USE OF RECOMBINANT HUMAN COAGULATION FACTOR VIII (ADVATE) TO TREAT HAEMOPHILIA A

REVIEW

HIGHLIGHTS

- Hemophilia A, also called factor VIII (FVIII) deficiency or classic hemophilia, is a genetic disorder caused by deficient or missing factor VIII, a major clotting protein. Thus, it is the majority of hemophilia cases (~85%), occurring at a frequency of roughly 1 out of 5,000 males worldwide.
- Currently, about 75% of the hemophilia community take a recombinant FVIII product. In which Octocog alfa antihaemophilic factor (trade name: Advate, rAHF-PFM) is a third-generation recombinant human full-length coagulation FVIII.
- The published data suggest that rAHF-PFM is safe and effective for the prevention and treatment of bleeding episodes and perioperative management in patients with hemophilia A.

ABSTRACT

Recombinant human coagulation factor VIII has become one of the major choices to treat hemophilia A since the introduction of plasma- or albumin-free purification technique. As the first third-generation recombinant human full-length coagulation factor VIII, Octocog alfa antihaemophilic factor (Advate, rAHF-PFM) has been gradually applied to treatment or prevention of bleeding episodes for patients with hemophilia A. To better recognize its efficacy and safety profiling as well as clinical applications in these patients, we systematically assessed all relevant studies. These studies indicated that rAHF-PFM is safe and effective for the prevention and treatment of bleeding episodes and preoperative management for patients with hemophilia A.

Bang-Shun He*, Yu-Qin Pan, Hong-Guang Xie
General Clinical Research Center, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, CHINA

*Corresponding author: hebangshun@163.com, **Tel.:** +86-25-52887029; **Fax:** +86-25-52269924

INTRODUCTION

Hemophilia A, an X chromosome-linked bleeding disorder, is caused by the mutations in the gene encoding coagulation factor VIII (also known as FVIII), which result in deficient or dysfunctional coagulant activity of the factor VIII. Hemophilia A, the majority of hemophilia cases (~85%), occurs at a frequency of roughly 1 out of 5,000 males worldwide [1,2]. According to a global survey across 109 countries conducted by the World Federation of Hemophilia (WFH), 142,205 people were diagnosed as hemophilia A, and 9,675 patients from China (http://www1.wfh.org/publications/files/pdf-1574.pdf). However, there were an estimated 80,000 - 120,000 patients with hemophilia in China (http://www.hemophilia.com.cn), but only 10,652 patients were registered online, of whom 9,055 (85%) were diagnosed with hemophilia A.

FVIII is a glycoprotein that acts as a cofactor responsible for the activation of factor X (FX) via activated factor IX (FIX), and therefore, deficient or dysfunctional FVIII can attenuate or abolish the formation of a normal clot at the site of injury [2-4] leading to various bleeding symptoms for affected patients. In clinical settings, the severity of hemophilia A is correlated negatively with the levels of FVIII activity. Therefore, hemophilia A can be categorized as mild, moderate, and severe, as measured with 5 - 40%, 1 - 5%, and <1% of the normal levels of the factor VIII, respectively. In China, patients were diagnosed as severe (25.7%), moderate (56.9%), and mild (17.4%), respectively, in an investigation of 926 cases [5].

Bleeding and bruising at the sites of the joints, soft tissues and muscles are two major clinical manifestations of hemophilia A. Therefore, it is not difficult to understand why patients with severe hemophilia have bleeding after injury, trauma or surgery, and spontaneous bleeding in the joints, muscles, and internal organs. In contrast, patients with moderate hemophilia may experience some bleeding episode only after injury, whereas patients with mild hemophilia may experience some bleeding episode after surgery or severe injury. However, addition of exogenous FVIII would be required for affected patients, regardless of knowledge of their severity of disease.

THE HISTORY OF TREATMENT OF HEMOPHILIA A

Alternative treatment with FVIII has long been the major treatment of choice for hemophilia A. This has evolved from the use of whole blood, fresh plasma, or cryoprecipitate to the use of highly concentrated and purified plasma-derived or recombinant relevant products. An important discovery in 1964 was that large amounts of FVIII concentrated in the fraction plasma cryoprecipitate made this replacement therapy of bleeding episodes more specific and effective than before [6], characterized by the transfusion from the infusion of fresh frozen plasma to the production of lyophilized concentrates of coagulation factors. Since then, the high-purity and more stably concentrated coagulation factors have been achieved by improving purification methods and adding additive chemicals. During the period of the 1970 - 1980s, the application of plasma-derived factor concentrates in therapy has reduced bleeding events and extended life expectancy of patients with hemophilia. Therefore, large quantity of such concentrates obtained from sufficiently pooled plasma from the donors is required to meet huge medical needs. Unfortunately, replace treatment of hemophilia with plasma-derived concentrates has resulted in dramatically increased infections of immunodeficiency virus (HIV), and consequently thousands of patients with hemophilia died of acquired immunodeficiency syndrome (AIDS) in the United States in the 1980 - 1990s due to widespread use of blood-derived commercial concentrates [7]. In addition, patients with hemophilia who received the replace treatment are at increased risk for infections of some other infectious diseases, such as hepatitis A (HAV), hepatitis B (HBV), or hepatitis C (HCV). However, the risk of viral transmission has been greatly reduced with the implementation of virucidal methods, such as pasteurization and nucleic acid amplification testing for the products of plasma-derived factor concentrates from human donated plasma [8, 9].

