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ARTICLE

Assessment of Relative Bioavailability of Moroctocog Alfa and Moroctocog Alfa (AF-CC) in Subjects With Severe Hemophilia A

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An open-label, single-dose, randomized, two-period, crossover study comparing the pharmacokinetics of factor VIII activity in plasma (FVIII:C) after administration of an albumin-free presentation of moroctocog alfa (test) and moroctocog alfa manufactured using the previous technique (reference) was conducted in 30 (25 evaluable) male subjects who had severe hemophilia A (FVIII:C < 1 IU/dL). Blood samples were collected for 48 h after administration of each dose. FVIII:C was assayed using a chromogenic substrate assay. The FVIII:C pharmacokinetic parameters were calculated using noncompartmental analysis. The presentations would be bioequivalent if the 90% confidence limits of the ratio of the geometric mean values of AUC_{inf} and recovery fell within the interval of 80–125%. The bioequivalence criteria were met. A total of 10 treatment-related adverse events were observed in a total of nine subjects. All were mild and none was determined to be related to administration of study medication.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✔ Few clinicians are aware of the studies required to support changes in formulation of coagulation factors nor the fact that the studies must be conducted in the rare population of subjects with severe hemophilia (FVIII:C less than 1% of normal) who must tolerate skipping standard treatment for the duration of the study, potentially exposing them to subtherapeutic FVIII activity and at risk for bleeding episodes.

WHAT QUESTION DID THIS STUDY ADDRESS?

✔ Demonstrated bioequivalence, based on factor VIII (FVIII) activity, of two presentations of moroctocog alfa in subjects with hemophilia, and described the other studies triggered by the change in formulation.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✔ In addition to life-cycle management issues, this study provides additional robust FVIII activity information that may be used to support development of smart tools to help physicians and patients manage use of FVIII. Patients and physicians using moroctocog alfa (AF-CC) can use the results from this study as prior pharmacokinetic parameter information to help them interpret their own FVIII:C data without having to undergo extensive sampling.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

✔ Knowledge of life-cycle management will allow clinicians to be better informed about drug supply challenges and knowledge of product specific FVIII activity will improve quality of drug utilization tools.

Recombinant technology has been used to develop synthetic molecules that have similar procoagulant activity as endogenous coagulation factors missing in patients with hemophilia, and thus eliminating the potential for transmission of human blood-borne viruses during treatment. Moroctocog alfa, also referred to as B-domain deleted recombinant blood coagulation factor VIII (BDDrFVIII), was first approved in 1997, and differs from endogenous factor VIII by the lack of the B-domain, which is not required for hemostatic activity.[1] Revisions to the manufacturing processes removed the requirement of albumin and the resulting drug product is designated moroctocog alfa (AF-CC).[2] Three distinct differences distinguish the albumin-free process. First, it completely removes the use of human serum albumin from the cell culture process. Second, the monoclonal antibody sepharose resin, used to immunoaffinity purify moroctocog alfa in the former process, was replaced by a peptide affinity resin using a chemically synthesized polypeptide affinity ligand (TN8.2), thereby eliminating a potential source of viral contamination. Finally, a virus-retaining filtration step has been added during purification to further enhance the viral safety profile. Although the process for manufacturing the drug substance was modified, the final drug product formulation is unchanged. The Chinese hamster ovary (CHO) cell line that produces moroctocog alfa (AF-CC) was isolated from CHO cells used in moroctocog alfa production. The

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factor VIII DNA construct is identical for moroctocog alfa (AF-CC) and moroctocog alfa. Biochemical characterization shows that drug substances produced by each manufacturing process are comparable with respect to structure and function. Moroctocog alfa (AF-CC) has been shown to be equivalent to moroctocog alfa on the basis of extensive bioanalytical and biochemical characterization and nonclinical evaluations. The first approval of moroctocog alfa (AF-CC) was in 2008 and it gradually replaced the previous formulation globally. This article describes one of the relative bioavailability studies performed as well as discusses the other studies required in the life cycle management of coagulation factors, some of which are still ongoing, as a result of the change in moroctocog alfa (AF-CC).

The objective of the current study was to determine the relative bioavailability of moroctocog alfa as manufactured using the revised, albumin-free method (AF-CC, test product) compared with moroctocog alfa as manufactured using the previous process (reference product) and evaluate bioequivalence.

