Received: 10 December 2020 | Revised: 19 February 2021 | Accepted: 26 February 2021

DOI: 10.1002/rth2.12506

rpth research & practice in thrombosis & haemostasis

STATE OF THE ART ISTH 2020

GPIbα is the driving force of hepatic thrombopoietin generation

Danielle Karakas BSc[1,2,3] | Miao Xu MD, PhD[4] | Heyu Ni MD, PhD[1,2,3,5,6,7]

[1]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

[2]Toronto Platelet Immunobiology Group, Toronto, ON, Canada

[3]Department of Laboratory Medicine, Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Toronto, ON, Canada

[4]Department of Hematology, Qilu Hospital, Cheeloo College of Medicine, Shandong University, Jinan, China

[5]Canadian Blood Services Centre for Innovation, Toronto, ON, Canada

[6]Department of Medicine, University of Toronto, Toronto, ON, Canada

[7]Department of Physiology, University of Toronto, Toronto, ON, Canada

Correspondence
Heyu Ni, Canadian Blood Services Centre for Innovation and Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital, University of Toronto, Room 421, LKSKI Keenan Research Center, 209 Victoria Street, Toronto, ON M5B 1W8, Canada.
Email: Heyu.Ni@unityhealth.to

Funding information
This work was supported in part by Canadian Institutes of Health Research Foundation grant (389035), Canadian Institutes of Health Research (CIHR Projects: MOP 119540, MOP 97918, MOP 68986 and MOP 119551), CIHR-Canadian Blood Services Partnership, grant-in-aid from the Heart and Stroke Foundation of Canada (Ontario), and the Canadian Foundation for Innovation

Handling Editor: Prof. Yotis Senis

Abstract

Thrombopoietin (TPO), a glycoprotein hormone produced predominantly in the liver, plays important roles in the hematopoietic stem cell (HSC) niche, and is essential for megakaryopoiesis and platelet generation. Long-standing understanding proposes that TPO is constitutively produced by hepatocytes, and levels are fine-tuned through platelet and megakaryocyte internalization/degradation via the c-Mpl receptor. However, in immune thrombocytopenia (ITP) and several other diseases, TPO levels are inconsistent with this theory. Recent studies showed that platelets, besides their TPO clearance, can induce TPO production in the liver. Our group also accidentally discovered that platelet glycoprotein (GP) Ibα is required for platelet-mediated TPO generation, which is underscored in both GPIbα$^{-/-}$ mice and patients with Bernard-Soulier syndrome. This review will introduce platelet versatilities and several new findings in hemostasis and platelet consumption but focus on its roles in TPO regulation. The implications of these new discoveries in hematopoiesis and the HSC niche, particularly in ITP, will be discussed.

KEYWORDS
Platelet, GPIbα and GPIIbIIIa, Thrombopoietin, Thrombocytopenia, Thrombosis, Antibodies

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2021 The Authors. *Research and Practice in Thrombosis and Haemostasis* published by Wiley Periodicals LLC on behalf of International Society on Thrombosis and Haemostasis (ISTH).

Essentials

- Thrombopoietin (TPO) regulates megakaryocyte differentiation and thus platelet production.
- Prevailing theory posits that TPO is constitutively produced and levels fine-tuned by platelet uptake.
- We identified that platelet glycoprotein (GP) Ibα drives liver TPO generation.
- This discovery has broad implications in liver biology, hematopoiesis, and thrombocytopenia.

1 | INTRODUCTION

Platelets, first identified 130 years ago, are small (1–2 μm) anuclear cells in the blood.[1,2] They are the second most abundant cells in circulation next to red blood cells, with an average range of 100–450 × 10^9/L in healthy adults. Platelets are produced from their precursor megakaryocytes in the bone marrow,[3,4] where immature larger proplatelets from megakaryocyte extensions are released into the blood due to local shear stresses, with each megakaryocyte generating an estimate of thousands of platelets.[5,6] Interestingly, recent studies suggest that platelets can also be produced from megakaryocytes in the lungs,[7,8] although the proportion of lung-derived platelets in blood and whether these platelets have unique functions, such as immune response in respiratory system infection,[9] remain to be further investigated.

Increasingly recognized for their versatility, platelets have implications in the immune system,[2,10,11] angiogenesis and liver regeneration,[2,12] and tumor metastasis,[13] among others, in addition to hemostasis.[14,15] At the site of vascular injury, subendothelial matrix proteins, such as collagen, are exposed, which is subsequently anchored with von Willebrand factor (VWF) and allows for glycoprotein (GP) Ibα interaction, mediating platelet tethering, particularly at high shear rates.[16-18] The transient VWF-GPIbα interactions slow platelets down, allowing for GPVI-collagen interactions for further platelet activation.[19] The activated platelets then express active conformations of integrins such as αIIbβ3 (GPIIbIIIa). Through interaction with ligands on vessel walls, these integrins can mediate firm platelet adhesion.[20-22] Following the first layer of adhesion, aggregation between adjacent platelets can occur through binding of fibrinogen to activated αIIbβ3.[22,23] Very interestingly, although the theory that fibrinogen is required for platelet aggregation has been prevalent for more than half a century, we found that platelet aggregation still occurs in mice lacking fibrinogen, and even more surprisingly in fibrinogen and VWF double-deficient, and fibrinogen/VWF/plasma fibronectin triple-deficient,[24,25] but not β3-deficient mice.[26] This clearly demonstrated the presence of a fibrinogen-independent pathway in platelet aggregation.[18,27-30] In addition to platelet adhesion and aggregation (ie, platelet accumulation—the first wave of hemostasis), activated platelets also express phosphatidylserine and negatively charged surfaces,[31,32] which markedly facilitates thrombin generation and blood coagulation (ie, the second wave of hemostasis). Furthermore, platelets may contribute to a newly termed "protein wave of hemostasis" through release of their α-granule components such as fibronectin.[27] Although clearance of these aggregated platelets/thrombi (platelet consumption) are not fully understood, macrophage and reticuloendothelial system (RES), as demonstrated in immune-mediated thrombocytopenia, are likely the important contributors.[33-35]

Platelet generation and clearance must be well controlled to avoid thrombosis and bleeding disorders. First described in 1958 as a humoral factor promoting platelet production, thrombopoietin (TPO) is the physiological regulator of megakaryocyte differentiation,[36-38] and is crucial for maintenance of the hematopoietic stem cell (HSC) niche. TPO is a glycoprotein hormone produced primarily by the liver (>65% of circulating levels).[39] Adequate circulating TPO levels are important for the maintenance of hemostasis; however, the mechanism of regulation in its entirety remains unclear. This review will discuss recent discoveries pertaining to TPO regulation and their respective implications in hematopoiesis as well as immune-mediated thrombocytopenias such as immune thrombocytopenia (ITP).

