

# Advances in Clotting Factor Treatment for Congenital Hemorrhagic Disorders

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## Abstract

During the last 50 years, clotting factor replacement has evolved from the use of frozen plasma in the 1950s, through the serendipitous discovery of cryoprecipitate in the 1960s and the development of purified clotting factors in the 1970s and 1980s, to the era of recombinant clotting factors beginning in the 1990s. The dawn of the new millennium has seen the refinement of recombinant factor (rF) VIII with enhanced safety via the elimination of plasma-derived culture media or product stabilizers. During the last decade of the 20th century, a cure for hemophilia through gene therapy became a possibility. This was, in part, facilitated by availability of large (dogs) and small (mice) animal models for hemophilia A and B. Although this review will focus primarily on clotting factor replacement, the reader may refer to recent discourse on gene therapy for hemophilia.<sup>1</sup>

## Evolution of FVIII Replacement Therapy for Hemophilia A

It is difficult to quantify which advance—the heat inactivation of plasma-derived clotting factor (pdCF) or the development of recombinant factor (rF) VIII—has had a greater impact on the safety of clotting factors for patients with hemophilia. While heat treatment (and subsequently pasteurization and solvent-detergent treatment) has virtually eliminated the risk of plasma-transmitted, potentially fatal viruses, the development of recombinant clotting factors (rCFs) has allayed most fears of pathogen transmission. The excitement over rFVIII was initially tempered by early suspicions that hemophiliacs using rFVIII, especially previously untransfused patients (PUPs), were at an increased risk of inhibitor development. Early postmarketing surveillance<sup>2,3</sup> suggested that risk of persistent inhibitor development is not greater than that for pdFVIII users. Recently, a retrospective comparison study has again resurrected a new suspicion that rFVIII preparations<sup>4,5</sup> induce neutralizing antibodies at a higher rate than pdFVIII. As it may be difficult to conduct prospective randomized studies in PUPs, intensive surveillance studies may be the only mechanism to corroborate this information.

The realization that the first generation of rFVIII (Recombinate [Baxter], Bioclote [Baxter], Kogenate [Bayer], Helixate [Bayer]) stabilizers, primarily human plasma-derived albumin, contained a human blood derivative fueled the new quest to develop a “human plasma-free” rFVIII. The next step toward that goal was licensure in 2000 of the first “albumin-free” rFVIII, Kogenate FS (Bayer). This so-called second or next generation rFVIII incorporated a filtration step to essentially remove the human pd-albumin from the supernatant and add sucrose as the principle stabilizer. Shortly thereafter, another second-generation rFVIII, Refacto (Wyeth), was licensed in the United States in early 2001.

During the same period of time, the hemophilia community experienced a profound shortfall in the supply of rFVIII. The reasons for the shortage are not entirely clear, but both the increasing demand and an abrupt alteration in the supply of at least one rFVIII manufacturer/distributor certainly influenced the situation.

The rFVIII shortage resurrected questions regarding the necessity and virtue of rFVIII over pdFVIII, as many prescriptions for rFVIII were changed to pdFVIII during that

## Keywords

Clotting factor, recombinant clotting factor, rFVIII, hemophilia

time. Clearly, the safety profiles of pdFVIII of the current era are virtually unblemished; however, the prospect, even if only theoretical, of a new and possibly resistant pathogen entering the plasma pool haunts many patients who have witnessed or themselves survived past catastrophes.

Although the ideal of rFVIII availability for the global hemophilia community is worthwhile, for many parts of the world the goal of widespread availability and use of rFVIII is economically unreachable. Thus, the importance of continued safety efforts for pdFVIII is arguably as important as emerging safety advances for rFVIII.

Along with these efforts, the hemophilia community in developed nations has further advocated for a “third generation” or “new category” of rFVIII manufactured, processed, and formulated without the addition of human or animal plasma reagents. The aim was supported by a major medical advisory group in the United States.<sup>6</sup> The first rFVIII of its kind, Advate (Baxter Bioscience), was recently licensed in the United States. The manufacture of Advate uses serum-free media in its cell culture, a disaccharide stabilizer and a 3-step purification process, including an affinity chromatography step. For added safety, a solvent-detergent treatment step is incorporated after purification. At the time of this writing, another plasma/albumin-less rFVIII manufactured by Wyeth is in phase II/III clinical trials. This product, preliminarily known as “Refacto AF,” uses a similar serum-free media in its cell culture, a disaccharide stabilizer, and a ligand-affinity filtration step during purification. The characteristics of all rFVIII and ultra-high purity pdFVIII products, licensed and available in the United States are listed in Tables 1 and 2, respectively.