METHODS

Study population

This study was conducted in compliance with the Declaration of Helsinki, with all International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, as well as local regulatory requirements. The final protocols, amendments, and informed consent documentation were reviewed and approved by the independent Ethics Committees at each of the investigators' centers: Hôpital Hotel Dieu, Lyon, France, Emory University, Atlanta, GA, St. Vincent's Hospitals and Health Services, Indianapolis, IN, UMDNJ – Robert Wood Johnson Medical School, New Brunswick, NJ, COMIRB, Aurora, CO, University Health Center, Detroit, MI, Georgetown University Medical Center, Washington, DC, UNC Chapel Hill, Chapel Hill, NC, Banner Health Research Institute, Phoenix, AZ, University of Pennsylvania IRB, Philadelphia, PA. A signed and dated, written, informed consent was required before any screening procedures were done.

Thirty male subjects with previously treated severe hemophilia A (FVIII activity below 1 IU/dL), with a mean $\pm$ SD age of 27 $\pm$ 14 years (min-max 12–70 years), weight of 72 $\pm$ 12 kg (min-max 41–103 kg) were enrolled in the study. Eligibility criteria permitted subjects with FVIII activity equal to 1 IU/dL to enroll, but all of those who enrolled were shown to have less than 1 IU/dL prior to dosing. Eight subjects were adolescents, aged 12–17 years. Most subjects were white (25/30), two were black, and three were Hispanic. All subjects had extensive exposure to prior FVIII replacement therapies (more than 250 exposure days). Subjects had to have a negative test for FVIII inhibitor using the Bethesda Inhibitor Assay ($<$0.6 Bethesda units/mL), at the local laboratory at screening, and had to be able to comply with a 5-day washout from FVIII-containing products prior to each administration of study product.

Study product

The test product was moroctocog alfa (AF-CC), manufactured using the albumin-free process (ReFacto AF, Pfizer, Collegeville, PA). The reference product was moroctocog alfa (ReFacto, Pfizer) manufactured using the process containing albumin.[2] Each subject received single doses of 50 IU/kg as 2-min infusions of each of the study medications in a randomized fashion. The dose was calculated on the basis of the subject's actual body weight as measured at the screening visit and on the labeled potency of the study drug. After completion of the first study period and a minimum washout of 5 days, subjects received the alternate treatment.

Study methods

This study was an open-label, single-dose, randomized, two-period, crossover study and was consistent with the guidance for the conduct of pharmacokinetic studies of FVIII replacement factors and the guidance for the assessment of bioequivalence.[3,4] During a screening visit, which was between 1 and 14 days prior to the first study drug administration, medical history was assessed, subjects underwent a complete physical examination, including assessment of vital signs and collection of blood to perform chemistry and hematology tests. Samples were also collected to measure FVIII activity (FVIII:C) and for the presence of inhibitors to FVIII in plasma. These were assayed at a laboratory local to the investigators.

During each of the two study periods associated with study drug administration, samples were collected for FVIII:C pharmacokinetics as well as testing for inhibitors to FVIII, antibodies to FVIII, and antibodies to CHO (the cell line used to produce moroctocog alfa) and TN8.2 (the ligand used to purify moroctocog alfa). Blood samples for determination of FVIII:C were collected before each infusion and then 0.25, 0.5, 1, 3, 6, 9 (or 12), 24, 28 (or 30), 32 (or 36), and 48 h after the start of the infusion. The actual times of sample collection were collected and used in the pharmacokinetic parameter calculations.

Undiluted plasma samples were analyzed at a central laboratory for FVIII:C using a validated chromogenic substrate assay. The assay method was based on the Coatest Factor VIII Kit (Chromogenix, Sweden) adapted to the microplate technique.[5] The chromogenic substrate assay is based on the two-stage principle. In stage one, activated factor X (Xa) is generated with factor VIII:C as a cofactor. In stage two, factor Xa is determined by the use of a synthetic chromogenic substrate (S-2222) in the presence of a thrombin inhibitor (I-2581) to prevent hydrolysis of the substrate by thrombin. The reaction is stopped with acid and the release of paranitroaniline, which is proportional to the FVIII:C content is measured photometrically at 405 nm against a reagent blank. The method allows 25 samples to be assayed in one microplate against the mean of the fourfold replicated standard curve. One of the samples must be the moroctocog alfa plasma reference. Changes from the kit were: factor IXa+X reagent was reconstituted with 12 mL of purified water, actin (Baxter, Deerfield, IL) was used instead of the phospholipid included in the kit and Michaelis buffer was used instead of the kit buffer. The lower limit of quantitation was 0.007 IU/mL and the upper limit of quantitation was 0.900 IU/mL. Interassay precision relative standard deviation (%RSD) was 19% (FVIII:C 0.02 to 0.144 IU/mL) and 4% (FVIII:C above 0.144 IU/mL).