2 | PREVAILING VIEWS OF TPO REGULATION

Major achievement in understanding the mechanisms regulating platelet production (thrombogenesis) was made following the identification of the cognate receptor[40] c-Mpl and subsequent cloning of TPO.[41] In thrombopoiesis, TPO binds to c-Mpl expressed on megakaryocytes and its HSC progenitors to stimulate their expansion and differentiation.[4,42-44] However, TPO is not required for megakaryocyte platelet shedding or proplatelet production.[45,46] In addition to its crucial role in thrombopoiesis,[47,48] TPO is required for the maintenance of the hematopoietic stem cell (HSC) niche.[49,50] Both $TPO^{-/-}$ and $Mpl^{-/-}$ mice have decreased numbers of erythroid and myeloid progenitors,[47] and loss of TPO signaling is associated with bone marrow failure and thrombocytopenia, as TPO supports HSC quiescence during adult hematopoiesis.[51,52] TPO can be expressed by multiple cell types such as osteoblasts, megakaryocytes, and stromal cells, and has recently been found in human and rat brain,[53] with an interesting observed protective effect against apoptosis in neural and endothelial cells.[54] However, contributions from hepatocytes above all are specifically critical for HSC maintenance and quiescence.[55]

The regulation of circulating steady-state TPO levels has long been thought to be constitutively produced by the liver[39] and regulated through platelet/megakaryocyte internalization and degradation via the c-Mpl receptor.[37] TPO in circulation binds the extracellular portion of predimerized c-Mpl, likely resulting in a change

of dimer arrangement equilibrium, activating signaling cascades within the cell and thus receptor phosphorylation.[56] Phosphorylated c-Mpl is then internalized and undergoes proteasomal, via c-Mpl ubiquitination, or lysosomal degradation within the cell,[57,58] contributing to the downregulation of circulating TPO levels. Studies have shown that reappearance of cell surface c-Mpl is due to recycling of the internalized receptor rather than receptor reserve intracellular storage,[56,59] allowing for further TPO internalization. In support of constitutive theory, TPO levels have been shown to be elevated in patients with congenital amegakaryocytic thrombocytopenia,[60] thrombocytopenia-absent radius,[61] and acquired aplastic anemia.[62] Moreover, mice with hemizygous deletion of the TPO gene showed no compensatory response from the wild-type (WT) locus. In each of these cases, TPO levels are inversely correlated to platelet counts, suggesting that TPO levels are dependent on or at least downregulated by platelet and megakaryocyte mass.

3 | PLATELET AND MEGAKARYOCYTE CLEARANCE: INADEQUATE UNDERSTANDING OF TPO REGULATION IN ITP

As it was once thought that platelet/megakaryocyte mass solely regulates circulating TPO levels,[37,39] it therefore follows that regulation of platelet clearance is imperative for homeostatic maintenance. In a healthy adult, ≈10^{11} platelets are produced and removed per day, with the spleen and liver as key organs mediating the mechanisms of platelet clearance.[33,35,63,64] However, environmental or genetic factors can lead to dysfunctional platelet production and clearance, causing thrombocytopenia.[65] Inherited thrombocytopenias include genetic defects leading to abnormal platelet size and function and/or impaired platelet production (ie, micro-, normo-, macrothrombocytopenia),[66] and malignancy-associated thrombocytopenias arise due to underlying chronic conditions (ie, cancer).[67] ITP arises from immune destruction of one's own platelets (autoimmune)[33,63,65] or response to allogenic platelet antigens (alloimmune) as seen in fetal and neonatal alloimmune thrombocytopenia (FNAIT) or posttransfusion purpura (PTP).[68-74]

The spleen is a major site for immune-mediated platelet clearance, including primary or secondary ITP, which can occur after infection or drug use.[35,65] IgG autoantibodies are thought to be the predominant mediators of platelet destruction in the spleen[35,75] and have been found in the bone marrow of patients with ITP, likely interfering with megakaryopoiesis and thrombopoiesis.[76-78] Cytotoxic T cells (CTLs) and the complement system may also play a role in ITP but CTLs may more effectively decrease platelets via targeting megakaryocytes in the bone marrow.[79-81] Recently, anti-αvβ3 autoantibodies, as seen in chronic ITP, have shown to have a selective inhibitory impact on megakaryocyte migration,[82] contributing to a decreased rate of thrombopoiesis in addition to platelet destruction. Glycoprotein Ib-IX (GPIb-IX) complex and GPIIbIIIa (αIIbβ3), the platelet receptors for VWF and fibrinogen, are the two most abundantly expressed platelet surface receptors and the most common autoantibody targets seen in ITP.[33] Following opsonization, platelet destruction was thought to occur in the RES (eg, spleen) through binding of the Fc portion of antibodies to FcγRIIa and FcγRIIIa on macrophages.[34,35,83,84]

Interestingly, however, we and our colleagues found that anti-GPIbα-mediated ITP may be different from those with anti–GPIIbIIIa-mediated ITP,[85-88] and further reported that anti-GPIbα and some anti-GPIIbIIIa antibodies may cause platelet downstream signaling, granule neuraminidase surface translocation and enzymatic removal of terminal surface sialic acids.[63,89,90] In addition to Fc—Fc receptor interactions, we found that anti-GPIbα antibodies can induce platelet activation in an Fc-independent manner by intracellular signaling cascades, which is similar to that induced by VWF binding.[63,91] Certain patient anti-GPIIbIIIa antibodies were also observed to cause platelet activation and desialylation, likely attributable to the binding of the Fc portion to FcγRIIa on the platelets and/or the adjacent platelets, and signaling through its tyrosine-based activation motif.[92] Anti-GPIbα-antibody binding, subsequent activation and desialylation lead to platelet clearance via non-Fc receptors in the liver, of which the Ashwell-Morell receptor (AMR) is a contributor.[63] More recently, it has become apparent that antibody epitope and binding affinity appears to be critical in anti-GPIbα antibody-mediated platelet clearance. Certain antibodies targeting non–N-terminus regions such as the mechanosensory domain (MSD), which cause receptor clustering, fail to induce platelet activation and Fc-independent platelet clearance.[93] However, it was reconciled that higher-affinity antibodies can exert a pulling force under shear, causing unfolding of the MSD domain, and are therefore more likely to cause GPIbα-mediated Fc-independent hepatic clearance.[94,95] Moreover, it is unclear whether sialic acid content influences autoantibody epitope or binding affinity and therefore subsequent mechanisms of clearance, and thus requires further study.

Recent studies have highlighted the importance of platelet glycoprotein modifications in liver-mediated platelet clearance.[63,96,97] Platelet glycoproteins are decorated by complex carbohydrate N-linked glycans (N-glycans) and mucin-type O-linked glycans (O-glycans) that are "capped" by sialic acids. Platelets are a source of endogenous neuraminidase,[63,96] which upon stimulation by cold storage, sepsis, or senescence, for example,[98] cause desialylation and subsequent rapid platelet clearance. Moreover, St3gal4$^{-/-}$ mice lacking the α2,3-sialyltransferase IV (ST3GalIV) exhibit rapid platelet clearance and thrombocytopenia.[96] The AMR, a multimeric endocytic receptor found on hepatocytes and more recently Kupffer cells,[99] binds to terminal galactose (Gal) or N-acetylgalactosamine (GalNAc) of glycans[100] and is reported to be a major contributor in the rapid clearance of desialylated platelets. The AMR exhibits higher affinity and preference for tetra- or triantennary ligands, as seen on N-glycans.[96] However, it was recently found that desialylation of O-glycans on GPIbα drive receptor signaling and platelet clearance through surface expression of neuraminidase, which is postulated to then desialylate platelet N-glycans, allowing for the possibility

of AMR-mediated clearance.[96,101] Thus, the AMR is involved in the clearance of platelets in senescence, sepsis, or cold storage.

Alongside the AMR, Kupffer cells have become increasingly recognized for their requirement in platelet clearance. Upon Kupffer cell depletion, aged platelets were reported to accumulate, and bleeding was increased due to impaired hemostatic ability of aged platelets.[97,99,102] αMβ2/Mac-1 on Kupffer cells was reported to interact with clustered GPIb to rapidly clear cold-stored platelets upon their transfusion.[102] It was also shown that hepatocyte microvilli can be found in the liver lumen,[103] and proposed that desialylated platelets interact with the AMR on hepatocyte protrusions, to be captured and cleared by Kupffer cell C-type lectin domain family 4 member F.[97] Others alternatively propose that such hepatocyte microvilli interactions are fleeting and rare and that platelet clearance occurs by direct Kupffer cell interactions.[99] It has been shown that while AMR deficiency on Kupffer cells alone has little impact on platelet clearance, macrophage galactose lectin is required in tandem with the AMR for sufficient senescent platelet phagocytosis.

While it has become evident that Kupffer cells play an important role in the clearance of aged platelets, the effects of immune-mediated thrombocytopenia and the site of platelet or megakaryocyte clearance (ie, spleen vs liver or bone marrow) on TPO levels in blood or the local environment have not been adequately addressed and require future investigation.

