tagraxofusp had modest clinical activity in most patients with MDS and AML in these studies, there was robust activity in patients with BPDCN (78% major response rate in the pilot study).[5] This led to the pivotal phase 1/2 study, by Pemmaraju et al, for patients with BPDCN, which demonstrated 90% overall response rate in frontline setting and 67% in relapsed/refractory setting, ultimately helping to lead to drug approval specifically for patients with BPDCN.[6]

Given the markedly different results with CD123-tageted monotherapy with this agent in AML vs BPDCN, several questions have been raised: (1) Do these divergent results have to do with the molecular heterogeneity of AML vs BPDCN? (2) Does the quantitative or qualitative CD123 expression level matter (universally overexpressed in all BPDCN cases vs not in all cases of AML)? (3) Are there different therapeutic gradients/windows, dosing parameters, or differential organ compartment responses to consider in AML vs BPDCN? (4) Is there a treatment resistance pathway with tagraxofusp monotherapy in AML that may not be present in BPDCN? This paper from Xiao et al is very helpful in addressing these questions. This study suggests consideration of a focused targeted approach specifically in the subset of patients with pDC-AML. Based on work of Togami et al, the authors discovered a novel resistance pathway, via an acquired downregulation in diphthamide synthesis pathway (*DPH1*). In experimental models, the restoration of *DPH1* pathway expression occurred after administration of the hypomethylating agent azacytidine in combination with tagraxofusp.[7] Based on this work, Lane and Pemmaraju have initiated a novel phase 1/2 clinical trial for patients with AML and high-risk MDS in an ongoing study with 2 arms (tagraxofusp + azacytidine; tagraxofusp + azacytidine + venetoclax) (NCT03113643). It will be of great interest to analyze the results of this trial for responses in *RUNX1*-mutated/pDC-AML patients. Future clinical studies of pDC-AML will certainly focus on the emerging field of CD123-targeting agents. Notably, there are several other drugs approaches in active clinical trials, including novel conjugated agents, bispecific agents, and chimeric antigen receptor T-cell therapies specifically targeting CD123.

Based on this study, pDC-AML$^{+/-}$ *RUNX1* mutations should be considered for full subcategory status in the next World Health Organization reclassification. In the latest World Health Organization 2016 reclassification by Arber et al, AML with mutated *RUNX1* mutation is listed as a provisional entity.[8]

In summary, this study by Xiao et al confirms a new subentity of AML, pDC-AML; presents a potential novel mechanistic/mutational profile with a strong association with *RUNX1* mutations; and outlines a new therapeutic avenue for investigation with CD123-targeted agents in AML. It provocatively raises the question: Is targeting pDC-AML as easy as CD 1-2-3?

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THROMBOSIS AND HEMOSTASIS

Comment on Mailer et al, page 1392

XPoRting (poly)phosphates limits thrombosis

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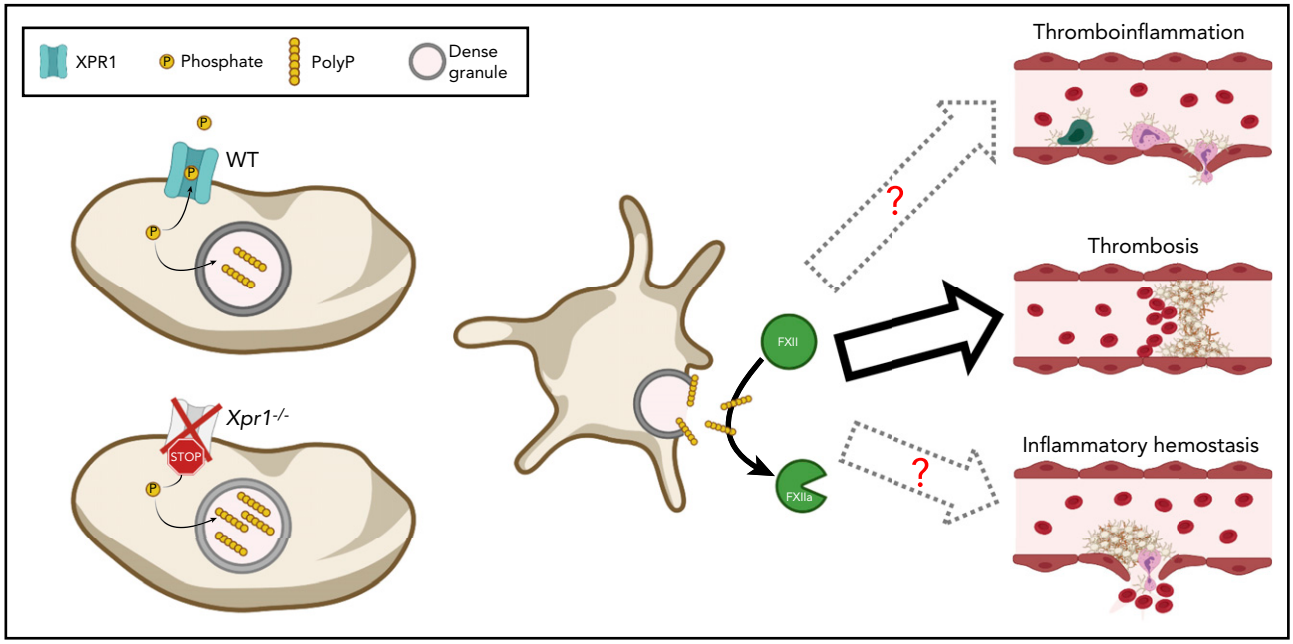
In this issue of *Blood*, Mailer et al demonstrate that the xenotropic and polytropic retrovirus receptor 1 (XPR1) serves as a phosphate transporter that controls platelet polyphosphate content and thereby modulates thrombosis.[1]