Samples to assess the development of antibodies, both neutralizing and non-neutralizing to FVIII:C, were collected prior to administration of study drug and at the final evaluation, 4 to 7 days after administration of the second dose. These were assayed using validated enzyme-linked immunosorbent assay (ELISA) methods. Assessment of the presence of inhibitors (neutralizing anti-FVIII:C antibodies) was performed using the Bethesda Inhibitor assay (BIA).[6] Samples were positive if the concentration of inhibitor was greater than or equal to 0.6 BU/mL. Low titer inhibitors (<5 BU/mL) were subsequently analyzed using the Nijmegen modification of the BIA. Samples were collected to test for anti-TN8.2 and anti-CHO antibodies prior to the administration of the first dose as well as during the last study visit. These were assayed using validated ELISA methods. Samples were considered positive if the ratio of the absorbance of the assay sample, compared with that of a normal absorbance based on a pool of normal sera, exceeded the cutoff value of 2 (for anti-TN8.2) and 2.1 (for anti-CHO antibodies). All bioanalytical work, except for the analysis of anti-TN8.2, was performed at a central laboratory (Biovitrum, Stockholm, Sweden). The anti-TN8.2 samples were analyzed at Pfizer (formerly Wyeth Research, Pearl River, NY).

Pharmacokinetic parameters were calculated for each subject using the FVIII:C and the actual sample collection times. In clinical care settings, because of the immediate link to need and response to treatment, FVIII:C activity is conventionally reported in units of IU/dL. Although no conversion of FVIII:C data was made for calculation of most pharmacokinetic parameters, FVIII:$C_{0.5h}$, FVIII:C_{pre} and graphical presentation of FVIII:C are reported as IU/dL. Noncompartmental analysis parameters were calculated. A pharmacokinetic parameter that is unique to hemophilia, recovery, was also calculated using the FVIII:C values reported prior to and at 0.5 h after the start of the infusion and the weight observed on the day of study drug administration, using the equation:

$$\text{Recovery} = \left(\text{FVIII} : C_{0.5h} - \text{FVIII} : C_{pre}\right) / (\text{dose}/\text{weight})$$

FVIII:C measurements were summarized for all subjects at each nominal time as were pharmacokinetic parameters for each presentation using descriptive statistics, including the number of observations, mean, standard deviation, minimum, maximum, and 95% confidence intervals (CI). The statistical comparison of the selected parameter estimates between the two preparations was performed by using an analysis of variance for a two-period crossover design. Additionally, 90% confidence limits for the test-to-reference ratios of the primary parameter estimates (AUC_T, AUC_{inf}, and recovery) were constructed on the log scale using the two-treatment, one-sided test procedure. The two preparations were judged to be bioequivalent if the 90% confidence limits fell within the bioequivalence interval of 80–125%. The PK parameters estimation and bioequivalence testing were performed using WinNonlin Professional (Pharsight, Mountain View, CA).

Safety assessments were performed regularly throughout the study from screening until collection of the final specimen.

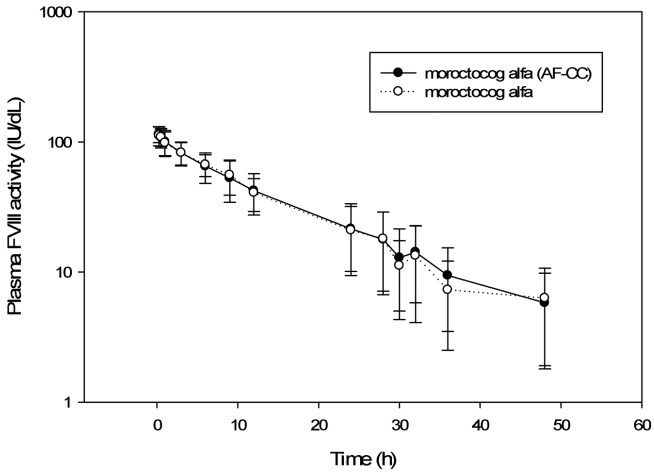

Figure 1 FVIII activity after single 50 IU/kg doses of moroctocog alfa and moroctocog alfa (AF-CC) in adolescent and adult men with severe hemophilia A.