The virtue of the B-domain of the FVIII molecule in natural biology and in rFVIII preparations has created discussion in recent years. Toole et al<sup>7</sup> observed that the B-domain could be eliminated from the FVIII molecule without sacrificing procoagulant activity. This finding facilitated a more efficient gene transfer into host cells for recombinant FVIII

production and, subsequently, early gene therapy trials. B-domain-less rFVIII expression in transfected cells is known to be more efficient than for the “full length” transcript. The first “B-domain-deleted” rFVIII was licensed in the United States in early 2001.

The case for the B-domain lies in its putative chaperone protein-binding function and its potential role in thrombin activation. As it pertains to rFVIII products only the latter would potentially carry clinical significance. Although no substantiated difference in the clinical efficacy of full-length and B-domain-deleted rFVIII has been codified, at least 1 anecdotal report implies that the efficacy of B-domain rFVIII may be inferior in patients with hemophilia A.<sup>8</sup> Whether the perceived difference in clinical efficacy is real or a function of assays used to measure activity is not entirely known. To date, no prospective, direct comparison of clinical efficacy between full-length rFVIII and B-domain-deleted rFVIII has occurred.

What the future holds for rFVIII technology will likely focus less on pathogen safety, now that the manufacturing process has virtually eliminated the use of human or animal plasma-derived reagents. Future challenges to optimize rFVIII will concentrate on more efficient expression in cell culture, a longer duration of biological activity of the infused molecules, protection from alloantigenicity, and a more convenient route of administration.

As mentioned above, the omission of the FVIII B-domain enhances expression of rFVIII 20-fold.<sup>9</sup> In addition, reducing the binding of translated rFVIII to chaperone proteins may increase secretion from the endoplasmic reticulum and thus increase expression. Early attempts at substituting the BiP binding region of FVIII with homologous residues from FV saw an increase in secretion at the expense of procoagulant cofactor function. Swaroop et al<sup>10</sup> found that more specific, site-directed mutagenesis of Phe309 to Ala or Ser led to enhanced secretion and retention of cofactor function.

**Table 1.** Licensed, Available rFVIII in the United States\*

Brand name	Manufacturer	Cell culture/medium	rFVIII molecule	Purification/viral reduction	Initial/final stabilizer(s)	Activity <sup>†</sup>	Reconstitution volume
<b>First generation</b>							
Recombinate	Baxter	CHO/FCS	Full length	Filtration/IA/IE	rvWF, hu-pd-Alb	>4,000	10 mL/vial
<b>Second generation</b>							
Kogenate FS	Bayer	BHK/PPM	Full length	Filtration/IA/IE/SD	hu-pd-Alb/sucrose	4,000	2.5 mL/vial
(Helixate Next Gen)	Bayer for Aventis	BHK/PPM	Full length	Filtration/IA/IE/SD	hu-pd-Alb/sucrose	4,000	2.5 mL/vial
Refacto	Wyeth	BHK/FCS	B-domain-deleted	Filtration/IA/IE	hu-pd-Alb sucrose	13,000	4 mL/vial
<b>Third generation</b>							
Advate	Baxter	CHO/serum-free	Full length	Filtration/IA/IE/SD	rvWF, trehalose/trehulose	4,000-10,000	5 mL/vial

\*Adapted from Kasper CK, Costa E, Silva M. Registry of clotting factor concentrates, update of June 2001. *Hemophilia Bulletin*. 2001.

<sup>†</sup>Specific activity=IU/mg protein excluding albumin.

rFVIII=recombinant factor VIII; CHO=Chinese hamster ovary; FCS=fetal calf serum; IA=immunoaffinity; IE=ion-exchange; rvWF=recombinant von Willebrand factor; hu=human; pd=plasma-derived; Alb=albumin; BHK=baby hamster kidney; PPM=plasma protein mixture; SD=solvent/detergent treatment.

**Table 2.** Licensed pdFVIII in the United States\*

Brand name	Manufacturer	Purification	Viral reduction	Activity <sup>†</sup>	Reconstitution volume
<b>Intermediate purity</b>					
Humate P	Aventis Behring	Multiple precipitation	P	36	20 mL/vial
Koate DVI	Bayer	Precip. + size chrom	Heat/SD	50	5–10 mL/vial
<b>High purity</b>					
Alphanate	Grifols	Heparin AC	Heat/SD	140	5–10 mL/vial
<b>Ultra-high purity</b>					
Hemofil M	Baxter	IA/IE	SD	>2,000	10 mL/vial
Monarc M	Baxter for ARC	IA/IE	SD	>2,000	10 mL/vial
Monoclate P	Aventis Behring	IA	P	>3,000	2.5–10 mL/vial

\* Adapted from: Kasper CK, Costa E, Silva M. Registry of clotting factor concentrates, update of June 2001. *Hemophilia Bulletin*. 2001.