4 | PLATELET-MEDIATED LIVER TPO GENERATION AS AN EMERGING MECHANISM OF REGULATION

In line with the prevailing theory of constitutive liver expression regulated by c-Mpl internalization, TPO levels should be inversely proportional to platelet/megakaryocyte mass. However, patients with ITP exhibit TPO levels lower than expected with no significant difference from healthy controls,[104] and patients with essential thrombocythemia typically have unexpectedly elevated TPO levels.[105] Interestingly, hepatic TPO synthesis has also been shown to be elevated in times of acute inflammatory response[106] due to interleukin (IL)-6 secretion from Kupffer cells.[107] Moreover, in addition to the liver, reports showed that marrow stromal cells produce TPO in response to thrombocytopenia in both mice and humans.[108,109] This suggests platelet/megakaryocyte mass is not exclusively responsible for maintenance of TPO levels, and more complex mechanisms of TPO regulation exist.

It was reported that aged platelets with reduced sialic content on their glycoproteins stimulate TPO synthesis through the hepatic AMR by Janus kinase 2—signal transducer and activator of transcription proteins 3 signaling.[64] However, there was no significant difference of plasma TPO concentrations between the WT and AMR$^{-/-}$ mice, suggesting that the AMR may not be the sole receptor responsible for platelet-mediated TPO generation.[64] Additionally, it has been shown that serotonin (5-hydroxytryptamine [5-HT]), a neurotransmitter known to largely be stored in platelet-dense granules and released upon activation, promotes megakaryocyte proplatelet formation in vitro, and inhibition of 5-HT causes markedly reduced TPO levels in vivo.[110] These data suggest that 5-HT may increase platelet number and subsequent opportunity for platelet-mediated TPO generation. However, the extent of contribution from this mechanism to platelet-mediated TPO generation is unclear. While it was previously shown that 5-HT does not induce TPO generation but proliferation of cultured hepatocytes,[111] whether varying 5-HT levels can increase platelet counts globally in their in vivo models requires future investigation.

5 | PLATELET GPIbα IS REQUIRED FOR PLATELET-MEDIATED HEPATIC TPO GENERATION

In 2015 and 2018, we reported the previously unknown link between platelet GPIbα and circulating TPO levels in mice and humans.[112,113]

GPIbα$^{-/-}$ mice, like patients with Bernard-Soulier syndrome (BSS), are macrothrombocytopenic. As existing theory stated that TPO levels inversely correlate to platelet/megakaryocyte mass, we expected GPIbα-deficient mice to have increased TPO levels. Unexpectedly, we observed approximately a twofold decrease in the sera and plasma TPO levels of GPIbα$^{-/-}$ compared to WT littermates and β3$^{-/-}$ mice. The decreased TPO levels were also seen in patients with BSS, demonstrating that GPIbα deficiency may lead to lower circulating TPO levels.

Since GPIbα$^{-/-}$ platelets are larger, it was plausible that decreased TPO levels could be due to increased c-Mpl number on platelet surface and thus enhanced clearance of circulating TPO. While more c-Mpl was found on GPIbα$^{-/-}$ platelets, this is counteracted by a significant decrease in platelet number in GPIbα$^{-/-}$ mice, resulting in an overall net similar c-Mpl number to WT mice. Thus, the observed TPO levels are not due to enhanced clearance by the GPIbα$^{-/-}$ mice. Moreover, we measured whether GPIbα$^{-/-}$ platelets could functionally internalize more TPO compared to WT, but found no significant difference, therefore demonstrating that lower TPO levels in GPIbα$^{-/-}$ mice are not due to enhanced TPO clearance.

To determine whether GPIbα$^{-/-}$ mice possessed lower hepatic constitutive production or a disruption in platelet-mediated TPO synthesis, we assessed whether WT platelets transfused to GPIbα$^{-/-}$ mice could rescue TPO levels, and found that WT but not GPIbα$^{-/-}$ platelets significantly raised TPO from baseline. Hepatic TPO mRNA also consistently increased upon the transfusion of WT but not GPIbα$^{-/-}$ platelets. We also confirmed that there was no difference in IL-6 levels, a proinflammatory cytokine previously shown to induce TPO synthesis.[106] Thus, both mechanisms of regulation, including TPO internalization/degradation by the c-Mpl receptor on platelets/megakaryocytes and platelet-induced production by the liver, occur simultaneously, and that platelet GPIbα contributes significantly to the de novo synthesis of hepatic TPO and thus circulating TPO levels.

A previous elegant study identified that desialylated platelets drive hepatic TPO generation through the AMR.[64] Interestingly, however, we observed that desialylation and asialofetuin, an asialoglycoprotein receptor blocker, did not significantly alter the inability GPIbα-deficient platelets to induce hepatic TPO generation in vitro.[113] It is also important to note that GPIbα$^{-/-}$ platelets display increased desialylation due to their larger size compared to WT and their compensative glycosylation mechanism. One potential explanation is that desialylated GPIbα is the binding partner of the AMR, which is plausible considering that GPIbα is the most heavily glycosylated platelet surface antigen, containing ≈60% of total platelet sialic acid content.[114] Moreover, a number of groups converge on the finding that the AMR is required for platelet clearance,[97,99,100,102] further consolidating its interaction with platelets. However, increased binding to hepatocytes upon GPIbα$^{-/-}$ platelet desialylation was observed, suggesting that the AMR may not exclusively bind GPIbα.[113] Additionally, as there are no N-glycans on murine GPIbα, it is unlikely that the AMR is a significant binding partner. This is confirmed by the finding that desialylated GPIbα epitopes do not significantly bind the AMR, and desialylation was found to be dispensable in our studies.[113] Altogether, this evidence supports that GPIbα-mediated TPO generation may occur through an AMR-independent pathway. While one explanation has suggested that glycans on GPIbα are needed for rapid AMR-dependent clearance, and in the absence of GPIbα, other platelet glycoproteins act as counterreceptors for the AMR, Aspgr1$^{-/-}$ mice do not display significantly lower TPO levels,[64] highlighting that GPIbα is indispensable for this process, whereas desialylation only enhances the process.

To test which portion of GPIbα is required between the ligand-binding domain or cytoplasmic portion, we examined TPO levels in IL4Rα/GPIbα transgenic (tg) mice where the extracellular portion of GPIbα is replaced by the α subunit of the IL-4 receptor, maintaining platelet intracellular integrity. Consistent with our GPIbα$^{-/-}$ model, IL4Rα/GPIbα-tg mice also had lower plasma and serum TPO levels. Furthermore, we coated recombinant murine GPIbα onto platelet-sized silica beads and found that these coupled beads were able to stimulate hepatic TPO generation in vitro, signifying that the extracellular domain of GPIbα but not other platelet components is required for the pathway of TPO production. However, the hepatocellular signaling pathways leading to TPO synthesis following GPIbα stimulation are currently unclear and require further investigation (Figure 1).

This finding may present previously unknown perspectives in the understanding of TPO regulation in disorders such as ITP, where patients exhibit lower TPO levels than expected in proportion to their platelet/megakaryocyte mass. In these patients, it has been speculated that platelet GPIbα may be unable to stimulate hepatic TPO generation due to antibody blocking contact with the potential GPIbα cognate receptor(s). This theory was recapitulated in our studies as we reported that platelets opsonized with anti-GPIbα antibodies were unable to stimulate hepatic TPO generation likely attributable to antibody blocking. This was observed with both polyclonal anti-GPIbα antibodies[31] and monoclonal antibody NIT G against the GPIbα N-terminus. However, conflicting data reported hepatic TPO mRNA to be upregulated following the in vivo immunization of high-dose anti-GPIbα antibody but unaltered following low-dose immunization with the same anti-GPIbα antibody.[115] Antibody binding to GPIbα that does not hinder the N-terminus can likely still allow for stimulation of the GPIbα hepatic cognate receptor for TPO generation if platelets are localized to the liver for clearance. It cannot be excluded that platelet activation, complement activation, or mechanosensory signaling mediated by epitope specificity, binding affinity, and/or antibody titers determines whether Fc-independent clearance occurs, thus diverting platelets away from the liver, disallowing for platelet-mediated TPO generation.