RESULTS

Twenty-nine of the 30 subjects enrolled in the study received both formulations and completed all study procedures. One subject withdrew after receiving only one treatment (moroctocog alfa (AF-CC)), as he had more than two bleeding episodes during the first study period. Five subjects reported five hemophilia-related adverse events. Four of these were observed after moroctocog alfa (AF-CC), including two episodes of ecchymosis and one episode each of arthralgia and joint disorder. One hemophilia-related adverse event, joint disorder, was reported after treatment with moroctocog alfa. All were classified as mild or moderate and no action was taken for three of these events, while additional factor replacement was required for the joint disorders. Nine subjects reported 10 treatment-emergent adverse events, but all were considered mild and not drug-related. There was no difference in those reported after the two different study medications. All tests for anti-FVIII antibodies, inhibitors, anti-CHO, and anti-TN8.2 were negative.

Pharmacokinetic data from five subjects were excluded from the PK analysis for the following reasons: one subject did not complete the study, one subject had an unreported history of low-titer FVIII inhibitor, and several blood samples from three separate subjects were inadvertently thawed during shipment to the analytical site. The FVIII:C parameters reported were based on the results of 25 individuals as is the bioequivalence assessment.

The mean $\pm$ standard error FVIII:C vs. time profiles are shown in **Figure 1**. The pharmacokinetic parameters are shown in **Table 1** and the bioequivalence assessment is shown in **Table 2**. The relative bioavailability of moroctocog alfa (AF-CC) compared with moroctocog alfa was 98.1% (90% CI: 92.2–104.3%) and 100.6% (90% CI: 97.6%, 103.6%) based on AUC_{inf} and recovery respectively. The CIs were within the acceptance range for bioequivalence of 80–125%.[4]

Table 1 Mean ± SD (min, max) Factor VIII pharmacokinetic parameters after 50 IU/kg single dose in patients with hemophilia A aged 12–70 years ($n = 25$)

Parameter	Moroctocog alfa	Moroctocog alfa AF-CC
C_{max} (IU/dL)	115 ± 16 (86, 145)	116 ± 17 (82, 146)
AUC_T (IU.h/mL)	14.5 ± 4.6 (6.8, 23.0)	14.5 ± 5.2 (6.5, 24.0)
AUC_{inf} (IU.h/mL)	15.6 ± 5.6 (7.0, 28.5)	15.4 ± 6.0 (6.7, 29.3)
$t_{½}$ (h)	10.9 ± 4.5 (4.6, 22.2)	9.9 ± 3.2 (3.9, 19.2)
MRT (h)	15.5 ± 5.2 (8.0, 28.6)	14.6 ± 4.6 (6.8, 28.1)
CL (mL/h/kg)	3.69 ± 1.48 (1.75, 7.13)	3.84 ± 1.69 (1.70, 7.50)
V_{ss} (mL/kg)	51.1 ± 8.5 (37.1, 69.5)	49.9 ± 9.1 (36.5, 70.7)
Recovery (IU/dL per IU/kg)	2.30 ± 0.32 (1.72, 2.89)	2.32 ± 0.35 (1.64, 2.91)

Table 2 Bioequivalence metrics

Parameter	Ratio of LS means	90% confidence interval
AUC_T	99.5%	93.8–105.6%
AUC_{inf}	98.1%	92.2–104.3%
Recovery (IU/dL per IU/kg)	100.6%	97.6–103.6%

DISCUSSION AND CONCLUSION

Relative bioavailability studies are among the most frequently conducted studies in life cycle management of approved products. The study design is straightforward and the results are usually of little interest to researchers or prescribers because the studies are performed in healthy volunteers and confirm pharmacokinetic parameters already characterized. However, for coagulation factors used in the treatment of patients with hemophilia, the introduction of a new manufacturing process and the necessary relative bioavailability studies represent higher hurdles than is usually the case in the introduction of the new presentations that make up life cycle management.

