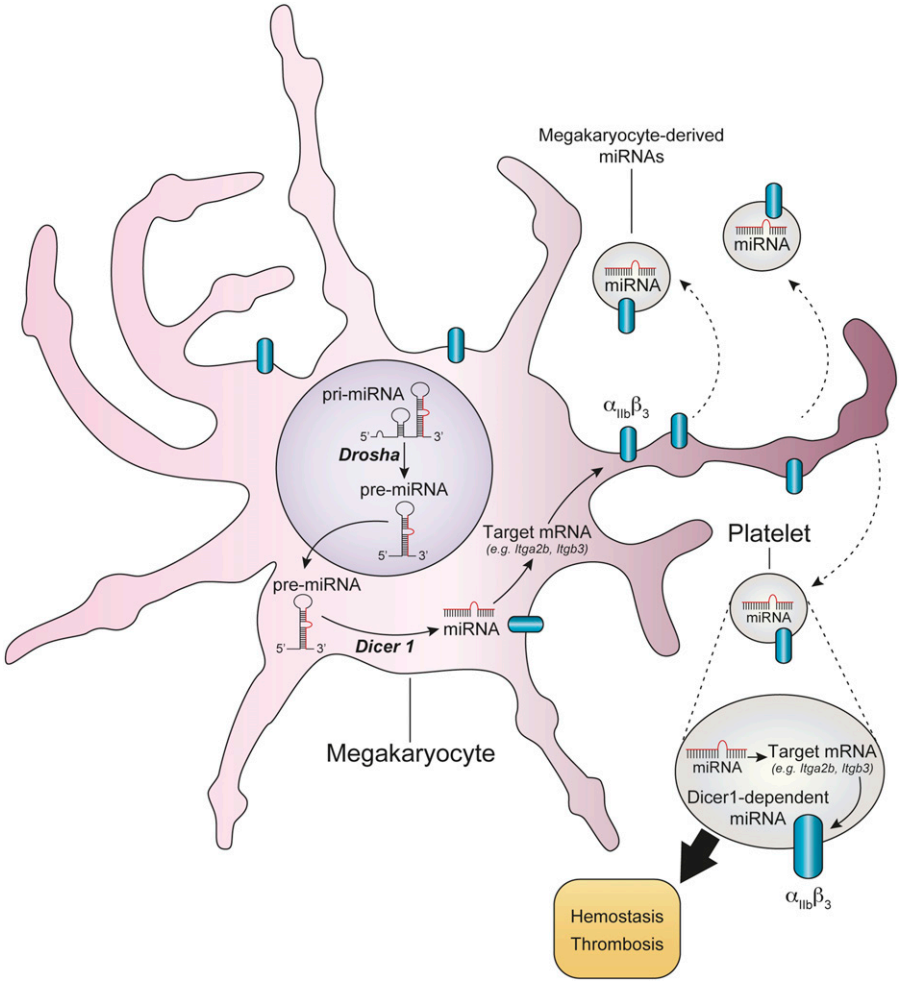

Dicer1-dependent generation of miRNAs modulate mRNA expression and functions of platelets. MicroRNA precursors (pre-miRNA) in the megakaryocyte nucleus are processed by Drosha and are then exported in the cytoplasm, where they undergo further trimming by Dicer1. Upon recognition of their mRNA targets, functional miRNAs trigger mRNA degradation or translational repression. Dicer1-dependent miRNAs modulate mRNA profiles in platelets, such as the mRNAs coding for Itgb3 (β_3) and Itgb2 (α_{IIb}). The ablation of Dicer1 in platelets leads to overexpression of $\alpha_{IIb}\beta_3$ protein at the platelet surface and heightened platelet reactivity. The modulation of the platelet mRNA repertoire by microRNAs can occur in the megakaryocyte and might potentially also take place in platelets once in the blood circulation or during their storage. pri-miRNA, primary miRNA.

The findings in this study have repercussions for understanding platelet functions in a multitude of ways. MicroRNAs in platelets can be encapsulated in small membrane vesicles, called "microparticles." Because platelet-derived microparticles mediate transfer of their cargo between platelets and other cells, such as endothelial cells and leukocytes,[7,9] this suggests that the (dys)regulation of miRNA content in platelets might also affect other cellular lineages. Furthermore, these observations are supported by previous studies on human platelets that miRNA content varies in different disease states and phenotypes, such as race, and correlates with platelet reactivity.[4-7] MicroRNAs may also regulate platelet functions in contexts other than thrombosis, such as immunity. Hence, miR-148a regulates platelet signaling induced by immune complexes in mice.[10] In sum, in addition to the potential of platelet miRNAs as biomarkers in pathogenesis, this study establishes that, generated via Dicer1, they define the hemostatic and thrombotic functions of platelets.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● CLINICAL TRIALS AND OBSERVATIONS

