Schematic of the effects of Flt3 inhibition in FLT3-ITD+ AML leading to resistance to Flt3 inhibitors, as well as the effects of dual inhibition with Flt3 and HDAC inhibitors. Dual inhibition leads to an increase of acetylated p53 and improved eradication of FLT3-ITD+ AML cells.

REFERENCES

1. Long J, Jia M-Y, Fang W-Y, et al. FLT3 inhibition upregulates HDAC8 via FOXO to inactivate p53 and promote maintenance of FLT3-ITD+ acute myeloid leukemia. *Blood.* 2020;135(17):1472-1483.

2. Dansen TB, Burgering BM. Unravelling the tumor-suppressive functions of FOXO proteins. *Trends Cell Biol.* 2008;18(9):421-429.

3. Lin S, Ptasinska A, Chen X, et al. A FOXO1-induced oncogenic network defines the AML1-ETO preleukemic program. *Blood.* 2017;130(10):1213-1222.

4. Zhang J, Gu Y, Chen B. Mechanisms of drug resistance in acute myeloid leukemia. *OncoTargets Ther.* 2019;12:1937-1945.

5. Karantanou C, Godavarthy PS, Krause DS. Targeting the bone marrow microenvironment in acute leukemia. *Leuk Lymphoma.* 2018;59(11):2535-2545.

6. Estey EH. Acute myeloid leukemia: 2013 update on risk-stratification and management. *Am J Hematol.* 2013;88(4):318-327.

7. Smith CC, Wang Q, Chin CS, et al. Validation of ITD mutations in FLT3 as a therapeutic target in human acute myeloid leukaemia. *Nature.* 2012;485(7397):260-263.

8. Zhang H, Savage S, Schultz AR, et al. Clinical resistance to crenolanib in acute myeloid leukemia due to diverse molecular mechanisms. *Nat Commun.* 2019;10(1):244.

9. Traer E, Martinez J, Javidi-Sharifi N, et al. FGF2 from marrow microenvironment promotes resistance to FLT3 inhibitors in acute myeloid leukemia. *Cancer Res.* 2016;76(22):6471-6482.

10. West AC, Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. *J Clin Invest.* 2014;124(1):30-39.

FLT3 inhibitor alone and reduced leukemia-initiating capacity, as measured in secondary transplantation assays. Last, the authors demonstrate that inhibition of HDAC8 and the respective tyrosine kinase may also be beneficial in cells harboring other activating tyrosine kinase mutations.

In summary, this paper suggests a novel pathway of resistance to TKIs via upregulation of HDAC8. Combination of FLT3 inhibitors with HDAC8 inhibitors may be a feasible strategy for elimination of leukemic stem cells in AML. Although it has been shown in different cancers that HDAC inhibition can overcome therapy resistance by different mechanisms, it remains to be seen whether the other HDACs may also contribute to therapy resistance in a similar FOXO1/3-p53–dependent manner. Certain HDAC inhibitors have entered clinical trials or clinical medicine in several hematological cancers. However, the future will show whether the development of inhibitors of other cunning fox(o)es, which promote leukemic progression, can make the hare,

that is, HDAC and other leukemic stem cell targets, finally, run away.

Conflict-of-interest disclosure: D.S.K. declares no competing financial interests. ■

