombocytopenia [60].

Amifostine is another molecule that has been found to improve thrombopoiesis. It is a cytoprotective agent that also has the effect of increasing hematopoiesis by mechanisms that remain unclear [61]. In one ITP study, all 24 amifostine-treated patients responded and 22 of 24 achieved a platelet count above $100 \times 10^9/L$ [62]. In another study, amifostine was associated

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### Table I

#### Novel treatments for immune thrombocytopenia (ITP)

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Mechanism of action</th>
<th>Efficacy</th>
<th>Adverse effects</th>
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<tr>
<td><strong>Therapies targeting T-cell co-stimulation</strong></td>
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<tr>
<td>Toralizumab (IDEC-151) [44]</td>
<td>Humanized anti-CD154 Antibody</td>
<td>Toralizumab (phase I): 20 patients with ITP, 3 of 5 with highest dose had sustained response</td>
<td>Thromboembolic events [49,51]</td>
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<td>Ruplizumab (huSc8) [45]</td>
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<td>Combined analysis of phase I/II studies of toralizumab and ruplizumab showed 24% overall response</td>
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<td><strong>Therapies targeting FcR binding and signaling</strong></td>
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<td>GMA161 [50]</td>
<td>Humanized anti-human FcγRIII antibody</td>
<td>Efficacy in transgenic murine model of ITP</td>
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<tr>
<td>Rzrolimupab [52]</td>
<td>25 recombinant human anti-RhD monoclonal antibodies</td>
<td>Multicenter phase I/II dose-escalation study showed 62% overall response in the group who received the highest dose</td>
<td>Hemolysis</td>
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<td>SM101 [56]</td>
<td>Soluble FcγRIIB</td>
<td>Dose-escalation part of a phase Ib, randomized clinical trial in ITP: 0–25% required rescue mediation in the highest dose group compared with 42% on placebo. Sustained responses ($&gt;50 \times 10^9/L$) were observed after one cycle</td>
<td>No dose limiting toxicity or serious adverse events observed</td>
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<td>R788 [20]</td>
<td>Syk inhibitor</td>
<td>Open-label, single-arm cohort dose-escalation study: 8/16 patients, had a significant response and 4 had transient responses</td>
<td>Mild adverse effects–gastrointestinal symptoms and increased liver enzymes</td>
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<td><strong>Therapies targeting platelet production</strong></td>
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<tr>
<td>Avatrombopag (ESS01) [58]</td>
<td>Small molecule TPO receptor agonist</td>
<td>Multicenter, randomized, double-blind, placebo-controlled phase II study: 52.8% response rate</td>
<td>Mostly mild adverse effects - fatigue, headache, epistaxis</td>
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<td>23A11 [60]</td>
<td>Murine anti-VPAC1 monoclonal IgG1 antibody, improves thrombopoiesis</td>
<td>Efficacy in murine models of ITP</td>
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<tr>
<td>Amifostine</td>
<td>Improve thrombopoiesis</td>
<td>In one study, all 24 patients showed a response and 22/24 achieved platelet counts $&gt; 100 \times 10^9/L$ [62]. In another study, all 17 patients had achieved a platelet count response that persisted for 2 months after discontinuation [63]</td>
<td>Dizziness, nausea, vomiting, fatigue, and mild hypocalcemia</td>
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with a normalization of the platelet count in 17 ITP patients. Responses persisted for 2 months after the medication was discontinued [63]. Novel treatments are shown in table 1.

**Future directions**

**Targeting the neonatal FcR**

Reducing the half-life of pathologic IgG is another potential method for treating autoimmune disease. The function of the neonatal FcR (FcRn) is to recycle IgG, thus effectively increasing its half-life [64]. Blocking FcRn may increase clearance of the pathogenic (and normal) IgG. In a murine model of myasthenia gravis, anti-FcRn mAb reduced the serum IgG concentration by approximately 40% and improved disease manifestations [65].

**Interference with TNF signaling**

TNF neutralizing therapies such as etanercept and infliximab have improved the treatment of certain autoimmune disorders [66,67]. Etanercept has been effective in a small number of ITP patients [68]. Recent discoveries of other ligands in the TNF family such as BAFF (B-cell activating factor belonging to the TNF family) and APRIL (a proliferation-inducing ligand) may also be promising for novel therapeutic targets. BAFF and APRIL are secreted by monocytes, dendritic cells, macrophages, and T-cells in response to inflammatory mediators [69]. They are required for B-cell survival during maturation in the spleen, and excess BAFF levels may lead to survival of autoreactive B-cells, autoantibody production, and development of autoimmune disease [70–72]. Studies of a humanized mAb that blocks BAFF have been effective in rheumatoid arthritis and systemic lupus erythematosus (SLE) [73,74]. Belimumab, a humanized mAb that blocks soluble BAFF, is now licensed for the treatment of SLE in several countries [75]; however, only a modest response has been reported for hematological manifestations of SLE [76]. Atacicept, a humanized fusion protein that binds both BAFF and APRIL, has had less success [74,77]. The utility of these agents in ITP is not yet known.

**Induction of immune tolerance**

The mechanisms of antibody response to autoantigens could reveal new targets for therapy. IgG responses that are driven primarily by CD4+ helper T-cells are associated with an anamnestic response to platelet autoantigens and loss of peripheral T-cell tolerance [78,79]. T-cells recognize short peptide antigens displayed on MHC class II molecules by antigen presenting cells [14] and subsequently stimulate B-cells to produce IgG [80,81]. Re-induction of tolerance can be achieved by exposing T-cells to soluble synthetic peptides containing MHC class II restricted epitopes, which can lead to the expansion of regulatory T-cells and deletion of antigen-specific memory cells [82].

Sukati et al. successfully mapped epitopes of GPIIIa that could stimulate T-cells from 31 ITP patients by synthesizing a panel of 86 overlapping peptides of GPIIIa [83]. While there was variability in the pattern of stimulation by peptides within and between patients, dominant sequences that induced proliferation were identified. Many of these sequences were predicted to have low affinity for restricting MHC molecules, suggesting that an inefficient presentation of self-peptides rather than antigen specificity may cause loss of tolerance [84,85]. These peptides may eventually form the basis of vaccine therapy in ITP [83].

Treatment with TPO receptor agonists may re-induce tolerance by increasing exposure to platelet antigens. In a retrospective study of 31 chronic ITP patients treated with either romiplostim or eltrombopag, Ghadaki et al. observed 9 patients who achieved a durable platelet count response after discontinuing the medication, including 3 patients who met all criteria for a TPO receptor agonist-induced remission. A progressive decline in platelet autoantibody titre was documented in one patient with ongoing exposure to the drug [86]. Another case series showed similar findings [87]. Durable remission after exposure to TPO receptor agonists may also be explained by improved regulatory T-cell function [28].

**Conclusion**

ITP is a heterogeneous disorder that is often associated with relapses and refractoriness to multiple therapies. Because of this, an individualized rather than an empiric approach to management may be beneficial. Given the significant morbidity associated with over-treatment of the disease, advancements in the management of ITP are necessary to improve the health and quality of life of patients. Novel agents that target specific steps in the mechanistic pathways may lead to more definitive treatments.

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