In the last two decades, blood-borne transmission of hepatitis viruses or HIV has been overcome mostly by the greatly improved safety of plasma-derived products [10]. On the other hand, with the rapid progress made in DNA technology, the recombinant FVIII (rFVIII) concentrates have been developed as a safer alternative to plasma-derived products since the FVIII-coding gene was cloned in 1984 [11-13] and the rFVIII can be produced by genetic engineering [14]. Three years later, the first clinical research study about the efficacy of the first-generation full-length rFVIII product was reported for two patients with hemophilia A [9]. The first- and second- generation products were prepared in a mammalian cell system, some of which contained human plasma proteins due to the use of human- or animal-derived materials, and therefore risk of transmission of pathogenic agents still exists, in particular previously unknown pathogens [15]. In contrast, the third-generation products do not use any additional animal or human proteins in the process of the production, almost eliminating all potential risk of transmission of blood-borne pathogens [16]. Accordingly, rFVIII concentrates, in particular their third-generation products (Table 1), have been proved to be safer alternatives to plasma-derived products.

Product (trade name)	Generation	Marketed year	Manufacturer	FVIII structure
Recombinate®	First	1992	Baxter Healthcare, USA	Full-length
KogenateFS®/Kogenate Bayer	Second	2000	Bayer Healthcare, Germany	Full-length
HelixateFS®/Helixate NexGen®	Second	--	CSL Behring, USA	Full-length
Xyntha®/Refacto AF®	Third	2008	Wyeth Pharmaceuticals, USA	B-domain deleted
Advate®	Third	2003	Baxter Healthcare, USA	Full-length

Table 1. Characteristics of currently available recombinant factor VIII concentrates
....

THE THIRD-GENERATION RECOMBINANT ADVATE

The plasma- and albumin-free, Octocog alfa antihaemophilic factor (trade name: Advate, rAHF-PFM) is a third-generation recombinant human full-length coagulation FVIII, without any human or animal blood derived additives throughout cell culture and formulation. rAHF-PFM, a dimeric glycoprotein that constitutes 2,332 amino acid residues with a molecular mass of approximately 280 kDa, has a similar amino acid sequence to that of human plasma derived FVIII. It is secreted by the CHO cells transfected with FVIII gene, and is purified from the culture medium by preparation chromatography (Figure 1) [17]. Further studies have documented that lyophilized rAHF-PFM is stable under the different conditions (e.g., 92% residual factor VIII activity at 5℃ for 30 months, 80% at room temperature for 18 months, and 84% at 40℃ for 3 months) [18] and is also stable during the period of continuous infusion [19]. In patient care, lyophilized rAHF-PFM is extensively used for the prevention and control of hemorrhagic episodes and perioperative management in patients with hemophilia A.