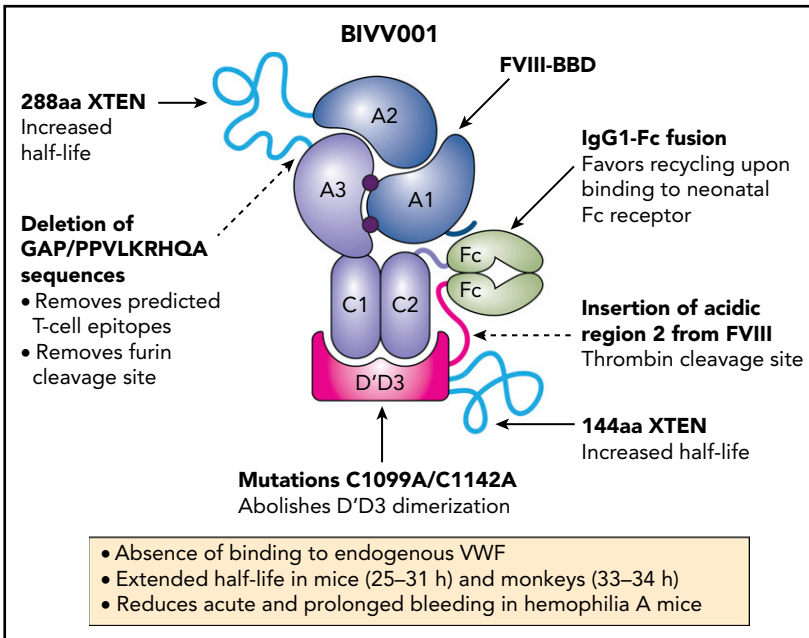### THROMBOSIS AND HEMOSTASIS

A molecular jewel for hemophilia A treatment

Julie Rayes[1] and Sébastien Lacroix-Desmazes[2] | [1]University of Birmingham; [2]INSERM

In this issue of *Blood*, Seth Chhabra et al describe the engineering of a new therapeutic chimera composed of B domain–deleted (BDD) factor VIII (FVIII), FVIII-binding domain of von Willebrand factor (VWF), Fcγ1 fragment, and XTEN polypeptides.[1] The molecule, referred to as rFVIIIFc-VWF-XTEN or BIVV001, has a prolonged half-life that is independent from endogenous VWF and is hemostatically competent.

Hemophilia A is a rare X-linked inherited hemorrhagic disorder resulting from deficiencies in procoagulant FVIII. Prevention or treatment of bleeding episodes is achieved by use of FVIII-derived products or bypassing agents. Expectedly, no FVIII-surrogate drug

BIVV001

- **288aa XTEN** Increased half-life
- **Deletion of GAP/PPVLKRHQA sequences**
  - Removes predicted T-cell epitopes
  - Removes furin cleavage site
- **FVIII-BBD**
- **IgG1-Fc fusion** Favors recycling upon binding to neonatal Fc receptor
- **Insertion of acidic region 2 from FVIII** Thrombin cleavage site
- **144aa XTEN** Increased half-life
- **Mutations C1099A/C1142A** Abolishes D'D3 dimerization

- Absence of binding to endogenous VWF
- Extended half-life in mice (25–31 h) and monkeys (33–34 h)
- Reduces acute and prolonged bleeding in hemophilia A mice

Engineering of a new therapeutic FVIII chimera BIVV001. BIVV001, or rFVIIIFc-VWF-XTEN, results from the fusion of BDD-FVIII with the D'D3 region of VWF, the Fc fragment of human IgG1 and 2 XTEN polypeptides. The engineered molecule lacks the capacity to bind endogenous VWF and has a prolonged half-life. To achieve this, several modifications were implemented: (1) 2 mutations in C1099A and C1142A were introduced in the D'D3 domain of VWF to prevent its dimerization; (2) fusion of the VWF propeptide D1D2 to the D'D3 domain to secure D'D3 optimal folding and to increase its affinity for FVIII (not depicted on the figure); (3) insertion of a 288-amino-acid (aa)-long XTEN between the A2 and A3 domains of FVIII and of a 144-aa-long XTEN between D'D3 and Fc domains to extend the half-life of FVIII; (4) insertion of the FVIII a2 thrombin site to allow optimal FVIII dissociation from D'D3 at the time of activation by thrombin; (5) removal of 3 aa residues (GAP) from the FVIII/XTEN junction and of 9 aa (PPVLKRHQR) from the N-terminal end of the FVIII B domain linker to (i) eliminate predicted major histocompatibility complex II–binding T-cell epitopes and (ii) remove the furin cleavage site between the heavy and light chains of FVIII to ensure its production as a single polypeptide; (6) expression of the FVIII-XTEN-Fc portion as a first chain and of the D'D3-XTEN-Fc portion as a second chain, both held together by disulfide bonds in the Fc region. The goal here is to stabilize the binding of the D'D3 domain to FVIII, to prevent binding of FVIII to the endogenous VWF, and to allow binding of the chimeric molecule to the FcRn. Binding to the FcRn prevents the complex from lysosomal degradation and fosters recycling toward the circulation. BIVV001 is secreted as a single-chain FVIII rather than as a heterodimer. It has a prolonged half-life, is hemostatically functional, and corrects bleeding time in hemophilia A mice.