Although regulatory guidance for nonbiological medications allows sponsors to request waivers for medications administered intravenously, because of the concern for the development of antidrug antibodies and neutralizing antibodies, such may not be the case of many biological products. Per regulatory guidance, significant changes in manufacturing processes of coagulation factor replacement products require assessment of immunogenicity and pharmacokinetics to assure the similarity of the active ingredient made using the new process to the active ingredient made by the former process.[3] In addition to assessing relative bioavailability of new formulations, clinical studies to document efficacy and safety in adults and children with hemophilia may also be required.[7–9]

There are several differences between pharmacokinetic studies for other biological products and those for coagulation factor replacement products. The latter are not assessed in healthy volunteers. Results obtained in the presence of normal FVIII activity concentrations (50 to 150 IU/dL) would be difficult to extrapolate to individuals where endogenous FVIII concentrations are less than 1 or 2 IU/dL. Patients participating in bioavailability studies must refrain from regular administration of replacement factor prior to study drug administration and those requiring factor to treat bleeding episodes are removed from studies. Hemophilia A is a rare disease, occurring in 1 in 5,000 male births, with those who have severe disease (FVIII activity less than 1 IU/dL) and most in need of replacement making up 60% of individuals with hemophilia A.[10] Because of the rarity of the disease, adolescent patients are frequently included in bioavailability studies, undergoing extensive sampling, just as adults do. Recommended sampling times are dictated by regulatory guidance.[3] In addition to reporting conventional pharmacokinetic parameters, studies of coagulation factors also report a parameter called recovery.[11] Recovery is inversely related to volume of distribution and is a measure of the anticipated increase in coagulation capability that is achieved with dosing of factor replacement products. Patients with lower than average recovery, such as young children, will require higher doses to achieve a target FVIII:C activity compared with patients with higher than average recovery, such as older, heavier patients.[12] Finally, relative bioavailability studies are important sources of pharmacokinetic information for coagulation factor replacement products. The dense sampling performed allows for precise assessment and robust determination of pharmacokinetic parameters that is more difficult in clinical trials or when individuals are being evaluated by their caregivers to optimize dosing. The pharmacokinetic results of these studies are of interest to prescribers as they help their patients optimize dosing or develop tools that may be used to optimize individual therapy.[13] The goal of factor replacement is to treat bleeding episodes, referred to as on-demand treatment, as well as to prevent bleeding episodes by giving before and during a surgical procedure, for example, or by giving regularly, so that spontaneous bleeding episodes may be prevented.[14] When administered for prophylaxis, the half-life is used to design regimens that keep the trough factor VIII activity above a target value. Patients with shorter $t_{½}$ may require more frequent dosing, while those with longer $t_{½}$ may be able to dose less frequently.[15] The values observed in this study, 9.9 h and 10.9 h, are similar to those reported by Shafer *et al.*[18] and the variability in half-life, coefficient of variation of 41% and 32%, respectively, within the range observed by others as well.

In addition to comparing formulations resulting from changes in the manufacturing process, it is also common practice for relative bioavailability studies to be performed comparing FVIII:C exposure obtained with the new presentation to that of the plasma-derived coagulation factor or to other recombinant coagulation factors replacement products. The current study is one of five comparative bioavailability studies performed for moroctocog alfa and its successor product moroctocog alfa (AF-CC). A summary of the pharmacokinetic parameters from these studies is shown in **Table 3**. The first study compared two different formulations made using the original process, containing albumin, to a factor VIII replacement product that was derived from plasma.[16] The second compared moroctocog alfa made using the original process with the recombinant full-length factor VIII replacement product, octocog alfa,[17] while the third study compared moroctocog alfa (AF-CC) with octocog alfa.[7] The fourth study demonstrated bioequivalence of FVIII activity measured after administration of moroctocog alfa (AF-CC) as a single injection of a dual-chamber presentation and as the combined contents of two vials.[18] Because of the

Table 3 Mean ± SD pharmacokinetic parameters from bioavailability studies of moroctocog alfa