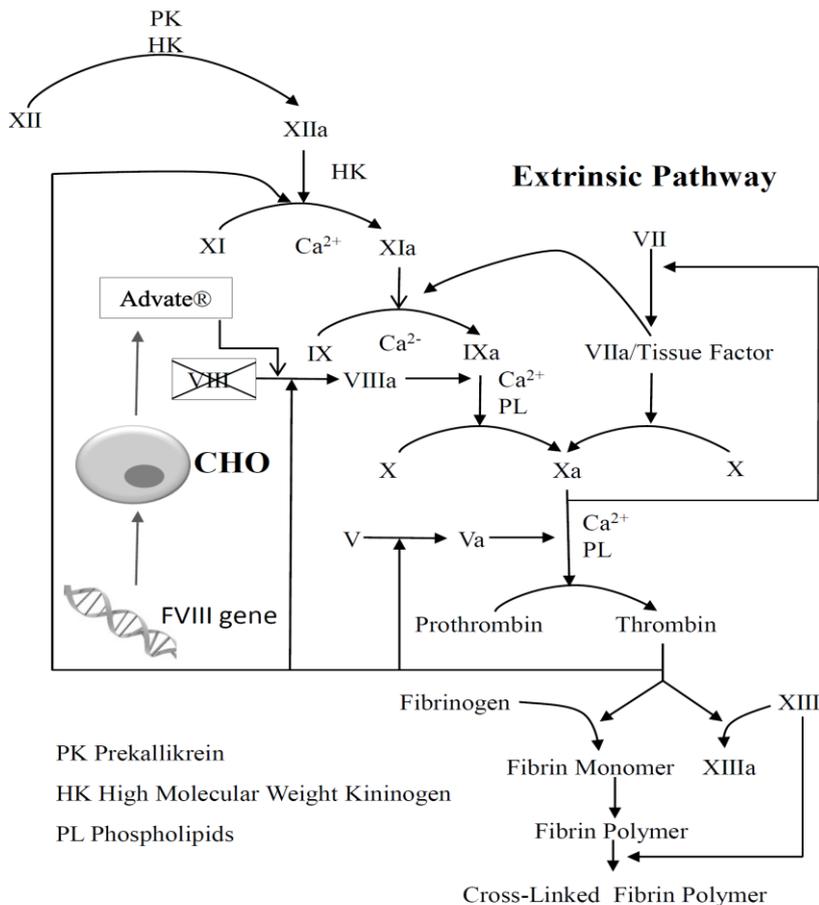**Figure 1. The use of rAHF-PFM (Advate) for hemophilia A patients by the substitution of endogenous coagulation factor VIII.**
....

The pharmacokinetic profile of rAHF-PFM is similar to that of Recombinate (a first-generation recombinant factor VIII antihaemophilic factor) [20], as estimated with area under the plasma concentration versus time curve (AUC), adjusted recovery, plasma half-life (t½), peak drug plasma concentrations (Cmax), mean residence time (MRT), and volume distribution at the steady-state (Vss) [17,21]. Further clinical studies have revealed that AUC, incremental recovery, and terminal phase t½ are lower and clearance is higher in infants and children than in adolescents and adults, as summarized in Table 2 [21-24]., and that the intrapatient variability of rAHF-PFM pharmacokinetics is uniformly less than the interpatient variability by comparison with short- and long-term exposure [24]. Patients receiving the same dosage of rAHF-PFM may have varying levels of FVIII. For infants or young children, the elimination rate of rFVIII (t½ or clearance) is associated with the annual incidence of all joint bleeds [23].; moreover, t½ and the intervals of dosing had a larger effect on FVIII trough concentrations and time per week with FVIII < 1 IU/dl than in vivo recoveries (IVR) and infused dose per kg. In addition, the relationship between the pharmacokinetics of rAHF-PFM and body weight is thought to be useful for the dose adjustments of rAHF-PFM to achieve adequate rAHF-PFM target levels [25]. Recently, Valentino et al reported that BAX 855, a pegylated form of unmodified rAHF-PFM, provided a longer duration of protection from haemarthrosis than did pretreatment with unmodified rAHF-PFM by improving the pharmacokinetic and pharmacodynamics properties [26].

Reference	Age (yrs)	N	AUC(0-48h) (IU.h/dL)	Recovery (IU/dL/kg)	Half-life (h)	Cmax (IU/dL)	MRT (h)	Vss (dL/kg)	CL (dL//h/kg)
Shapiro et al.[52]	0.08-2	8	1217 (312)	2.11 (0.51)	8.59 (1.34)	105 (26)	--	0.43 (0.09)	0.043 (0.009)
	2-12	55	1254 (468)	1.96 (0.48)	9.95 (1.91)	99 (27)	--	0.53 (0.12)	0.045 (0.015)
	12-16	28	1410 (527)	2.29 (0.57)	12.27 (3.61)	115 (29)	--	0.55 (0.11)	0.040 (0.014)
	> 16	76	1717 (497)	2.54 (0.60)	12.16 (3.02)	128 (30)	--	0.48 (0.10)	0.032 (0.010)
Blanchette et al.[53]	< 6 (3.1 ± 1.5)	52	1208 (391)	1.88 (0.42)	9.71 (1.89)	95.0 (22.4)	12.2 (3.1)	51.4 (12.3)	0.044 (0.014)
		47	1236 (401)	1.90 (0.43)	9.88 (1.89)	95.6 (23.3)	12.5 (3.1)	51.4 (12.9)	0.043 (0.014)
Dipaola et al.[54]	35.8 (19–72)	17	1380 (420)[a]	110.4 (25.3)	13.6 (3.8)	118 (25)	16.3 (5.0)	0.617 (0.018)	0.040 (0.014)
Tarantino et al.[55]	18 (10–65)	30	1534 (436)	2.4 (0.5)	12.0 (4.3)	120 (26)	15.69 (6.21)	0.47 (0.10)	0.03 (0.01)
Product information [b]	--	30	1534 (436)	2.41 (0.50)	11.98 (4.28)	120 (26)	15.68 (6.21)	0.47 (0.10)	0.03 (0.01)