Another report showed that ITP patients with anti-GPIb autoantibodies had no difference in circulating TPO levels compared to patients with anti-GPV or anti-GPIIb/IIIa autoantibodies.[116] This can be attributed to differences that epitope, such as N-terminus blocking, and/or binding affinity may cause on platelet clearance and subsequent TPO generation. Moreover, platelet counts, which control TPO clearance, were not included and normalized in this study. If platelet counts drastically vary between patient cohorts the extent of circulating TPO internalization will thus differ and skew the analysis of TPO levels across the patient groups. Therefore, platelet counts should be included in prospective clinical studies to provide normalization in accurately determining the difference in circulating TPO levels across patients with various platelet autoantibodies.

In addition to thrombopoiesis, TPO is required for hematopoiesis and HSC niche maintenance,[52] and thus, adequate circulating levels are imperative. Studies have shown abnormal megakaryocyte membrane demarcation system (MDS) morphology, decreased platelet release, and impaired megakaryocyte localization in GPIbα deficient mice.[117,118] Moreover, anti-GPIbα antibodies suppress megakaryocyte growth,[78] and cause reduced GPIbα surface expression on generated platelets,[115] likely decreasing subsequent TPO generation and thus further impairing megakaryopoiesis. However, whether decreased circulating TPO levels due to GPIbα deficiency significantly impairs hematopoiesis and the HSC niche in patients with ITP or BSS, for example, remains unknown and warrants further study.

6 | ISTH 2020 REPORT

Abstracts presented at ISTH 2020 present novel insights toward the importance of understanding homeostatic regulation of circulating TPO levels.[119-121] Platelet shedding, the final step in megakaryopoiesis, occurs in the vascular niche, where damage is well documented in ITP patients.[122,123] However, it is unclear if this is due to circulating autoantibodies or chronically lower TPO levels that decrease megakaryopoiesis, or both. An ISTH Congress 2020 abstract showed that TPO has a protective effect on endothelial

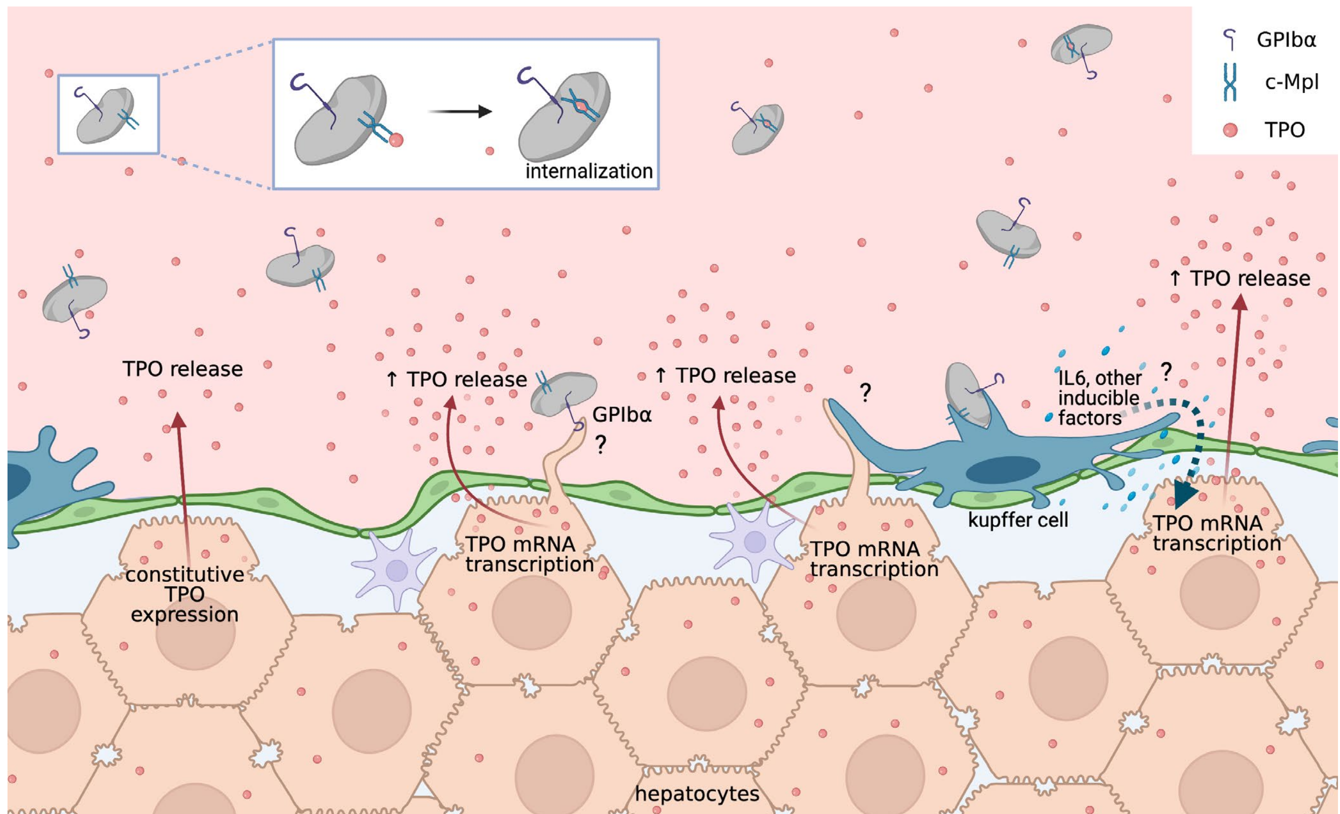

FIGURE 1 Glycoprotein Ibα (GPIbα) is the driving force of hepatic thrombopoietin (TPO) generation. TPO is constitutively produced by liver hepatocytes and internalized and degraded by platelet and megakaryocytes via the c-Mpl receptor. Although the detailed mechanism is currently unknown, GPIbα is required for platelet-mediated TPO generation. The interactions between platelet GPIbα and hepatocyte protrusions and Kupffer cells may contribute to this process. Following phagocytosis of GPIbα-positive platelets, Kupffer cells may interact with hepatocyte protrusions or release of soluble factors, such as interleukin-6 that directly or indirectly stimulate TPO generation

progenitor cells (EPCs) from patients with ITP, promoting their tubal formation and migration capacity compared to untreated ITP EPCs in vitro.[119,120] It is well accepted that a healthy bone marrow microenvironment is conducive for megakaryopoiesis, which occurs through direct interaction of TPO and megakaryocyte c-Mpl receptor. Interestingly, this abstract uncovers a potential role of TPO in promoting the bone marrow microenvironment, thereby introducing a mechanism in which TPO indirectly mediates megakaryopoiesis. Moreover, these data consolidate the notion that low platelet counts in ITP are not solely due to increased platelet destruction by autoantibodies against platelet surface glycoproteins like GPIbα, but also faulty megakaryopoiesis due to decreased GPIbα-mediated TPO generation. Future studies are needed to determine the impact of low circulating TPO levels on the bone marrow microenvironment, and therefore megakaryopoiesis, in vivo.

Furthermore, our group presented an abstract detailing a novel method for the detection of ITP antibodies. The current gold standard is the monoclonal antibody immobilization of platelet antigens (MAIPA) assay. However, MAIPA requires fresh donor blood and patient platelets, and the assay takes 3 to 4 days to perform and is prone to false negatives/positives, making it less convenient for clinicians in their treatment. Our group has developed a novel flow cytometry–based assay to detect patient antiplatelet autoantibodies, in which GPIIbIIIa or GPIbα was oriented upward-coupled to antifouling organosilane self-assembled-monolayer (SAM) beads.[121] As the beads allow for a higher antigen density than found on the platelet surface, our assay was found to be more sensitive than MAIPA in our preliminary experiments, taking only 3 to 4 hours to complete compared to 3 to 4 days. Importantly, the SAM demonstrated superior antifouling properties, which almost completely eliminates any nonspecific interactions, allowing analytical assays to be performed without a blocking step, such as bovine serum albumin. Comparisons between MAIPA and our assay are ongoing but show promise in the realm of treatment for ITP. This new technique should be very useful for ITP autoantibody detection and contribute to understanding whether anti-GPIbα antibodies, particularly those with epitopes in close proximity to the VWF ligand-binding area, affect TPO induction in patients.