<sup>†</sup> Specific activity=IU/mg protein excluding albumin.

pdFVIII=plasma-derived factor VIII; P=pasteurization; SD=solvent/detergent treatment; AC=affinity chromatography; IA=immunoaffinity; IE=ion-exchange.

The prospect of prolonging the half-life of rFVIII rests in part on the ability to protect the molecule from proteolysis by activated protein C (APC) and thrombin, and by blocking catabolism by lipoprotein receptor-related protein (LRP).<sup>11</sup> These and other approaches to altering these complex interactions and to prolonging the survival of secreted FVIII, without compromising procoagulant activity nor increasing antigenicity, are eloquently reviewed by Saenko et al.<sup>11</sup>

## Evolution of FIX Replacement Therapy for Hemophilia B

Unlike FVIII, FIX is not present in appreciable amounts in cryoprecipitate or, for that matter, in whole plasma. The initial preparations of concentrates of FIX from plasma or the supernatant of cryoprecipitate were in combination with FX, FVII, FV, and prothrombin and called prothrombin complex concentrates (PCC) or FIX complex concentrates. Through the 1970s and 1980s these products were the mainstay for FIX replacement therapy for patients with hemophilia B. The emergence of blood-borne infections led to modifications in PCCs to attenuate potential viral contamination. It was not until the late 1980s and early 1990s that so-called high-purity FIX concentrates were licensed. These formulations had much higher specific activity of FIX in the range of 100–300 IU/mg protein. Assumptions that these high-purity products were to be less thrombogenic than PCCs were supported by several reports.<sup>12–14</sup>

The cDNA for FIX was cloned in the 1980s, the FIX gene was sequenced in the mid-1980s, and FIX was expressed in recombinant Chinese hamster ovary cells shortly after that. Almost a decade later the first and only rFIX with high specific activity was licensed in the United States, manufactured by Wyeth using a recombinant process that does not utilize stabilizers or culture media derived from human plasma. Unlike pdFIX preparations, the adjusted recovery of FIX activity following a bolus infusion was found to be lower than the typical 1 U/dL/Unit/kg infused, leading to a 20% increase in the unit dose recommendations by

the manufacturer. This was illustrated in a pharmacokinetic comparison trial reported by Ewenstein et al<sup>15</sup> that suggested a wide interpatient variability in FIX recovery, regardless of FIX source, but a significantly higher adjusted recovery for pdFIX. The mechanism for this discrepancy is not entirely understood but may be due to differences in posttranslational modification between pdFIX and rFIX, as suggested by White et al.<sup>16</sup> As such, cogent arguments supporting the use of pdFIX revolve around cost, while those in favor of rFIX cite potential added safety. Table 3 lists the characteristics of high-purity pdFIX and rFIX preparations available in the United States.

## Clotting Factors for the Treatment of Hemophilic Inhibitors

Treatment of bleeding episodes in patients with hemophilic inhibitors remains a challenge. Early clotting factor products designed to bypass the neutralized FVIII or FIX, as shown in Table 3, were of low or intermediate purity, containing FVII, FV, FX, FII, FIX, and FVIII. Intentional activation during production of PCCs produces the so-called activated PCCs (aPCCs). Although aPCCs are thought to control acute hemorrhage more effectively, no definitive comparative efficacy trial has been conducted. Furthermore, no specific laboratory test predicts clinical efficacy. Occasionally, these products precipitate thrombi or thromboemboli<sup>17,18</sup> and must be used with caution.

Most FVIII inhibitors do not have appreciable cross-reactivity with porcine FVIII. In the 1980s, a high-purity porcine FVIII was developed to treat or prevent hemorrhage in patients with congenital or acquired hemophilia A.<sup>19–21</sup> Unfortunately, contamination of the product with porcine parvovirus has limited its availability and use. A recombinant version of porcine FVIII is currently under development.