Table 2. Pharmacokinetic parameters of Advate following a single infusion of 50 IU Kg^{-1} according to published literature. [a]AUC∞, area under the plasma concentration-time curve from zero to infinity; yrs, years; [b]Advate PI 08022013

As for the application of rAHF-PFM for the prophylaxis and treatment of bleeding episodes in patients with moderate or severe hemophilia A, accumulated data have suggested the efficacy of rAHF-PFM. Five prospective clinical research studies on efficacy of rAHF-PFM were pooled to show that 88% of 1,724 bleeding episodes were categorized as 'excellent/good', and that 90% of bleeding episodes were managed with one or two infusions. In addition, patients who received prophylactic therapy and complied with at least the minimum prescribed doses and infusion intervals had a significantly lower frequency of bleeding episodes than those who were noncompliant to the therapy [27]; moreover, a post hoc analysis has also revealed that compared with on-demand therapy, both standard factor (F) VIIII prophylaxis and pharmacokinetic-tailored prophylaxis with FVIII significantly reduced annualized joint bleeding rate [28]. In addition, a meta-analysis based on 120 patients revealed overall median annualized bleeding rate (ABR) was 2.0 [29]. All the released studies indicated that rAHF-PFM has acceptable effectiveness for on-demanded or prophylaxis treatment for hemophilia A in adults and children.

The safety of rAHF-PFM is also concerned in its clinical applications. Adverse events (AEs) and inhibitor are the two major aspects of its safety profiling. An updated study integrated analyses of 12 clinical interventional studies of rAHF-PFM used for haemophilia A, and showed that 93 AEs were reported to be related to use of rAHF-PFM in 45 of 418 patients (10.8%), of which most AEs were reported as FVIII inhibitors, pyrexia and headache, and 81.7% of the AEs were considered mild or moderated [30]. Moreover, another study based on a post-authorization safety study (PASS) global program has also demonstrated that 5 of 83 treatment-related AEs were reported to be serious AEs (with the exception of FVIII inhibitor development) in 5 patients (5/1188) [31]. These integrated studies showed the rarity of AEs after use of rAHF-PFM.

For patients with hemophilia A, the development of inhibitors remains the most serious complications that are frequently triggered by the treatment with FVIII replacement therapy. As reported, inhibitors occur in approximately 30% of individuals with severe hemophilia, and increase the risk of uncontrollable bleeding and disability, particularly arthropathy, making the treatment of bleeding episodes more difficult [32-34]. Regardless of the presence of little or no amount of any animal- or human-derived proteins during the process of production, FVIII inhibitors would be produced in patients with hemophilia A

treated with recombinant FVIII [35]. For rAHF-PFM, Tarantino et al. reported that approximately 1 of 108 previously treated patients was inhibitor-positive after 26-day exposure [21]. In contrast, Guenter et al. reported that the inhibitor was developed in 16 of 55 (29.1%) patients who received at least one infusion of rAHF-PFM, and that non-Caucasian ethnicity, and high intensity treatment were associated with high risk of inhibitor development [36]. Recently, a integrated analysis of safety data from 12 clinical interventional studies of rAHF-PFM for haemophilia A was reported, which documented that 4.06% (17/418) patients developed inhibitor [30], consistent with the results reported as 2% (21/1188) derived from a meta-analysis of ADVATE-PASS studies. In addition, none of 219 patients with low-titer inhibitors or inhibitor history, were found positive for a high-titer inhibitor during the period of study (an average of 196 days) [29]. Furthermore, in another study of 12 patients with high-titer inhibitors who were enrolled for received immune tolerance induction (ITI) by rAHF-PFM, a high incidence (75%) of tolerance for inhibitors was achieved, including 7 of 10 patients (70%) with high-titer inhibitors [37]. As summarized in Table 3, the accumulated clinical studies suggest that rAHF-PFM is a safe rFVIII for hemophilia A in patient care.