Comment on Santagostino et al, page 1761

A new era for hemophilia B treatment

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In this issue of *Blood*, Santagostino et al, in their phase 3 study, demonstrate efficacy and safety of recombinant fusion protein linking coagulation factor IX (FIX) with albumin (rIX-FP) which, along with the other 2 extended half-life FIX products, heralds a new era for the treatment of hemophilia B.[1]

We are in an era of safe effective treatment of hemophilia. Since the late 1980s, HIV and hepatitis C virus have been eliminated from plasma-derived factor products. Recombinant products have been available for hemophilia B for over 20 years. Prophylactic treatment to prevent bleeding has become standard of care.[2] Before 2014, the half-life of available FIX concentrates was around 18 to 27 hours,[3,4] thus necessitating factor infusion about twice weekly to sustain FIX trough levels above 1% and therefore decreasing the chance of spontaneous bleeding. Different technologies have emerged to prolong the half-life of FIX concentrate. The first extended half-life factor utilizing the neonatal Fc receptor (recombinant FIX [rFIX] Fc fusion protein [rFIXFc])[5] was US Food and Drug Administration (FDA) approved in March 2014. Results of a second protein, a recombinant glycoPEGylated FIX (nonacog β pegol), were reported by Collins et al.[6] This trial by Santagostino and colleagues investigates a rIX-FP and completes the last of 3 first-generation recombinant extended half-life FIX phase 3 studies (see table).[7] These products have the potential to revolutionize hemophilia treatment with dosing intervals increasing from twice a week to dosing every 7 to 21 days and increasing FIX trough levels.

The open-label phase 3 study presented in this issue demonstrates efficacy of rIX-FP to prevent bleeding episodes. rIX-FP is a recombinant fusion protein linking FIX with recombinant albumin. It utilizes the neonatal Fc receptor to recycle the factor after endocytosis,[8] a technology that has been shown to be safe and effective in other settings.[9] This modification results in a 4.3-fold increase in factor half-life as compared with a traditional recombinant FIX product (rFIX), equivalent to a half-life of 102 hours in this study. Weekly injections of 40 IU/kg resulted in a trough rIX-FP of 20% whereas every 14-day injections resulted in a trough of 12%. Like most modern factor studies, subjects had low bleeding rates and the treatment was well tolerated with a median annualized bleeding rate of 0.0. Furthermore, 99% of bleeding episodes were successfully treated with FIX-FP, 94% of bleeds with only a single dose. In this study of previously treated patients, no FIX inhibitors were detected throughout the study. It is noteworthy that subjects in the on-demand

Characteristics of 3 extended half-life FIX concentrates from recently completed phase 3 clinical studies

	rFIXFc[4]	glycoPEGylated FIX[5]	rIX-FP
Other name	Alprolix, eftrenonacog alfa	Nonacog beta pego	Albutrepenonacog alfa
Mechanism	• rFIX covalently fused to dimeric Fc domain of IgG1 • binding to neonatal Fc receptor utilizes endogenous IgG recycling pathways	• polyethylene glycol (PEG) moiety attached to FIX • PEG is cleaved off during FIX activation	• recombinant fusion protein linking FIX with albumin • albumin binds to neonatal Fc receptor and is protected from degradation
Study population	115 PTP's age 12-71 (median 30) baseline FIX ≤2%	67 PTP's age 13-65 (17 were <18 years) baseline FIX ≤2%	63 PTP's age 12-61 (mean 33, 7 were <18 years) baseline FIX ≤2%
Incremental recovery	0.92 (IU/dL)/(IU/kg)	2.0-3.0 (IU/dL)/(IU/kg)	1.27 (IU/dL)/(IU/kg)
Half-life (mean)	82.1 hours	**Receiving 10 IU/kg q7d** 93 hours (single dose) 107 hours (steady state) **Receiving 40 IU/kg q7d** 85 hours (single dose) 111 hours (steady state)	101.7 hours
Fold increase of half-life over regular half-life FIX	2.4 fold	4.8 fold[7]	4.3 fold
Trough levels (mean) [reporting methodology varied across trials]	**Time to 1 IU/dL** 11.2 d after 50 IU/kg dose	**Steady state trough levels:** • 10 IU/kg q7d: 8.5 IU/dL • 40 IU/kg q7d: 27.3 IU/dL	**Level >5 IU/dL** • 10 d after 25 IU/kg dose • 14 d after 50 IU/kg dose
Annual spontaneous bleeding rate on prophylactic dosing	1.0 (~45 IU/kg q 7d) 0.9 (100 IU/kg q ~12.5d)	**Median AsBRs:** 0.97 (10 IU/kg q 7d) 0.00 (40 IU/kg q 7d)	**Median AsBRs:** 0 for all groups **Mean AsBRs:** 0.83 (75 IU/kg q 14d) 0.56 (75 IU/kg q 10d) 0.65 (40 IU/kg q 7d)