7 | FUTURE DIRECTIONS

Sustained response in ITP is inconsistently achieved following tapering or withdrawal of pre-existing first-line therapies such as corticosteroids, intravenous immunoglobulin and anti-D.[124] Second-line therapies include splenectomy, rituximab, fostamatinib, and other

immunosuppressive drugs, and/or TPO receptor agonists, which act on c-Mpl as the therapeutic target stimulating downstream signaling pathways to increase megakaryocyte platelet generation, demonstrating an ≈80% long-term durability rate.[125] Current diagnostics are underdeveloped, however, platelet desialylation detected by *Ricinus communis* agglutinin I binding could be a promising marker of patients likely to be refractory to first-line treatments.[90,126] While these advances in ITP therapeutics offer potential solutions for suffering patients, there still lacks a gold standard of treatment that is efficacious across all patients. Our finding shows that platelet GPIbα is the driving force for liver TPO generation and is therefore important for the maintenance of homeostatic circulating TPO levels. Future studies should aim to elucidate the GPIbα cognate receptor, and identification of the downstream signal pathways in which platelet GPIbα stimulated de novo TPO synthesis, and whether other cells contribute to platelet-mediated TPO production in the liver should also be addressed. A comprehensive understanding of the mechanism behind GPIbα-mediated TPO generation may allow for discovery of novel therapeutics, such as GPIbα-anchored lipid rafts, that could potentially serve as TPO mimetics. Moreover, it is currently unclear why patients with ITP do not have higher TPO levels despite their significantly low platelet/megakaryocyte mass and less TPO clearance, and whether this is due to anti-GPIbα antibodies impairing platelet-mediated TPO generation. Prior studies indicate that antibody titers, epitope specificity, and/or affinity dictate the location of platelet clearance and the extent to which anti-GPIbα antibodies impact circulating TPO levels.[115,116] Larger clinical cohorts, normalized to platelet counts, are needed to understand the extent to which anti-GPIbα antibodies impact circulating TPO levels. Furthermore, whether these patients with anti-GPIbα antibodies will be exceptionally sensitive to and benefit from TPO therapy should be studied.[127]

Patients suffering from ITP can experience severe bleeding and are at continual risk for fatal hemorrhage along with comorbidities such as constant fatigue, increased risk of infection, and overall decreased quality of life.[128] These future directions will aid in optimizing therapies for these suffering patients, ultimately benefiting their quality of life, and reducing impacts on the health care system. Additionally, whether hematopoiesis and the HSC niche are impacted in patients with anti-GPIbα antibodies or in patients with BSS due to lower circulating TPO levels requires further exploration. Notably, anti-GPIbα antibodies can occur in both autoimmune disorders, such as ITP or drug-induced thrombocytopenia, and alloimmune disorders, such as PTP and FNAIT. The maternal anti–human platelet antigen-2 (located in the N-terminus of GPIbα) may cause severe FNAIT disease in fetuses and neonates, although its pathogenesis has been poorly understood. It is currently unknown whether these anti-GPIbα antibodies affect hematopoiesis and the HSC niche, including the possible interactions with mesenchymal stem cells.[129] These questions are important not only for basic science but also for diagnosis and therapies for patients, and therefore warrants further investigation.

ACKNOWLEDGMENTS

The authors thank June Li for her inspiration and help during the manuscript preparation. DK is a recipient of a Queen Elizabeth II (QE-II) Graduate Scholarship, and St. Michael's Hospital Research Training Centre Scholarship. MX is a recipient of a Young Taishan Scholar Foundation of Shandong Province state scholarship from the China Scholarship Council, an Ontario Trillium scholarship, and a graduate fellowship from the Canadian Blood Services Centre for Innovation. Figure 1 was created with BioRender.com.

AUTHOR CONTRIBUTIONS

D.K. prepared the manuscript and the figure; M.X. contributed to the original discovery, and edited the manuscript; H.N. is the principal investigator who supervised the research, and prepared the manuscript.

RELATIONSHIP DISCLOSURE

Some of the monoclonal antibodies are patented in the United States, Canada, and Europe (US Patent Application No. 12/082 686; Canadian Patent Application No. 2 628 900; European Patent Application No. 08153880.3).

ORCID

Heyu Ni https://orcid.org/0000-0002-7621-2945

REFERENCES

1. Coller B. Historical perspective and future directions in platelet research. *J Thromb Haemost*. 2011;9:374-395.
2. Xu X, Zhang D, Oswald B et al. Platelets are versatile cells: New discoveries in hemostasis, thrombosis, immune responses, tumor metastasis and beyond. *Crit Rev Clin Lab Sci*. 2016;53(6):409-430.
3. Radley J, Haller C. The demarcation membrane system of the megakaryocyte: a misnomer? *Blood*. 1982;60(1):213-219.
4. Kaushansky K. The molecular mechanisms that control thrombopoiesis. *J Clin Invest*. 2005;115(12):3339-3347.
5. Patel SR, Hartwig JH, Italiano JE Jr. The biogenesis of platelets from megakaryocyte proplatelets. *J Clin Invest*. 2005;115(12):3348-3354.
6. Junt T, Schulze H, Chen Z et al. Dynamic visualization of thrombopoiesis within bone marrow. *Science (New York, NY)*. 2007;317(5845):1767-1770.
7. Fuentes R, Wang Y, Hirsch J et al. Infusion of mature megakaryocytes into mice yields functional platelets. *J Clin Invest*. 2010;120(11):3917-3922.
8. Lefrançais E, Ortiz-Muñoz G, Caudrillier A et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. *Nature*. 2017;544(7648):105-109.
9. Li C, Li J, Ni H. Crosstalk between platelets and microbial pathogens. *Front Immunol*. 2020;11:1962.
10. Li C, Li J, Li Y et al. Crosstalk between platelets and the immune system: old systems with new discoveries. *Adv Hematol*. 2012;2012:1-14.
11. Semple J, Italiano J, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol*. 2011;11(4).
12. Lisman T, Porte RJ. Mechanisms of platelet-mediated liver regeneration. *Blood*. 2016;128(5):625-629.
13. Xu X, Yousef G, Ni H. Cancer and platelet crosstalk: opportunities and challenges for aspirin and other antiplatelet agents. *Blood*. 2018;131(16):1777-1789.

