Experience of Advate rAHF-PFM in previously untreated patients and minimally treated patients with haemophilia A

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Summary

We report a prospective trial of 55 previously untreated patients (PUPs) and minimally treated patients (MTPs) with severe/moderately severe haemophilia A (baseline factor VIII [FVIII] ≤2%) treated with a single FVIII replacement product. It was the objective of this study to evaluate the immunogenicity, efficacy, and safety of rAHF-PFM (Advate®). On-demand or prophylactic treatment regimens were determined at the discretion of the investigator. rAHF-PFM was also permitted for perioperative management. There were 633 bleeding episodes (BEs), including 517 treated, and 466 rated for efficacy. Haemostatic efficacy was considered excellent/good in 93% of 466 rated treatments. Of 517 treated BEs, 463/517 (90%) were managed with one (356/517 [69%]) or two infusions (107/517 [21%]). There were 27 surgeries. Intraoperative (n=22) and postoperative (n=25) haemostatic efficacies were considered excellent or good in 100% of rated surgeries. Related serious adverse events (SAEs) were inhibitor development in 16/55 (29.1%) subjects who received at least one infusion of rAHF-PFM. Non-serious, related adverse events (AEs) were few in number (14 in eight subjects). The odds ratio (OR [95% Confidence Interval, CI]) of developing inhibitors was significantly higher in subjects with a family history of inhibitor (4.95 [1.29–19.06]), non-Caucasian ethnicity (4.18, [1.18–14.82]), and intensive treatment at high dose (4.5 [1.05–19.25]) within ≤20 exposure days (EDs). In conclusion, rAHF-PFM was safe and effective for the management and perioperative coverage of PUPs/MTPs with severe/moderately severe haemophilia A. This report supports previous findings from studies in which family history of inhibitor, non-Caucasian ethnicity, and high intensity treatment were associated with high risk of inhibitor development.

Keywords

Haemophilia A, previously untreated patients, paediatric, minimally treated patients, factor VIII, factor VIII inhibitor

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Introduction

Haemophilia A is an X-linked, congenital deficiency of coagulation factor VIII (FVIII), requiring life-long replacement therapy with FVIII concentrate. The safety and efficacy of Advate® (Antihaemophilic Factor [Recombinant], Plasma/Albumin Free Method [rAHF-PFM]) in the treatment of bleeding episodes (BEs) has been established in studies of previously treated patients (PTPs) with at least 150 prior FVIII exposure days (EDs), and in pediatric PTPs with at least 50 prior FVIII EDs (1, 2). rAHF-PFM has also been demonstrated to be efficacious for perioperative haemostatic management (3). This study was designed to analyse haemostatic efficacy and overall safety of rAHF-PFM in previously untreated patients (PUPs) and minimally treated patients (MTPs), as well as assess risk factors for inhibitor development. The primary safety endpoint of the study was to determine inhibitor incidence since this is the most serious complication of treatment occurring after exposure to FVIII replacement therapy in approximately 30% of severe haemophilia A patients (4–7). The occurrence of inhibitors in PUPs may be seen as the natural response of the immune system to a foreign protein, whereas PTPs with >150 EDs are considered immunologically stable, and inhibitor development could be due to characteristics of an individual FVIII product (8–10).

Documentation of the clinical management of PUPs/MTPs is not only useful for the collection of data related to haemostatic efficacy, safety, and tolerability of FVIII treatment in a naïve population, but also in gaining additional experience of the natural history of inhibitor development.

Materials and methods

Description of study

This was a multicentre, open-label clinical study in PUPs and MTPs with severe or moderately severe haemophilia A (baseline FVIII ≤2%). Subjects underwent an evaluation of the immunogenicity, efficacy, and safety of rAHF-PFM according to one of the following three treatment regimens: on-demand treatment, a standard prophylactic regimen consisting of 25 to 50 IU/kg body weight, 3–4 times per week, or a modified prophylactic regimen (with dose and frequency at the discretion of the investigator). rAHF-PFM was also used for perioperative management, if necessary. The treatment regimen was determined by the investigator and could be changed at any time. Subjects who developed a confirmed high-titre inhibitor (>5 Bethesda Units [BU/ml]) or who developed a low-titre inhibitor and in whom bleeding could not be adequately managed by prophylactic or on-demand treatment with rAHF-PFM were eligible to undergo immune tolerance induction (ITI) with rAHF-PFM, according to regimens that were at the discretion of the investigators. Subjects were to be followed for 75 EDs or three years, whichever occurred first. The sponsor, on the recommendation of the study's Data Safety Monitoring Board, could have stopped the study at any time if there were unacceptable safety risks to study participants. Consistent with the Committee for Proprietary Medicinal Products (CPMP) guideline on studies of recombinant FVIII products regarding PTPs greater than 12 years old, the number of subjects intended for enrollment in this study was approximately 50 (7).