Reference	Characteristics of Patients	Exposure days (median)	No. of inhibitor tests (median)
Shapiro et al.* [30]	418 patients (median aged 18.7 yrs) from 12 interventional studies with FVIII levels <=2% of normal, including 55 PUPs/MTPs from all rAHF-PFM phase I-IV studies.	97.0 (1-709)	PTP:1/127(0.36%) PUP/MTP:16/55(29.1%)
Pollmann et al. [56]	152 patients, 69 % had severe HA, 116 (76.3%) patients aged≧16 yrs.	116 (1 – 642)	1/144 (0.69 %)
Auerswald et al. [57]	66 patients from 24 international sites, 55 (18 PUPs and 37 MTPs) received at least one infusion of rAHF-PFM.	498 (82 – 1,360)	16/55 (29.1%)
Zhang et al. [47]	58 patients, aged 7 - 53 (mean 24); weighing 47 - 75 (mean: 59.1) kg. Severe: 8(13.8%), moderately severe: 31 (53.5%), moderate: 14 (21.14%), HA: 5 (8.62%)	6 mos.	1/54 (1.85%)
Bacon et al. [58]	113 patients (>18 yrs: 76; < 18 yrs: 37; mild: 8, moderate: 4, severe: 101; prophylaxis: 71, OD: 42)	Most (>85%) > 100 EDs	1/113 (0.88%)
Shapiro et al.† [52]	234 patients (FVIII: C ≤2%) (median aged 14.7, range: 0.02- 72.7 yrs).	178 (10 – 598)	1/198 (0.51%)
Negrier et al. [59]	58 patients with age ≥5 years, baseline FVIII: C ≤2%, and ≥150 prior FVIII EDs	2 wks	0/65(0)
Blanchette et al. [53]	53 patients, aged 3.1 ± 1.5 yrs (< 3 yrs: 24; 3–5 yrs: 29) and 50 prior EDs; patients for OD: 5(9.4%), prophylaxis: 39(73.6%), both: 9(17.0%).	156 (14–384)	0/53(0)
Tarantino et al. [55]	Median aged 18 yrs, 96% of patients had baseline factor VIII <1%, and 108 received rAHF-PFM.	117	1/108(0.93%)

Table 3. Inhibitors derived from patients receiving rAHF-PFM according to the literature. HA: haemophilia A; PUP: previously untreated patient; IU, international unit; MTP: minimally treated patient; OD: on-demand; BI, bolus infusion; yrs, years; mos, months; wks: weeks; *Part of data were from Auerswald *et al.*, Shapiro *et al.*, Blanchette *et al.* and Tarantino *et al.* (2004); †Part of data were from Tarantino *et al.*, Blanchette *et al.* and Negrier *et al.*

CURRENT STATUS OF APPLICATIONS OF ADVATE IN CHINA

Hemophilia care in China has made rapid progress with the fast development of national economy and further improvement of healthcare conditions in the recent two decades. However, fundamental changes began in 1993 with the involvement of WFH to help set up a foundation of comprehensive care in China. In 2004, under the help of WFH, the Hemophilia Treatment Centers Collaborative Network of China (HTCCNC) constituting 6 centers (Beijing, Tianjin, Jinan, Hefei, Shanghai, and Guangzhou) was established to develop special strategies for improving hemophilia care, such as registration in Tianjin, nursing and prophylaxis in Guangzhou, laboratory diagnosis in Shanghai, and physiotherapy in Beijing. To date, the health care for hemophilia A in China has made greater achievements in the education for hemophilia A care [38,39], laboratory diagnosis [40-43], prenatal diagnosis [40], secondary prophylaxis [44], and comprehensive therapy [5].

Nowadays, like most other countries, replacement therapy of plasma-derived and rFVIII concentrates has been widely applied for patients with hemophilia A in China. However, plasma-derived FVIII concentrates produced by the China's companies possessed the major share of the marketed products, and patients with hemophilia A may be at increased risk of blood-borne infections. A retrospective study of Chinese patient cohort with hemophilia revealed that 12.62% of 926 patients were infected with HCV, and 0.22% HIV-positive [5], 4.5% - 10.4% HBsAg-positive, and 39.6% - 45.5% HCV-Ab-positive [45,46], rFVIII with lower risk of transmission of blood-borne infections has been accepted as the best choice for replacement therapy. Up to date, the rFVIII products that will be used in clinical settings in China are largely imported after official approval of the three categories of rFVIII (Kogenate FS®, in 2007; Advate® and Xyntha® in 2012). Currently, there are 2 available clinical research studies on rAHF-PFM in Chinese patients [5,47] due to the short history of use of rAHF-PFM. A multicenter prospective clinical study was reported by Zhang et al to evaluate the efficacy, safety and immunogenicity of rAHF-PFM in patients with

hemophilia A [47], in which 58 patients (8 severe, 45 moderate, and 5 mild) were enrolled for 6 months of treatment, and the response to the first rAHF-PFM treatment was grouped as either 'excellent' (82.8%) or 'improved' (17.2%) in all subjects, with an inhibitor of 4 Bethesda units measured in one patient at clinic visit on day 180 after discharge. A retrospective analysis of 1,226 Chinese patients with haemophilia reported that, of 102 patients treated with the third-generation rFVIII (Xyntha® or Advate®), inhibitors were measured in 3 patients (two from Advate® and one from both Advate® and Xyntha®) after rAHF-PFM treatment. In contrast, after 110 patients were treated with both plasma-derived FVIII and rFVIII, 8 were inhibitor-positive, but only one was derived from rAHF-PFM. The incidence of the inhibitors was ranked in an ascending order as follows: 2.9% for the third-generation FVIII (Advate® and Xyntha®), 10.5% for the first-generation FVIII, 11.0% for the second-generation FVIII, and 14.3% for plasma-derived FVIII. In general, the use of rAHF-PFM in Chinese patients with hemophilia A is well tolerated for the excellent safety and efficacy and low rate of inhibitor formation [48].