is as efficacious as FVIII in performing all of the hemostatic functions of FVIII. Hence, primary prophylaxis with FVIII products remains the treatment of choice for patients with severe hemophilia A.[2] However, the use of therapeutic FVIII meets with 2 major hurdles: (i) the short half-life of the molecule (ie, 12 to 19.7 hours) that imposes repeated administration of FVIII to ensure optimal joint health and patients' quality of life, and (ii) the immunogenicity of the molecule that leads to the development of neutralizing anti-FVIII immunoglobulin G (IgG) in ~30% of patients. With an estimated half-life of 33 to 34 hours in nonhuman primates, BIVV001 represents a breakthrough in the field.

Previous attempts to generate long-lasting FVIII molecules have exploited the Fc fusion or PEGylation technologies. Although the clinical use of extended half-life FVIII products has led to an at least 30% reduction in the frequency of IV injections,[2] half-life extension is limited (1.3- to 1.7-fold on average) and, in the case of Fc-fused products, is far from the expected 3-week-long half-life of human IgG. One of the reasons is that Fc-fused and PEGylated FVIII products retain their capacity to bind VWF, the chaperon of FVIII.[3] VWF protects FVIII from proteolytic degradation and unwanted activation, controls its catabolism by preventing its binding to catabolic receptors, transports FVIII to the bleeding sites, and reduces FVIII immunogenicity by reducing its endocytosis by antigen-presenting cells. The other side of the coin however is that VWF dictates FVIII half-life, notably by acting as a sink and driving the elimination of VWF-bound FVIII upon interaction with VWF-specific catabolic receptors. As a result, the half-life of FVIII-derived molecules able to bind VWF is not expected to exceed that of VWF (ie, 9 to 15 hours). This limitation is levered in BIVV001.

BIVV001 is the outstanding achievement of rational protein engineering (see figure). In the molecule, modification of the D'D3 region of VWF by mutation of cysteine residues prevents the formation of D'D3 dimers, whereas the *cis*-addition of the VWF propeptide D1D2 ensures optimal folding of D'D3 and highest affinity for FVIII. Fusion of the BDD-FVIII moiety and of the modified D1D2D'D3 moiety to the Fc fragment of human IgG1 further stabilizes the association between D'D3 and FVIII by virtue of the disulfide bonds between the Fc fragments. It also favors the binding of the molecule to the neonatal Fc receptor (FcRn), which guides the intracellular routing of the endocytosed molecules away from lysosomal degradation pathways and promotes recycling to the circulation.[4] Importantly, insertion of a 288-amino-acid-long polypeptide (referred to as XTEN) between the A2 and A3 domains of FVIII and of a 144 XTEN between the D'D3 and Fc domains confers increased solubility and reduces in vivo clearance. Insertion of the FVIII acidic region 2 (a2) thrombin cleavage site between the D'D3 and Fc domains ensures optimal release kinetics of activated FVIII. Last, removal of the PPVLKRHQR sequence N-terminal to the A3 domain eliminates the furin cleavage site between the heavy and light chains of FVIII, thus fostering production of FVIII as a single polypeptide. Together, iterative steps of rational molecular improvements have generated a technological jewel with normal procoagulant FVIII activity that does not bind to endogenous VWF and is endowed with a truly extended half-life in both mice and nonhuman primates. Of note, the achieved half-life is shorter than that of a fusion D'D3-Fc,[5] suggesting that a deeper understanding of FVIII biology should allow further improvement of its pharmacokinetics in the future. Of course, the half-life of BIVV001 is also much shorter than that of emicizumab, a recombinant bispecific antibody that mimics the cofactor function of activated FVIII. In contrast to emicizumab,[6] however, BIVV001 is expected to provide full protection under conditions of severe hemostasis challenge, such as trauma, major surgery, or strenuous physical activity.