At sites of vascular injury, activated platelets release their granule content, which amplifies the activation response and promotes local activation of the coagulation cascade resulting in the formation of a fibrin-rich clot that seals the wound. This concerted action of platelets and the coagulation system has to be regulated with high fidelity to enable normal hemostasis while preventing uncontrolled thrombus growth, which may lead to thrombotic vessel occlusion, ischemia, and infarction of vital organs. In the classic cascade or waterfall models of blood coagulation,[2] fibrin formation can be initiated through either of 2 converging cascades designated the extrinsic and intrinsic pathways. The essential function of the factor VIIa (FVIIa)/tissue factor complex–triggered extrinsic pathway is firmly established, and functional defects in these components severely



XPR1-mediated phosphate export reduces platelet polyP content and thereby limits platelet-dependent FXII activation and thrombosis. In the absence of functional XPR1, phosphates are no longer exported, which results in increased polyP levels in dense granules of platelets. Consequently, at sites of platelet activation, more polyP is released, which results in increased thrombosis as demonstrated by Mailer et al. It will be interesting to further investigate whether elevated polyP levels as a consequence of reduced XPR1 activity would also translate into increased thromboinflammation and/or might reduce inflammatory bleeding. This figure was created using BioRender.com.

compromise blood coagulation in vivo.[2] In sharp contrast, hereditary deficiency of FXII (Hageman factor), the protease that triggers the intrinsic pathway after being activated by polyanionic surfaces (contact activation), is not associated with spontaneous hemorrhage or excessive injury-related bleeding, which led to the hypothesis that FXII is not required for fibrin formation in vivo. This picture changed in 2005, when Renné et al[3] showed that FXII-deficient mice, although they display normal hemostasis, were profoundly protected from injury-induced occlusive thrombus formation in different thrombosis models. These findings provided the first experimental evidence that the mechanisms that drive thrombosis may in part be distinct from those required for hemostasis, which might allow pharmacologic prevention of thrombosis without increasing the bleeding risk. This was experimentally confirmed a few years later using the first specific FXII inhibitor, rHA-Infestin-4.[4] In addition to the intrinsic coagulation pathway, FXII also triggers the proinflammatory kallikrein-kinin system (KKS) that liberates the vasoactive peptide hormone bradykinin.[5] This dual function puts FXII in a central position at the interface between thrombotic and inflammatory pathways, which makes it a prime target to interfere with thromboinflammatory diseases such as ischemic stroke.[6]

The recognition of FXII as a key player in thrombotic and thromboinflammatory disease settings sparked intense interest in the mechanisms underlying its activation in vivo. Despite a long-recognized role of activated platelets in promoting FXII activation, it took until 2009 to identify platelet polyphosphate (polyP) as the underlying factor in this process.[7] Polyphosphates are linear polymers of orthophosphate that are stored in platelet-dense granules and released upon activation. Following this seminal discovery, several groups have identified that platelet polyP contributes to procoagulant and proinflammatory pathways. Polyphosphates can also be secreted from microorganisms and some immune cells and, depending on the polyP chain length, have been shown to promote plasmatic coagulation, modulate inflammation by inhibiting the complement system, and trigger bradykinin release,[5,8] and inhibition of polyP has been suggested as a novel antithrombotic and anti-inflammatory strategy.[8,9] Although platelet polyP is shorter than bacterial polyP and is thus considered to be less potent, it has been shown to increase endothelial cell permeability, promote the formation of neutrophil extracellular traps, and modulate fibrin clot structure,[5,8]