14. Xu XR, Carrim N, Neves MA et al. Platelets and platelet adhesion molecules: novel mechanisms of thrombosis and anti-thrombotic therapies. *Thromb J*. 2016;14(suppl 1):29.
15. MacKeigan D, Ni T, Shen C et al. Updated understanding of platelets in thrombosis and hemostasis: the roles of integrin PSI domains and their potential as therapeutic targets. *Cardiovasc Hematol Disord Drug Targets*. 2021;20(4):260-273.
16. Ruggeri Z. Platelets in atherothrombosis. *Nat Med*. 2002;8:11.
17. Lei X, Reheman A, Hou Y et al. Anfibatide, a novel GPIb complex antagonist, inhibits platelet adhesion and thrombus formation in vitro and in vivo in murine models of thrombosis. *Thromb Haemost*. 2014;111(02):279-289.
18. Wang Y, Gallant R, Ni H. Extracellular matrix proteins in the regulation of thrombus formation. *Curr Opin Hematol*. 2016;23(3):280-287.
19. Nieswandt B, Brakebusch C, Bergmeier W et al. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. *EMBO J*. 2001;20(9):2120-2130.
20. Ni H, Freedman J. Platelets in hemostasis and thrombosis: role of integrins and their ligands. *Transfus Apheresis Sci*. 2003;28(3):257-264.
21. Shen B, Zhao X, O'Brien K et al. A directional switch of integrin signalling and a new anti-thrombotic strategy. *Nature*. 2013;503(7474):131-135.
22. Jackson S. The growing complexity of platelet aggregation. *Blood*. 2007;109(12):5087-5095.
23. Ni H, Papalia J, Degen J, Wagner D. Control of thrombus embolization and fibronectin internalization by integrin alpha IIb beta 3 engagement of the fibrinogen gamma chain. *Blood*. 2003;102(10):3609-3614.
24. Ni H, Denis C, Subbarao S et al. Persistence of platelet thrombus formation in arterioles of mice lacking both von Willebrand factor and fibrinogen. *J Clin Invest*. 2000;106(3):385-392.
25. Reheman A, Yang H, Zhu G et al. Plasma fibronectin depletion enhances platelet aggregation and thrombus formation in mice lacking fibrinogen and von Willebrand factor. *Blood*. 2009;113(8):1809-1817.
26. Yang H, Reheman A, Chen P et al. Fibrinogen and von Willebrand factor-independent platelet aggregation in vitro and in vivo. *J Thromb Haemost*. 2006;4(10):2230-2237.
27. Wang Y, Reheman A, Spring CM et al. Plasma fibronectin supports hemostasis and regulates thrombosis. *J Clin Invest*. 2014;124(10):4281-4293.
28. Hou Y, Carrim N, Wang Y, Gallant RC, Marshall A, Ni H. Platelets in hemostasis and thrombosis: novel mechanisms of fibrinogen-independent platelet aggregation and fibronectin-mediated protein wave of hemostasis. *J Biomed Res*. 2015;29(6):437-444.
29. Dunne E, Spring CM, Reheman A et al. Cadherin 6 has a functional role in platelet aggregation and thrombus formation. *Arteriosclerosis Thrombosis Vasc Biol*. 2012;32(7):1724-1731.
30. Reheman A, Tasneem S, Ni H, Hayward C. Mice with deleted multimerin 1 and alpha-synuclein genes have impaired platelet adhesion and impaired thrombus formation that is corrected by multimerin 1. *Thromb Res*. 2010;125(5):e177-e183.
31. Li C, Piran S, Chen P et al. The maternal immune response to fetal platelet GPIbα causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. *J Clin Invest*. 2011;121(11):4537-4547.
32. Reddy E, Rand M. Procoagulant phosphatidylserine-exposing platelets in vitro and in vivo. *Front Cardiovasc Med*. 2020;7:15.
33. Li J, Sullivan J, Ni H. Pathophysiology of immune thrombocytopenia. *Curr Opin Hematol*. 2018;25(5):373-381.
34. Crow A, Lazarus A. Role of Fcgamma receptors in the pathogenesis and treatment of idiopathic thrombocytopenic purpura. *J Pediatr Hematol Oncol*. 2003;25(suppl 1):S1-8.
35. Aslam R, Kapur R, Segel G et al. The spleen dictates platelet destruction, anti-platelet antibody production, and lymphocyte distribution patterns in a murine model of immune thrombocytopenia. *Exp Hematol*. 2016;44(10):924-930.e1.
36. Kelemen E, Cserhati I, Tanos B. Demonstration and some properties of human thrombopoietin in thrombocythaemic sera. *Acta Haematol*. 2004;20(6):350-355.
37. Li J, Xia Y, Kuter D. Interaction of thrombopoietin with the platelet c-mpl receptor in plasma: binding, internalization, stability and pharmacokinetics. *Br J Haematol*. 1999;106(2):345-356.
38. Kaushansky K. Thrombopoiesis. *Semin Hematol*. 2015;52(1):4-11.
39. Qian S, Fu F, Li W, Chen Q, de Sauvage F. Primary role of the liver in thrombopoietin production shown by tissue-specific knockout. *Blood*. 1998;92(6):2189-2191.
40. Vigon I, Mornon J, Cocault L et al. Molecular cloning and characterization of MPL, the human homolog of the v-mpl oncogene: identification of a member of the hematopoietic growth factor receptor superfamily. *Proc Natl Acad Sci*. 1992;89(12):5640-5644.
41. Bartley T, Bogenberger J, Hunt P et al. Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. *Cell*. 1994;77(7):1117-1124.
42. de Sauvage FJ, Carver-Moore K, Luoh SM et al. Physiological regulation of early and late stages of megakaryocytopoiesis by thrombopoietin. *J Exp Med*. 1996;183(2):651-656.
43. Kaushansky K, Lok S, Holly RD et al. Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature*. 1994;369(6481):568-571.
44. Nakamura-Ishizu A, Matsumura T, Stumpf P et al. Thrombopoietin metabolically primes hematopoietic stem cells to megakaryocyte-lineage differentiation. *Cell Rep*. 2018;25(7):1772-1785.e6.
45. Choi E, Hokom M, Chen J et al. The role of megakaryocyte growth and development factor in terminal stages of thrombopoiesis. *Br J Haematol*. 1996;95(2):337-233.
46. Ito T, Ishida Y, Kashiwagi R, Kuriya S. Recombinant human c-Mpl ligand is not a direct stimulator of proplatelet formation in mature human megakaryocytes. *Br J Haematol*. 1996;94(2):387-390.
47. Alexander W, Roberts A, Nicola N, Li R, Metcalf D. Deficiencies in progenitor cells of multiple hematopoietic lineages and defective megakaryocytopoiesis in mice lacking the thrombopoietic receptor c-Mpl. *Blood*. 1996;87(6):2162-2170.
48. Carver-Moore K, Broxmeyer H, Luoh S et al. Low levels of erythroid and myeloid progenitors in thrombopoietin-and c-mpl-deficient mice. *Blood*. 1996;88(3):803-808.
49. Yoshihara H, Arai F, Hosokawa K et al. Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell*. 2007;1(6):685-697.
50. Kimura S, Roberts A, Metcalf D, Alexander W. Hematopoietic stem cell deficiencies in mice lacking c-Mpl, the receptor for thrombopoietin. *Proc Natl Acad Sci*. 1998;95(3):1195-1200.
51. Qian H, Buza-Vidas N, Hyland CD et al. Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell*. 2007;1(6):671-684.
52. de Graaf CA, Metcalf D. Thrombopoietin and hematopoietic stem cells. *Cell Cycle*. 2014;10(10):1582-1589.
53. Li L, Yi C, Xia W et al. c-Mpl and TPO expression in the human central nervous system neurons inhibits neuronal apoptosis. *Aging*. 2020;12(8):7397-7410.
54. Liang L, Li L, Wang J, Ye J, Yang L, Chong B et al., . Thrombopoietin Protects Neural Cells and Endothelial Cells from Apoptosis Via PI3K/AKT Pathway (confex.com). 62nd ASH Annual Meeting and Exposition; 2020 2020/12/07: ASH.
55. Decker M, Leslie J, Liu Q, Ding L. Hepatic thrombopoietin is required for bone marrow hematopoietic stem cell maintenance. *Science*. 2018;360(6384):106-110.
56. Varghese LN, Defour J-P, Pecquet C, Constantinescu SN. The thrombopoietin receptor: structural basis of traffic and activation by ligand, mutations, agonists, and mutated calreticulin. *Front Endocrinol*. 2017;8:59.