AsBR, annual spontaneous bleed rate; d, day; FIX, factor IX; PTP, previously treated patient; q, every. Professional illustration by Patrick Lane, ScEYEnce Studios.

arm had a 100% reduction in annualized spontaneous bleeding and full resolution of target joints.

An interesting facet to this study was the flexibility given to the treating physician. Subjects in the prophylaxis group received 35 to 50 IU/kg weekly. The exact entry dose was determined by the treating physician. If there was no spontaneous bleeding in the first 26 weeks on this weekly dosing, dose and dosing interval could be adjusted to 75 IU/kg every 10 to 14 days. Importantly, the physician could increase or decrease the dose received based on clinical assessment. This flexibility gave the study a real-life feel, allowing physicians to make clinical judgments. Conceivably, this real-world design could ease the application in clinical use.

One of the vexing problems with all chronic illnesses is patient treatment fatigue. Decreasing dosing intervals may improve this issue in hemophilia B, increase adherence to prophylactic treatment, and prevent bleeding and associated joint arthropathy. Another key element of extended half-life rFIX products is the potential for higher trough levels which decrease the risk for breakthrough bleeding. A desirable trough level in the past has been considered to be >1% due to the notion that, by converting someone with severe disease to a phenotypic moderate hemophilia, spontaneous bleeding could be prevented. With the advent of these longer-acting FIX products, aiming for a higher trough level has become feasible and we have to ask whether 1% is enough for our patients with hemophilia. People with moderate and mild hemophilia (FIX levels of 2%-30%) still potentially experience microbleeding and certainly trauma/activity-related bleeding that can lead to undesirable outcomes. Combining increased compliancy with higher trough level allows for the normalization of activity and increasing long-term joint and overall health. The economic impact of this new paradigm certainly has to be considered. We are truly entering a new era for hemophilia B treatment.

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● ● ● LYMPHOID NEOPLASIA

Comment on Juilland et al, page 1780

ATF3, a new player in DLBCL cell survival

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In this issue of *Blood*, Juilland and colleagues reveal the expression pattern and the role of different members of the activating transcription factor (ATF) family in survival of diffuse large B-cell lymphoma (DLBCL) cells.[1]

In adults, DLBCL is the most common lymphoid malignancy. It is a heterogeneous disease composed of multiple molecular subtypes that differ in their expression of hundreds of genes, their responsiveness to chemotherapy, and survival rates after chemotherapy.[2] The activated B-cell (ABC)–like subtype (ABC DLBCL) is the most aggressive form of DLBCL with the lowest cure rate. Initially, the constitutive activity of the nuclear factor–κB signaling pathway was identified as a major feature of ABC DLBCL.[3] However, recently, several independent studies have reported elevated expression levels and activity of Jun transcription factors in ABC DLBCL cell lines and clinical specimens.[4-6] Although Jun factors are primarily involved in regulating the cell cycle and apoptosis,[7] a large number of inducible genes contain Jun-binding sites in their promoters or enhancers and, therefore, they can be considered as Jun-target genes. But the complexity of this regulation starts with the fact that dimerization of Jun is required prior to its binding to DNA. Different Jun factors (c-Jun, JunB, and JunD) can form homodimers or heterodimers with proteins belonging to the FOS, ATF, and MEF families, creating the activator protein-1 (AP-1) transcription factor.[8] The composition of AP-1 complexes determines the genes that are regulated, either positively or negatively.

A report from Juilland and colleagues reveals novel and exciting findings regarding the role and molecular composition of AP-1 in DLBCL.[1] Using an unbiased biochemical approach, they identified ATF2, ATF3, and ATF7 as constitutive binding partners of Jun in lymphoma cells. Although Jun/ATF2 and Jun/ATF7 complexes were abundant in the majority of cell lines, ATF3 was exclusively expressed in cell lines derived from the ABC subtype of DLBCL (see figure). The clinical relevance of this observation was evaluated in patient biopsies. In a cohort of 350 DLBCL patients, the ATF3 messenger RNA level was significantly higher in the ABC vs the germinal center B-cell (GCB) subtype. Immunohistochemical analysis revealed strong nuclear ATF3 expression in tumors from ABC DLBCL patients. Altogether, these data raise