Mailer et al have added another piece to the platelet polyP puzzle by identifying XPR1 as a major phosphate exporter in platelets. XPR1 is expressed in murine and human platelets, and pharmacologic inhibition of this transporter in heterologous cells or on human platelets resulted in the accumulation of intracellular polyP. Similar results were obtained in mice lacking XPR1, specifically in megakaryocytes and platelets (*Xpr1*$^{fl/fl,\ Pf4\text{-}Cre}$), indicating that XPR1, by exporting excess phosphates, regulates homeostatic polyP levels in platelets. Of note, polyP levels were affected independently of their chain length because soluble and longer platelet polyP were affected equally. As a consequence of the elevated polyP content, the procoagulant activity of these platelets was increased, which translated into increased thrombus formation and fibrin deposition in vitro and in vivo. Furthermore, the authors identified FXII as the main target of platelet polyP, because FXII blockade abrogated the procoagulant effect of elevated platelet polyP levels. This FXII dependency is interesting, because platelet polyP has been proposed to be a weak

FXII activator, and its procoagulant effects were ascribed to accelerated FXI activation and blockade of the tissue factor pathway inhibitor rather than to FXII activation.[8]

Future studies, using either *Xpr1*$^{fl/fl,\ Pf4\text{-}Cre}$ mice, which have higher polyP levels, or mice lacking the inositol hexakisphopshate kinase 1 (*Ip6k1*$^{-/-}$), which have lower platelet polyP levels,[10] may help shed light on the specific role of platelet polyP in different thrombo-inflammatory disease settings. Mice with defective dense granule secretions that are thus unable to release platelet polyP are protected from arterial thrombosis as well as thrombo-inflammatory cerebral infarct progression after experimental ischemic stroke.[6] Although the antithrombotic protection is clearly a result of the abolished platelet adenosine 5′-diphosphate/adenosine triphosphate release, the reduced cerebral thromboinflammation may, at least in part, be a consequence of the abrogated polyP-induced FXII activation. FXII has been shown to facilitate various aspects of thromboinflammation, from modulating the KKS to fine-tuning neutrophil responses to neuroinflammation in the context of autoimmune encephalomyelitis.[5] It remains to be seen whether the platelet polyP-FXII axis also triggers these reactions and how it affects the KKS.

Another question arising from the work of Mailer et al is whether inhibiting XPR1 and thereby enhancing the procoagulant potential of platelets might be a suitable strategy for limiting excessive spontaneous or injury-related bleeding. XPR1-deficient mice did not display shortened tail bleeding times; however, these mice were healthy. Under inflammatory conditions, maintenance of vascular integrity and hemostasis depend on mechanisms that are in part distinct from those required for normal hemostasis.[6] It will be interesting to study the significance of platelet polyP and its regulation in models of thromboinflammation and inflammatory bleeding (see figure). Of note, mice with reduced platelet polyP levels had slightly prolonged tail bleeding times,[10] indicating that platelet polyP can contribute to hemostasis, perhaps even in an FXII-independent manner.[3,8,10] Because XPR1 inhibition would elevate platelet polyP levels, the procoagulant effect of such a treatment would be observed only at sites of vascular injury where platelets adhere and release their granule content, including polyP.

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THROMBOSIS AND HEMOSTASIS

Comment on Obermayer et al, page 1406

Natural IgM antibodies help fend off thrombosis

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It is tempting to view the immune system as a thrombosis driver,[1] but the article by Obermayer et al[2] in this issue of *Blood* provides a counterpoise through the discovery of an anticoagulant role for innate immunity. Their article linking circulating microvesicles with natural immunoglobulin M (IgM) antibodies is an intriguing story with high clinical significance.

Plasma microvesicles are a heterogeneous collection of membranous cell-derived microparticles released in response to inflammatory activation, cellular stress, or apoptosis. Typically, microvesicles express phosphatidylserine on their exterior, allowing for their identification in the laboratory by flow cytometry with Annexin V. Microvesicles also tend to express the membrane glycoproteins of their cell of origin, whether they are lineage markers (eg, of platelets, endothelial cells, neutrophils, monocytes) or other functionally significant glycoproteins. Consequently, there is now a sizeable number of studies identifying specific microvesicle phenotypes as biomarkers of disease. Microvesicles are already known to contribute to thrombosis by virtue of expressing negatively charged phospholipids that facilitate prothrombinase complex assembly. Some plasma microvesicles also express tissue factor, and experiments using intravital microscopy to visualize clot formation in preclinical models have highlighted the importance of these for thrombus propagation.[3] Furthermore, plasma tissue factor–expressing microvesicles are associated with thrombotic events in patients, as for example, in Behçet syndrome.[4] Until now, the existence of a specific endogenous means to counter the prothrombotic effect of microvesicles has not been realized.

Natural antibodies are those germline-encoded immunoglobulins that are expressed without the need for immunization and that contribute to innate humoral immunity. They are mainly of the pentavalent IgM class and not only serve