57. Märklin M, Tandler C, Kopp HG et al. C-Cbl regulates c-MPL receptor trafficking and its internalization. *J Cell Mol Med*. 2020;24:12491-12503.
58. Saur SJ, Sangkhae V, Geddis AE, Kaushansky K, Hitchcock IS. Ubiquitination and degradation of the thrombopoietin receptor c-Mpl. *Blood*. 2010;115(6):1254-1263.
59. Dahlen D, Broudy V, Drachman J. Internalization of the thrombopoietin receptor is regulated by 2 cytoplasmic motifs. *Blood*. 2003;102(1):102-108.
60. Ihara K, Ishii E, Eguchi M et al. Identification of mutations in the c-mpl gene in congenital amegakaryocytic thrombocytopenia. *Proc Natl Acad Sci*. 1999;96(6):3132-3136.
61. Ballmaier M, Schulze H, Strauss G et al. Thrombopoietin in patients with congenital thrombocytopenia and absent radii: elevated serum levels, normal receptor expression, but defective reactivity to thrombopoietin. *Blood*. 1997;90(2):612-619.
62. Schrezenmeier H, Griesshammer M, Hornkohl A et al. Thrombopoietin serum levels in patients with aplastic anaemia: correlation with platelet count and persistent elevation in remission. *Br J Haematol*. 1998;100(3):571-576.
63. Li J, van der Wal DE, Zhu G et al. Desialylation is a mechanism of Fc-independent platelet clearance and a therapeutic target in immune thrombocytopenia. *Nat Commun*. 2015;6:7737.
64. Grozovsky R, Begonja AJ, Liu K et al. The Ashwell-Morell receptor regulates hepatic thrombopoietin production via JAK2-STAT3 signaling. *Nat Med*. 2014;21(1):47-54.
65. Douglas B, Cines JBB, Liebman HA, Eline T. Luning Park. The ITP syndrome: pathogenic and clinical diversity | Blood | American Society of Hematology. *Blood*. 2009;113(26):6511-6521.
66. Balduini C, Melazzini F, Pecci A. Inherited thrombocytopenias-recent advances in clinical and molecular aspects. *Platelets*. 2017;28(1):3-13.
67. Kuter D. Managing thrombocytopenia associated with cancer chemotherapy. *Oncology (Williston Park, NY)*. 2015;29(4):282-294.
68. Kaplan C, Bertrand G, Ni H. Alloimmune thrombocytopenia. In Michelson A, Cattaneo M, Frelinger A, Newman P, eds. *Platelets*. Cambridge, Massachusetts: Academic Press; 2019.
69. Chen ZY, Oswald BE, Sullivan JA et al. Platelet physiology and immunology: pathogenesis and treatment of classical and non-classical fetal and neonatal alloimmune thrombocytopenia. *Ann Blood*. 2019;4:29.
70. Yougbaré I, Lang S, Yang H et al. Maternal anti-platelet β3 integrins impair angiogenesis and cause intracranial hemorrhage. *J Clin Invest*. 2015;125(4):1545-1556.
71. Yougbaré I, Tai W-S, Zdravic D et al. Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. *Nat Commun*. 2017;8(1):1-13.
72. Tiller H, Killie M, Husebekk A et al. Platelet antibodies and fetal growth: maternal antibodies against fetal platelet antigen 1a are strongly associated with reduced birthweight in boys. *Acta Obstet Gynecol Scand*. 2012;91(1):79-86.
73. Bussel J, Zabusky M, Berkowitz R, McFarland J. Fetal alloimmune thrombocytopenia. *N Engl J Med*. 1997;337(1):22-26.
74. Hawkins J, Aster R, Curtis B. Post-transfusion purpura: current perspectives. *J Blood Med*. 2019;10:405-415.
75. Harrington WJ, Minnich V, Hollingsworth JW, Moore CV. Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. *J Lab Clin Med*. 1951;38(1):1-10.
76. Shrestha S, Nazy I, Smith JW, Kelton JG, Arnold DM. Platelet autoantibodies in the bone marrow of patients with immune thrombocytopenia | Blood Advances | American Society of Hematology. *Blood Adv*. 2020;4(13):2962-2966.
77. Takahashi R, Sekine N, Nakatake T. Influence of monoclonal antiplatelet glycoprotein antibodies on in vitro human megakaryocyte colony formation and proplatelet formation. *Blood*. 1999;93(6):1951-1958.
78. Chang M, Nakagawa P, Williams S et al. Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal autoantibodies inhibit megakaryocytopoiesis in vitro. *Blood*. 2003;102(3):887-895.
79. Chow L, Aslam R, Speck E et al. A murine model of severe immune thrombocytopenia is induced by antibody- and CD8+ T cell-mediated responses that are differentially sensitive to therapy. *Blood*. 2010;115(6):1247-1253.
80. Olsson B, Andersson P, Jernås M et al. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. *Nat Med*. 2003;9(9):1123-1124.
81. Cheloff AZ, Kuter DJ, Al-Samkari H. Serum complement levels in immune thrombocytopenia: characterization and relation to clinical features | Blood | American Society of Hematology. *Blood*. 2019;134(suppl 1):1087.
82. Zeng D, Chen F, Wang S et al. Autoantibody against integrin α v β 3 contributes to thrombocytopenia by blocking the migration and adhesion of megakaryocytes. *J Thromb Haemost*. 2018;16(9):1843-1856.
83. Audia S, Santegoets K, Laarhoven A et al. Fcγ receptor expression on splenic macrophages in adult immune thrombocytopenia. *Clin Exp Immunol*. 2017;188(2):275-282.
84. Nimmerjahn F, Ravetch J. Fcgamma receptors as regulators of immune responses. *Nat Rev Immunol*. 2008;8(1):34-47.
85. Webster M, Sayeh E, Crow M et al. Relative efficacy of intravenous immunoglobulin G in ameliorating thrombocytopenia induced by antiplatelet GPIIbIIIa versus GPIbalpha antibodies. *Blood*. 2006;108(3):943-946.
86. Zeng Q, Zhu L, Tao L et al. Relative efficacy of steroid therapy in immune thrombocytopenia mediated by anti-platelet GPIIbIIIa versus GPIbα antibodies. *Am J Hematol*. 2012;87(2):206-208.
87. Peng J, Ma S, Liu J et al. Association of autoantibody specificity and response to intravenous immunoglobulin G therapy in immune thrombocytopenia: a multicenter cohort study. *J Thromb Haemost*. 2014;12(4):497-504.
88. Nieswandt B, Bergmeier W, Rackebrandt K, Gessner J, Zirngibl H. Identification of critical antigen-specific mechanisms in the development of immune thrombocytopenic purpura in mice. *Blood*. 2000;96(7):2520-2527.
89. Li J, Callum J, Lin Y, Zhou Y, Zhu G, Ni H. Severe platelet desialylation in a patient with glycoprotein Ib/IX antibody-mediated immune thrombocytopenia and fatal pulmonary hemorrhage. *Haematologica*. 2014;99(4):e61-e63.
90. Tao L, Zeng Q, Li J et al. Platelet desialylation correlates with efficacy of first-line therapies for immune thrombocytopenia. *J Hematol Oncol*. 2017;10(1):46.
91. Marshall S, Senis Y, Auger J et al. GPIb-dependent platelet activation is dependent on Src kinases but not MAP kinase or cGMP-dependent kinase. *Blood*. 2004;103(7):2601-2609.
92. Anderson G, van de Winkel J, Anderson C. Anti-GPIIb/IIIa (CD41) monoclonal antibody-induced platelet activation requires Fc receptor-dependent cell-cell interaction. *Br J Haematol*. 1991;79(1):75-83.
93. Liang X, Syed A, Russell S, Ware J, Li R. Dimerization of glycoprotein Ibα is not sufficient to induce platelet clearance. *J Thromb Haemost*. 2016;14(2):381-386.
94. Zhang X, Zhang W, Quach M, Deng W, Li R. Force-regulated refolding of the mechanosensory domain in the platelet glycoprotein Ib-IX complex. *Biophys J*. 2019;116(10):1960-1969.
95. Quach M, Dragovich M, Chen W et al. Fc-independent immune thrombocytopenia via mechanomolecular signaling in platelets. *Blood*. 2018;131(7):787-796.
96. Grozovsky R, Giannini S, Falet H, Hoffmeister KM. Regulating billions of blood platelets: glycans and beyond. *Blood*. 2015;126(16):1877-1884.