	Recovery (IU/dL per IU/kg)	V_{ss} (mL/kg)	CL (mL/h/kg)	$t_{½}$ (h)
Kessler[16] – formulation A	2.36 ± 0.34	Not reported	Not reported	15.4 ± 5.4
Kessler – Formulation B	2.43 ± 0.38	Not reported	Not reported	14.8 ± 5.6
Di Paola[17]	2.33 ± 0.33	58.6 ± 13.7	3.85 ± 1.86	13.0 ± 3.1
Recht[7]	2.35 ± 0.47	Not reported	4.51 ± 2.23	11.2 ± 5.0
Shafer[18] – dual chamber	1.96 ± 0.43	~52.8 ± 8.6*	~4.32 ± 2.05*	9.52 ± 3.02
Shafer – vials	2.02 ± 0.34	~49.2 ± 9.4*	~3.79 ± 1.99*	10.29 ± 2.95
Present study – Moroctocog alfa (AF-CC)	2.30 ± 0.32	51.1 ± 8.5	3.69 ± 1.48	10.9 ± 4.5
	2.32 ± 0.35	49.9 ± 9.1	3.84 ± 1.69	9.9 ± 3.2

Calculated from mean data reported.

availability of very specific regulatory guidance[3] for the conduct of pharmacokinetic assessment of factor VIII replacement products, all of these studies were conducted using a similar study design: all enrolled patients with hemophilia that was severe (baseline FVIII activity < 1 IU/dL) or moderately severe (baseline FVIII activity <2 IU/dL), all administered single doses of 50 IU/kg, all used FVIII activity in lieu of measuring drug concentrations, and all demonstrated bioequivalence and all collected samples at the same time.

Although it has been helpful to have had guidance so that robust pharmacokinetic assessments can be obtained for even the most modest of changes in formulations and presentations, innovative coagulation factor replacement products with longer half-lives[19] will necessitate different sampling schemes for relative bioavailability studies than those used for regular half-life replacement factors. It would also be helpful to have some flexibility in dose administration so that subjects would not have to forego regularly schedule prophylactic doses and could use smaller doses, perhaps in a multiple-dose setting.

In addition to documenting bioequivalence of new formulations of coagulation factors, clinical studies may also be required to demonstrate the safety and efficacy of the product and perhaps fulfill the requirement of obtaining more information in pediatric subjects that are required by regulatory authorities. In the case of ReFacto AF, four additional clinical studies were performed to support European registration. The first study[20] was a postapproval commitment, surveillance study assessing the incidence of inhibitors (neutralizing antibodies against FVIII) in 208 subjects who were followed as they switched from moroctocog alfa or another coagulation factor VIII product that they had been using to moroctocog alfa (AF-CC). The second and third studies were also postapproval commitment studies to assess the pharmacokinetics of FVIII:C as well as the safety and efficacy of moroctocog alfa (AF-CC) in pediatric subjects, in those with prior treatment with FVIII replacement products,[9] and those without prior treatment (NCT00950170). Finally, a registry study in minimal and not previously treated pediatric patients is ongoing in European countries. Unlike the case of small molecules, for biologicals, bioequivalence was simply one of the outcomes that needed to be satisfied for the new formulation. With the removal of albumin from the manufacturing process, although the product was analytically the same as that obtained using the previous process, demonstration of bioequivalence between moroctocog alfa and moroctocog alfa (AF-CC) was required. Demonstration of safety and efficacy in patients receiving product prepared through the new process was also required, as was continued monitoring for differences in immunogenicity.

This study was part of a submission to the European Medicines Agency (EMA), among other regulatory authorities, which supported the approval of moroctocog alfa (AF-CC). The results were published as part of the EMA Assessment Report (EPAR). [21] While publication as part of an EPAR is helpful in making information available, the study may not be found in literature searches, does not undergo peer review, and the data are not available for physicians, in particular for hemophilia treaters. Publications also offer interested readers the opportunity to interact with the investigator and through Letters to the Editor to offer commentary on the results.

In conclusion, this study demonstrated the bioequivalence of moroctocog alfa (AF-CC) to moroctocog alfa. The available pharmacokinetic data are supportive of the efficacy and safety studies already conducted, and may also be helpful for the development of tools to aid patients and prescribers in optimizing coagulation replacement therapy with moroctocog alfa (AF-CC).

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Conflict of Interest. All of the authors are employees and may be stock holders of Pfizer Inc.

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