THE FUTURE OF HEMOPHILIA A TREATMENT

Haemophilia A is a genetic deficiency in clotting factor VIII. Currently, the alternative treatment with FVIII is the major choice for hemophilia A. Gene therapy could be an ideal treatment for this disease ultimately. In the past decades, clinical trials based on gene therapy have achieved a goal in the production of the active factor VIII mediated by the virus vector [49]. In the future, the development of genome editing technology [50], in particular the improvement of precise genome editing, will provide a useful method for the haemophilia A gene therapy [51]., which will make an era for haemophilia A patients.

In conclusion, rAHF-PFM has been shown to be safe and effective for the prevention and treatment of bleeding episodes and perioperative management in patients with hemophilia A. As prophylactic treatment regimen, rAHF-PFM is more effective in preventing bleeding episodes than on-demand therapy. In China, rAHF-PFM has been officially approved for patients with hemophilia A for two years according to its clinical safety and effectiveness in patient care.

CONFLICT OF INTEREST
The author declares no competing interests.

ACKNOWLEDGEMENT
None.

REFERENCES

[1]   Hoyer LW. Hemophilia A . N Engl J Med, 1994,330(1):38-47.

[2]   Bolton-Maggs PH, Pasi KJ. Haemophilias A and B . Lancet, 2003,361(9371):1801-1809.

[3]   Shen BW, Spiegel PC, Chang CH, et al. The tertiary structure and domain organization of coagulation factor viii . Blood, 2008,111(3):1240-1247.

[4]   Goodeve AC, Peake IR. The molecular basis of hemophilia A: Genotype-phenotype relationships and inhibitor development . Semin Thromb Hemost, 2003,29(1):23-30.

[5]   Xuan M, Xue F, Fu R, et al. Retrospective analysis of 1,226 chinese patients with haemophilia in a single medical centre . J Thromb Thrombolysis, 2013.

[6]   Pool JG, Gershgold EJ, Pappenhagen AR. High-potency antihaemophilic factor concentrate prepared from cryoglobulin precipitate . Nature, 1964,203(312.

[7]   Lee CA. The best of times, the worst of times: A story of haemophilia . Clin Med, 2009,9(5):453-458.

[8]   From the centers for disease control and prevention. Blood safety monitoring among persons with bleeding disorders--United States, May 1998-June 2002 . JAMA, 2003,289(5):541-543.

[9]   White GC, 2nd, McMillan CW, Kingdon HS, et al. Use of recombinant antihemophilic factor in the treatment of two patients with classic hemophilia . N Engl J Med, 1989,320(3):166-170.

[10]   Mannucci PM. Back to the future: A recent history of haemophilia treatment. Haemophilia, 2008,14 (Suppl 3):10-18.

[11]   Toole JJ, Knopf JL, Wozney JM, et al. Molecular cloning of a cDNA encoding human antihaemophilic factor . Nature, 1984,312(5992):342-347.

[12]   Gitschier J, Wood WI, Goralka TM, et al. Characterization of the human factor VIII gene. Nature, 1984,312(5992):326-330.

[13]   Vehar GA, Keyt B, Eaton D, et al. Structure of human factor VIII. Nature, 1984,312(5992):337-342.

[14]   Wood WI, Capon DJ, Simonsen CC, et al. Expression of active human factor viii from recombinant DNA clones . Nature, 1984,312(5992):330-337.

[15]   Meeks SL, Josephson CD. Should hemophilia treaters switch to albumin-free recombinant factor VIII concentrates . Current opinion in hematology, 2006,13(6):457-461.

[16]   Franchini M, Lippi G. Recombinant factor VIII concentrates . Semin Thromb Hemost, 2010,36(5):493-497.

[17] Ding P, Yang Y, Cheng L, et al. The relationship between seven common polymorphisms from five DNA repair genes and the risk for breast cancer in northern Chinese women. PLoS One, 2014,9(3):e92083.

[18] Parti R, Schoppmann A, Lee H, et al. Stability of lyophilized and reconstituted plasma/albumin-free recombinant human factor VIII (Advate rAHF-PFM). Haemophilia, 2005,11(5):492-496.