97. Li Y, Fu J, Ling Y et al. Sialylation on O-glycans protects platelets from clearance by liver Kupffer cells. *Proc Natl Acad Sci U S A*. 2017;114(31):8360-8365.
98. Jansen AJ, Josefsson EC, Rumjantseva V et al. Desialylation accelerates platelet clearance after refrigeration and initiates GPIbalpha metalloproteinase-mediated cleavage in mice. *Blood*. 2012;119(5):1263-1273.
99. Deppermann C, Kratofil RM, Peiseler M et al. Macrophage galactose lectin is critical for Kupffer cells to clear aged platelets. *J Exp Med*. 2020;217(4):e20190723.
100. Grewal P, Uchiyama S, Ditto D et al. The Ashwell receptor mitigates the lethal coagulopathy of sepsis. *Nat Med*. 2008;14(6):648-655.
101. Wang Y, Chen W, Zhang W et al. Desialylation of O-glycans on glycoprotein Ibα drives receptor signaling and platelet clearance. *Haematologica*. 2020;106(1):220-229.
102. Hoffmeister K, Felbinger T, Falet H et al. The clearance mechanism of chilled blood platelets. *Cell*. 2003;112(1):87-97.
103. Warren A, Le Couteur DG, Fraser R, Bowen DG, McCaughan GW, Bertolino P. T lymphocytes interact with hepatocytes through fenestrations in murine liver sinusoidal endothelial cells. *Hepatology*. 2006;44(5):1182-1190.
104. Kosugi S, Kurata Y, Tomiyama Y et al. Circulating thrombopoietin level in chronic immune thrombocytopenic purpura. *Br J Haematol*. 1996;93(3):704-706.
105. Griesshammer M, Hornkohl A, Nichol J et al. High levels of thrombopoietin in sera of patients with essential thrombocythemia: cause or consequence of abnormal platelet production? *Ann Hematol*. 1998;77(5):211-215.
106. Kaser A, Brandacher G, Steurer W et al. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. *Blood*. 2001;98(9):2720-2725.
107. Kraakman MJ, Lee MKS, Al-Sharea A et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. *J Clin Invest*. 2017;127(6):2133-2147.
108. McCarty J, Sprugel K, Fox N, Sabath D, Kaushansky K. Murine thrombopoietin mRNA levels are modulated by platelet count. *Blood*. 1995;86(10):3668-3675.
109. Sungaran R, Chisholm O, Markovic B, Khachigian L, Tanaka Y, Chong B. The role of platelet alpha-granular proteins in the regulation of thrombopoietin messenger RNA expression in human bone marrow stromal cells. *Blood*. 2000;95(10):3094-3101.
110. Yang M, Liang E, Ye J, Chong BH, Li L, eds. The Role of 5-HT on Proplatelet Formation and Thrombopoietin Production (confex.com). 62nd ASH Annual Meeting and Exposition; 2020 2020/12/05: ASH.
111. Liang EY, Ye JY, Zhou LX, Li C, Chong BH, Yang M, Platelet-derived serotonin stimulates TPO production from bone marrow mesenchymal stromal cells but not hepatocytes. *Blood*. 2015;126(23):4773.
112. Xu M, Ma L, Carrim N et al. Platelet GPIba is important for thrombopoietin production and thrombopoietin-induced platelet generation | Blood | American Society of Hematology. *Blood*. 2015;126(23):12.
113. Xu M, Li J, Neves M et al. GPIbα is required for platelet-mediated hepatic thrombopoietin generation. *Blood*. 2018;132(6):622-634.
114. Li R, Emsley J. The organizing principle of the platelet glycoprotein Ib-IX-V complex. *J Thromb Haemost*. 2013;11(4):605-614.
115. Morodomi Y, Kanaji S, Won E, Ruggeri Z, Kanaji T. Mechanisms of anti-GPIbα antibody-induced thrombocytopenia in mice. *Blood*. 2020;135(25):2292-2301.
116. Porcelijn L, Schmidt D, van der Schoot C, Vidarsson G, de Haas M, Kapur R. Anti-glycoprotein Ibα autoantibodies do not impair circulating thrombopoietin levels in immune thrombocytopenia patients. *Haematologica*. 2020;105(4):e172-e174.
117. Bianchi E, Norfo R, Pennucci V, Zini R, Manfredini R. Genomic landscape of megakaryopoiesis and platelet function defects. *Blood*. 2016;127(10):1249-1259.
118. Dütting S, Gaits-Iacovoni F, Stegner D et al. A Cdc42/RhoA regulatory circuit downstream of glycoprotein Ib guides transendothelial platelet biogenesis. *Nat Commun*. 2017;8(1):1-13.
119. Jiao XZX, Li Z, Zhang Y, Ye J. Role of thrombopoietin in rescuing vascular niche of ITP patients - ISTH Congress Abstracts. *Res Pract Thromb Haemost*. 2020;4 (Suppl 1).
120. Zeng YJX, Li Z, Zhang Y, Ye J. Thrombopoietin rescues megakaryopoiesis, reduces hemorrhage via protecting bone marrow endothelial function of chemotherapy-treated hematological malignancies patients - ISTH Congress Abstracts. International Society on Thrombosis and Haemostasis; 2020: Res Pract Thromb Haemost. 4 (Suppl 1). https://abstracts.isth.org/abstract/thrombopoietin-rescues-megakaryopoiesis-reduces-hemorrhage-via-protecting-bone-marrow-endothelial-function-of-chemotherapy-treated-hematological-malignancies-patients/. Accessed April 5, 2021.
121. Neves M, Arad A, Wang Y, Zhu G, Galant R, Thompson M et al. Development of a flow cytometry–based assay for the detection of ITP autoantibodies - ISTH Congress Abstracts. ISTH 2020 Congress. 2020. 4 (Suppl 1). https://abstracts.isth.org/abstract/development-of-a-flow-cytometry-based-assay-for-the-detection-of-itp-autoantibodies/. Accessed April 5, 2021.
122. Malara A, Abbonante V, Di Buduo C, Tozzi L, Currao M, Balduini A. The secret life of a megakaryocyte: emerging roles in bone marrow homeostasis control. Cellular and molecular life sciences. *Cell Mol Life Sci*. 2015;72(8):1517-1536.
123. Kong Y, Hu Y, Zhang X et al. Association between an impaired bone marrow vascular microenvironment and prolonged isolated thrombocytopenia after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2014;20(8):1190-1197.
124. Provan D, Arnold D, Bussel J et al. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817.
125. Kuter D. Thrombopoietin and thrombopoietin mimetics in the treatment of thrombocytopenia. *Ann Rev Med*. 2009;60(1):193-206.
126. Li J, Sullivan J, Ni H. Is platelet desialylation a novel biomarker and therapeutic target in immune thrombocytopenia? *J Cell Immunol*. 2020;2(1).6–14.
127. Smith R, Zandi S, Liederman Z, Xu M, Lim G, Ghaffar H editors et al., New clinicopathologic finding in a patient with acquired amegakaryocytic thrombocytopenia treated with thrombopoietin receptor agonist therapy. International Society on Thrombosis and Haemostasis; 2018;2(suppl 1). Dublin, Ireland.
128. Frederiksen H, Christiansen C, Nørgaard M. Risk and prognosis of adult primary immune thrombocytopenia. *Exp Rev Hematol*. 2012;5(2):219-228.
129. He Y, Xu L, Feng F et al. Mesenchymal stem cell deficiency influences megakaryocytopoiesis through the TNFAIP3/NF-κB/SMAD pathway in patients with immune thrombocytopenia. *Br J Haematol*. 2018;180(3):395-411.

How to cite this article: Karakas D, Xu M, Ni H. GPIbα is the driving force of hepatic thrombopoietin generation. *Res Pract Thromb Haemost*. 2021;5:e12506. https://doi.org/10.1002/rth2.12506