Imploring the extrinsic coagulation pathway to bypass neutralizing alloantibodies in the intrinsic pathway forms the basis of using FVII or FVIIa to treat hemorrhage. Currently,

**Table 3.** Licensed High-Purity FIX and rFIX in the United States\*

Brand	Manufacturer	Purification	Viral reduction	Activity <sup>†</sup>	Reconstitution volume
Alphanine SD	Grifols	IE/Carb ligand	SD	210	10 mL/vial
Mononine	Aventis Behring	AC	NaSN/nF	>190	2.5–10 mL/vial
Recombinant					
Benefix	Wyeth	recombinant	nF	≥200	5–10 mL/vial

\* Adapted from: Kasper CK, Costa E, Silva M. Registry of clotting factor concentrates, update of June 2001. *Hemophilia Bulletin*. 2001.

<sup>†</sup> Specific activity=IU/mg protein excluding albumin.

FIX=factor IX; rFIX=recombinant FIX; IE=ion-exchange; Carb=carbohydrate; SD=solvent/detergent treatment; AC=affinity chromatography; NaSN=sodium thiocyanate; nF=nanofiltration.

one product licensed in the United States, Europe, and Japan is NovoSeven, a recombinant FVIIa manufactured by Novo Nordisk. Through clinical trial experience in surgery,<sup>22</sup> home therapy for hemorrhage,<sup>23</sup> and a large compassionate-use trial,<sup>24</sup> rFVIIa has established itself as one of the front-line agents to control bleeding in patients with congenital or acquired hemophilia with inhibitors. Although dose-finding studies were part of rFVIIa development, hemophilia treaters have continued to search for the optimal dose schedule.<sup>25</sup> Unlike aPCCs or PCCs, rFVIIa is a recombinant product, formulated without plasma-derived stabilizers. However, like aPCCs or PCCs, no laboratory tests have correlated with clinical effectiveness, and thromboemboli have been reported after rFVIIa use.<sup>22</sup> The comparative efficacy, safety, and thrombogenicity between PCCs, aPCCs, and rFVIIa are unknown. However, at least 1 clinical trial is in progress to address these outstanding issues.

Strategies to reduce the titer of neutralizing antibodies in hemophilia patients with inhibitors, including high- or low-dose FVIII, multimodal immunosuppressive therapy, and immunoadsorption therapy, are considered in 3 landmark studies<sup>26-28</sup> and 2 registry reports.<sup>29,30</sup> The dose intensity of FVIII in immune tolerance regimens is the subject of an intercontinental clinical trial currently underway.

### The Treatment of von Willebrand Disease

The estimated prevalence of inherited von Willebrand disease (vWD) is 1%, making the diagnosable disorder approximately 50 times more prevalent than hemophilia. Unlike hemophilia, most afflicted with vWD have relatively mild clinical manifestations, and most may not realize their affliction. Perhaps because of the unfamiliarity of many clinicians with vWD, many patients may have “subdiagnostic” vWD as opposed to “subclinical” vWD. This may be best illustrated in recent studies<sup>31-34</sup> suggesting that a considerable proportion of women with menorrhagia fit the diagnostic criteria for vWD.

Nevertheless, awareness of vWD is increasing, reflected in increased enrollment of patients at hemophilia treatment centers in the United States and abroad.<sup>35</sup> As new cases of vWD are often diagnosed around the context of planned surgical procedures, one may speculate that the use of von Willebrand factor (vWF) clotting preparations is also increasing. With that, one may further estimate the scope

and impact of this increased recognition by quantifying the use of vWD-specific clotting factors. At the Comprehensive Bleeding Disorders Center in Peoria, IL, the number of registered patients with vWD and the amount of vWD-specific clotting factor prescribed has nearly doubled in the past 3 years.

The treatment of bleeding associated with vWD was improved with the discovery of cryoprecipitate in the 1960s.<sup>36</sup> Cryoprecipitate was considered frontline treatment until the 1990s and is still used by some clinicians today. Several plasma-derived FVIII replacement products of intermediate or high purity are known to contain considerable titers of vWF monomer and multimers. These agents—Humate P (Aventis Behring), Alphanate (Grifols), and Koate DVI (Bayer)—are listed in Table 2. Only Humate P has a US Food and Drug Administration indication for use in vWD.

Another important advance in the treatment of vWD (and mild hemophilia A) emerged in the late 1970s with the discovery that the synthetic vasopressin analog 1-deamino-8-d-arginine vasopressin (ddAVP; DDAVP, Aventis) administered intravenously in patients with moderate or mild hemophilia led to an increase in plasma FVIII and vWF activity.<sup>37</sup> This fortuitous finding was subsequently applied to patients with type I vWD and, hence, is now widely used for most types of vWD (excluding type IIb and platelet-type vWD) via intravenous or intranasal injection.<sup>38,39</sup> As suggested recently by Federici et al,<sup>40</sup> the response to ddAVP in patients with vWD may be “disease-severity dependent.” Therefore, all patients should be given a challenge dose including documentation of the laboratory response before prescribed use.