[19] Fernandez M, Yu T, Bjornson E, et al. Stability of advate, antihemophilic factor (recombinant) plasma/albumin-free method, during simulated continuous infusion. Blood Coagul Fibrinolysis. 2006,17(3):165-171.

[20] Shapiro AD. Anti-hemophilic factor (recombinant), plasma/albumin-free method (Octocog-alpha; Advate) in the management of hemophilia A. Vascular health and risk management, 2007,3(5):555-565.

[21] Tarantino MD, Collins PW, Hay CR, et al. Clinical evaluation of an advanced category antihaemophilic factor prepared using a plasma/albumin-free method: Pharmacokinetics, efficacy, and safety in previously treated patients with haemophilia A. Haemophilia, 2004,10(5):428-437.

[22] Blanchette VS, Shapiro AD, Liesner RJ, et al. Plasma and albumin-free recombinant factor VIII: Pharmacokinetics, efficacy and safety in previously treated pediatric patients. J Thromb Haemost, 2008,6(8):1319-1326.

[23] Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. J Thromb Haemost, 2009,7(3):413-420.

[24] Bjorkman S, Blanchette VS, Fischer K, et al. Comparative pharmacokinetics of plasma- and albumin-free recombinant factor VIII in children and adults: The influence of blood sampling schedule on observed age-related differences and implications for dose tailoring. J Thromb Haemost, 2010,8(4):730-736.

[25] Bjorkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor viii: The relationships of pharmacokinetics to age and body weight. Blood, 2012,119(2):612-618.

[26] Valentino LA, Cong L, Enockson C, et al. The biological efficacy profile of Bax 855, a pegylated recombinant factor VIII molecule. Haemophilia, 2015,21(1):58-63.

[27] Shapiro A, Gruppo R, Pabinger I, et al. Integrated analysis of safety and efficacy of a plasma- and albumin-free recombinant factor VIII (rAHF-PFM) from six clinical studies in patients with hemophilia A. Expert Opin Biol Ther, 2009,9(3):273-283.

[28] Valentino LA, Reyes CM, Ewenstein B, et al. Advate prophylaxis: Post hoc analysis of joint bleeding episodes. Haemophilia, 2014,20(5):630-638.

[29] Romanov V, Marcucci M, Cheng J, et al. Evaluation of safety and effectiveness of factor VIII treatment in haemophilia a patients with low titre inhibitors or a personal history of inhibitor. Patient data meta-analysis of rafh-pfm post-authorization safety studies. Thromb Haemost, 2015,114(1):56-64.

[30] Shapiro AD, Schoenig-Diesing C, Silvati-Fidell L, et al. Integrated analysis of safety data from 12 clinical interventional studies of plasma- and albumin-free recombinant factor VIII (rAHF-PFM) in haemophilia A. Haemophilia, 2015, 21(6):791-8.

[31] Iorio A, Marcucci M, Cheng J, et al. Patient data meta-analysis of post-authorization safety surveillance (PASS) studies of haemophilia A patients treated with rAHF-PFM. Haemophilia, 2014,20(6):777-783.

[32] Ingerslev J, Freidman D, Gastineau D, et al. Major surgery in haemophilic patients with inhibitors using recombinant factor VIIa . Haemostasis, 1996,26 (Suppl 1):118-123.

[33] Leissinger CA. Prevention of bleeds in hemophilia patients with inhibitors: Emerging data and clinical direction. Am J Hematol, 2004,77(2):187-193.

[34] Lusher JM. Inhibitor antibodies to factor VIII and factor IX: Management. Semin Thromb Hemost, 2000,26(2):179-188.

[35] Franchini M, Tagliaferri A, Mengoli C, et al. Cumulative inhibitor incidence in previously untreated patients with severe hemophilia a treated with plasma-derived versus recombinant factor VIII concentrates: A critical systematic review. Critical reviews in oncology/hematology, 2012,81(1):82-93.

[36] Auerswald G, Thompson AA, Recht M, et al. Experience of Advate rAHF-PFM in previously untreated patients and minimally treated patients with haemophilia A. Thromb Haemost, 2012,107(6):1072-1082.

[37] Valentino LA, Recht M, Dipaola J, et al. Experience with a third generation recombinant factor VIII concentrate (Advate) for immune tolerance induction in patients with haemophilia A. Haemophilia, 2009,15(3):718-726.

[38] Chen L, Sun J, Hilliard P, et al. 'Train-the-trainer': An effective and successful model to accelerate training and improve physiotherapy services for persons with haemophilia in China. Haemophilia, 2013, 20(3):441-5.

[39] Sun J, Hilliard PE, Feldman BM, et al. Chinese hemophilia joint health score 2.1 reliability study. Haemophilia, 2013, 20(3):435-40.