Whether the increase in pharmacokinetics of BIVV001 is accompanied by a reduction in its immunogenicity remains to be determined. The predicted T-cell epitope PPVLKRHQR present in the C-terminal

region of the B domain of the commercially available rFVIIIFc, Eloctate (patent CA2841066A1), has been removed in BIVV001. In addition, BIVV001 contains 2 XTEN polypeptides that, in essence, are devoid of T-cell epitopes and are claimed to reduce the immunogenicity of the molecules they are fused to.[7] Furthermore, the binding of FVIII to the D'D3 domain of VWF reduces its endocytosis by antigen-presenting cells in vitro,[8] and the presence of VWF in FVIII products was proposed to reduce FVIII immunogenicity in vivo.[9] Taken together, this evidence suggests that the immunogenicity of BIVV001 should not be greater, and may be lower, than that of Eloctate (clinical trial #NCT02196207). Whether D'D3 alone may prevent FVIII uptake to a similar extent as the entire VWF molecule is unknown. Importantly, although the endocytosis of FVIII by antigen-presenting cells is necessary to initiate neutralizing anti-FVIII humoral immune responses, it is also a prerequisite to the induction of active FVIII-specific immune tolerance and to the maintenance of established immune tolerance. Future studies will need to investigate whether BIVV001 shows an altered immunological profile as compared with FVIII products with physiological affinities toward VWF. Will BIVV001 be able to induce FVIII-specific tolerance as we believe is spontaneously happening in ~70% of severe patients during standard FVIII replacement therapy?[10] Will BIVV001 allow maintenance of protective tolerance in hemophilia A patients who have developed active immune tolerance to therapeutic FVIII, either spontaneously or after immune tolerance induction by high-dose FVIII?

Conflict-of-interest disclosure: J.R. declares no competing financial interests. S.L.-D. is the recipient of a research grant from Sanofi Genzyme and Sobi. ∎

REFERENCES

1. Seth Chhabra E, Liu T, Kulman J, et al. BIVV001, a new class of factor VIII replacement for hemophilia A that is independent of von Willebrand factor in primates and mice. *Blood.* 2020;135(17): 1484-1496.

2. Mannucci PM. Benefits and limitations of extended plasma half-life factor VIII products in hemophilia A. *Expert Opin Investig Drugs.* 2020;29(3):303-309.

3. Hartholt RB, van Velzen AS, Peyron I, Ten Brinke A, Fijnvandraat K, Voorberg J. To serve and protect: The modulatory role of von Willebrand factor on factor VIII immunogenicity. *Blood Rev.* 2017;31(5): 339-347.

4. Blumberg RS, Lillicrap D; IgG Fc Immune Tolerance Group. Tolerogenic properties of the Fc portion of IgG and its relevance to the treatment and management of hemophilia. *Blood.* 2018;131(20):2205-2214.

5. Yee A, Gildersleeve RD, Gu S, et al. A von Willebrand factor fragment containing the D'D3 domains is sufficient to stabilize coagulation factor VIII in mice. *Blood.* 2014; 124(3):445-452.

6. Lenting PJ, Denis CV, Christophe OD. Emicizumab, a bispecific antibody recognizing coagulation factors IX and X: how does it actually compare to factor VIII? *Blood.* 2017; 130(23):2463-2468.

7. Podust VN, Balan S, Sim BC, et al. Extension of in vivo half-life of biologically active molecules by XTEN protein polymers. *J Control Release.* 2016;240:52-66.

8. Dasgupta S, Repessé Y, Bayry J, et al. VWF protects FVIII from endocytosis by dendritic cells and subsequent presentation to immune effectors. *Blood.* 2007;109(2): 610-612.

9. Peyvandi F, Mannucci PM, Garagiola I, et al. A randomized trial of factor VIII and neutralizing antibodies in hemophilia A. *N Engl J Med.* 2016;374(21):2054-2064.

10. Varthaman A, Lacroix-Desmazes S. Pathogenic immune response to therapeutic factor VIII: exacerbated response or failed induction of tolerance? *Haematologica.* 2019; 104(2):236-244.