Technological advances in the replacement of vWF have lagged behind those for FVIII or FIX. vWD is a more complex, larger molecule that undergoes extensive post-translational modification and multimerization. Thus, the technical considerations in developing a recombinant vWF are considerably more involved than for FVIII or FIX. Having said that, the cDNA or vWF has been cloned<sup>41</sup> and the transcript translated in stable transfectants that produce rFVIII.<sup>42,43</sup> If indeed an rvWF is producible, other physiological considerations would need to be clarified. For example, would rvWF require its natural passenger,

FVIII, for efficient recovery and clearance? Nevertheless, it would appear that the steps to arrive at a usable recombinant vWF are not out of reach.

### Treatment of Rare Bleeding Disorders

Thousands of patients suffer from what are termed “rare” (perhaps more correctly termed “more rare”) bleeding disorders. For virtually every procoagulant, fibrinolytic, and anti-fibrinolytic element described, a corresponding deficiency state has also been described. For treatment of hemorrhage in these disorders, physicians often rely on frozen plasma. With the exception of FXI, FXIII, and fibrinogen, for which plasma concentrates are under development, plasma continues to be the only medicinal source of replacement of FV, FII, and FX and other more rare coagulation protein deficiencies. A recent survey-based registry of over 200 hemophilia treatment centers<sup>44</sup> summarizes a broad clinical experience for patients with more rare bleeding disorders.

An increasing number of anecdotal reports suggest that rFVIIa affords adequate hemostasis for patients with rare bleeding disorders, including patients with inherited or acquired deficiencies of FXI,<sup>45,46</sup> FII,<sup>47</sup> FV,<sup>48</sup> and platelet function.<sup>49</sup> In these cases, it is thought that a local thrombin “burst” is generated at the platelet plug by tissue factor-independent binding of rFVIIa to the platelet surface, bypassing the need for factors or cofactors earlier in the coagulation cascade. How, exactly, this process facilitates hemostasis in patients with thrombocytopenia or platelet dysfunction is unclear.

### The Case of rCFs Versus pdCFs: The Different Plane of Points and Counterpoints

Should the medical community of hemophilia treaters, clotting factor manufacturers, and patients strive for the exclusive use of rCFs? The question stems from issues of feasibility (regarding disorders rarer than hemophilia), safety (regarding the potential and reality of emerging resistant pathogens) and economics (regarding whether all national economies can afford rCFs). As stated in the previous paragraph, for several bleeding disorders, more rare than hemophilia, plasma remains the only replacement product.

Are pdCFs completely safe from pathogen transmission? The medical vernacular and clotting factor manufacturers use terms such as “virtually” and “essentially no risk” when speaking of product safety. Rightly so, no one is willing to give a guarantee for fear of “potential future pathogens.” The pathogen safety history for rCFs, thus far, is untarnished. However, the recent report of “probable transmission of vCJD [variant Creutzfeldt-Jakob disease] to a blood recipient”<sup>50</sup> has already stirred the attention of the British government as well as the global hemophilia community. Few would argue that worldwide availability of “plasma-free” rFVIII and rFIX would improve safety and eliminate the risk of future unknown pathogens, such as that which causes vCJD, for patients with hemophilia A or B.

Despite the major advances in clotting factor technology, most patients with hemophilia in underdeveloped countries do not have access to optimal, or even any, clotting factor treatment for hemorrhagic episodes, much less for prophylaxis. Currently several pdFVIII and rFVIII preparations are simultaneously marketed in North America, Europe, and Japan. In these and many other countries, there remains heavy reliance on the availability of plasma and/or pdCFs to meet the needs of hemophilia patients. The unfortunate likelihood is that the supplication for all patients, worldwide, to have rCFs will not be realized.<sup>51,52</sup>

### Summary

Advances in the treatment of congenital and acquired bleeding disorders has leaped from the use of stored plasma to the refinement of pure individual rCFs in less than 4 decades. Improving the biological profiles of naturally occurring proteins is a formidable task. Nevertheless, the future will hopefully see the realization of safe clotting factors, accessible to all in need, conveniently administered, with substantially longer half-lives, and less or no immunogenicity.

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