[40] Dai J, Lu Y, Ding Q, et al. The status of carrier and prenatal diagnosis of haemophilia in China. Haemophilia, 2012,18(2):235-240.

[41] Hua BL, Yan ZY, Liang Y, et al. Identification of seven novel mutations in the factor VIII gene in 18 unrelated chinese patients with hemophilia A. Chin Med J, 2010,123(3):305-310.

[42] He Z, Chen J, Xu S, et al. A strategy for the molecular diagnosis in hemophilia A in Chinese population. Cell Biochem Biophys. 2013, 65(3):463-72.

[43] Ding QL, Lu YL, Dai J, et al. Characterisation and validation of a novel panel of the six short tandem repeats for genetic counselling in Chinese haemophilia a pedigrees. Haemophilia, 2012,18(4):621-625.

[44] Wu R, Luke KH, Poon MC, et al. Low dose secondary prophylaxis reduces joint bleeding in severe and moderate haemophilic children: A pilot study in China. Haemophilia, 2011,17(1):70-74.

[45] Shi J, Zhao Y, Wu J, et al. Safety and efficacy of a sucrose-formulated recombinant factor VIII product for the treatment of previously treated patients with haemophilia A in China. Haemophilia, 2007,13(4):351-356.

[46] Zhang L, Zhao Y, Sun J, et al. Six-month clinical observation on safety and efficacy of a full-length recombinant factor VIII for on-demand treatment of Chinese patients with haemophilia A. Haemophilia, 2011,17(3):538-541.

[47] Zhang L, Zhao Y, Sun J, et al. Clinical observation on safety and efficacy of a plasma- and albumin-free recombinant factor VIII for on-demand treatment of Chinese patients with haemophilia A. Haemophilia, 2011,17(2):191-195.

[48]   Xuan M, Xue F, Fu R, et al. Retrospective analysis of 1,226 Chinese patients with haemophilia in a single medical centre. J Thromb Thrombolysis, 2014,38(1):92-97.

[49]   Nathwani AC, Davidoff AM, Tuddenham EGD. Advances in gene therapy for hemophilia. Human gene therapy, 2017,28(11):1004-1012.

[50]   Ormond KE, Mortlock DP, Scholes DT, et al. Human germline genome editing. Am J Hum Genet, 2017, 101(2):167-176.

[51]   Barbieri EM, Muir P, Akhuetie-Oni BO, et al. Precise editing at DNA replication forks enables multiplex genome engineering in eukaryotes. Cell, 2017:S0092-8674(0017)31256-31254.

[52]   Shapiro A, Gruppo R, Pabinger I, et al. Integrated analysis of safety and efficacy of a plasma- and albumin-free recombinant factor VIII (rAHF-PFM) from six clinical studies in patients with hemophilia A. Expert Opin Biol Th, 2009,9(3):273-283.

[53]   Blanchette VS, Shapiro AD, Liesner RJ, et al. Plasma and albumin-free recombinant factor VIII: Pharmacokinetics, efficacy and safety in previously treated pediatric patients. J Thromb Haemost, 2008,6(8):1319-1326.

[54]   Di Paola J, Smith MP, Klamroth R, et al. Refacto (r)(1) and advate (r)(2): A single-dose, randomized, two-period crossover pharmacokinetics study in subjects with haemophilia A. Haemophilia, 2007,13(2):124-130.

[55]   Tarantino M, Abshire T, Shapiro A, et al. Global clinical evaluation of an rfVIII prepared using a plasma/albumin-free method, Advate rAHF-PFM. Pediatr Res, 2004,55(4):286a-286a.

[56]   Pollmann H, Klamroth R, Vidovic N, et al. Prophylaxis and quality of life in patients with hemophilia A during routine treatment with Advate   [antihemophilic factor (recombinant), plasma/albumin-free method]. in Germany: A subgroup analysis of the Advate PASS post-approval, non-interventional study. Ann Hematol, 2013,92(5):689-698.

[57]   Auerswald G, Thompson AA, Recht M, et al. Ranging from birth to 47 months of age. Thromb Haemostasis, 2012,107(6):1072-1082.

[58]   Bacon CL, Singleton E, Brady B, et al. Low risk of inhibitor formation in haemophilia A patients following en masse switch in treatment to a third generation full length plasma and albumin-free recombinant factor VIII product (Advate(r)). Haemophilia, 2011,17(3):407-411.

[59]   Negrier C, Shapiro A, Berntorp E, et al. Surgical evaluation of a recombinant factor VIII prepared using a plasma/albumin-free method: Efficacy and safety of Advate in previously treated patients. Thromb Haemost, 2008,100(2):217-223.