The primary safety endpoint was the percentage of subjects who developed an inhibitor to FVIII. Secondary endpoints were the percentage of subjects who had adverse events (AEs) deemed possibly or probably related to treatment with rAHF-PFM, and the percentage of subjects who developed antibodies to Chinese hamster ovary (CHO) cell protein, murine immunoglobulin G (IgG), or human von Willebrand factor (VWF). Also analysed were potential risk factors for inhibitor development.

Efficacy outcome measures were the number of rAHF-PFM infusions required to achieve adequate haemostasis for all BEs; the overall haemostatic efficacy rating of rAHF-PFM for all treated BEs; the annualised bleeding rate, incremental recovery of rAHF-PFM determined at study visits, intra- and post-operative haemostatic efficacy rating, and the percentage of actual to predicted blood loss during surgery.

Efficacy assessments

Haemostatic efficacy for BEs was evaluated by the subject's legally authorised representative (for home infusion) or the investigator (for infusions at the clinic). Intraoperative and postoperative assessments of efficacy were evaluated by the operating surgeon and the investigator, respectively. For efficacy rating scales, refer to online supporting information.

Laboratory assessments

Inhibitor testing by the Nijmegen assay at the Baxter Central Laboratory was to be performed at screening, then after 5 ± 1, 10 ± 1, 15 ± 1, and 20 ± 1 EDs, and thereafter every 10 ± 3 EDs or 3 months ± 7 days from the last inhibitor test, whichever came first. If inhibitor formation was observed, epitope mapping of the inhibitory antibody was performed on remaining plasma. For additional information on epitope mapping, refer to the online supplemental material (available online at www.thrombosis-online.com). Subjects were to be evaluated for *in vivo* incremental recovery (IR) throughout the study. Part way through the study it was noted that IRs determined at the Baxter Laboratory in Round Lake were markedly lower than expected. Following an in-depth investigation and with the concurrence of the US Food and Drug Administration (FDA), it was decided to have the subsequent testing of FVIII activity conducted at the Department of Medical and Chemical Laboratory Diagnostics, Medical University Vienna, Austria. Due to the systematic underestimation of the peak levels, the results obtained at the Baxter Round Lake Laboratory are not meaningful and cannot be used for the interpretation of recoveries. Due to the small number of evaluable IR values from the Medical University Vienna Laboratory, IR results will not be presented in this report.

FVIII gene mutation and human leukocyte antigen (HLA) genotype testing was performed in the central laboratory at DRK-Blood Donor Service, Institute of Transfusion-Medicine/Immunology/ Haematology, Frankfurt, Germany. The laboratory techniques used for mutation analysis are described in the online supplemental material (available online at www.thrombosis-online.com).

Serum was assayed for the presence of antibodies to heterologous proteins which are present in trace quantities in the study product (i.e. CHO protein, murine IgG, and VWF) using proprietary enzyme immunoassays.

Subjects

Eligible subjects were PUPs and MTPs <6 years of age with severe (FVIII level < 1%) or moderately severe haemophilia A (FVIII level 1–2%) determined at baseline. Subjects with a detectable inhibitor to FVIII, a known hypersensitivity to rAHF-PFM, or a history of exposure to FVIII other than rAHF-PFM were excluded from the study. Subjects may have had up to three infusions of rAHF-PFM within 28 days prior to enrolment to treat a BE and three infusions between enrolment and the first IR infusion. Infusions of rAHF-PFM received prior to the start of the study were factored into the calculation of EDs.

Statistical methods

Efficacy and safety data were summarised by medians (min and max) and means (standard deviations [SD]). Incidence of inhibitor development was summarised by percentages (95% confidence intervals [CI]), and the median time to inhibitor development was calculated. The cumulative incidence of inhibitor development, including all, high and low titre, was presented in a post-hoc plot showing percentage inhibitor development by the number of EDs prior to inhibitor incidence. Individual analyses of incidence of inhibitor development for the PUPs and MTPs were provided as post-hoc analyses. Immunogenicity to heterologous protein was analysed by linear regression with antibody titre (Y) as the dependent variable and time (X) as the independent variable per subject. A Fisher exact test was used for a post-hoc calculation to assess association between geographic location with regards to prescription of a prophylactic regimen and age of enrolment. The examination of initial treatment regimens and calculations of annualized bleeding rates for all subjects and by age of enrolment into the study were conducted as post-hoc analyses.

Risk factor analysis

To investigate potential risk factors of inhibitor development, post-hoc logistic regression analyses for putative risk factors were performed. The analysis of risk factors was performed on an immunogenicity analysis set (N=50) which included all those developing an inhibitor and all those who were inhibitor-free with at least 10 EDs.

Genetic risk factor information such as family history of inhibitors and race/ethnicity were collected at the time of enrolment. FVIII mutations considered high risk for inhibitor development were large deletion; intron inversion; nonsense; splice site; and frameshift mutations (small deletions/insertions) resulting in stop codons with no prior documentation of partial correction of reading frame. Mutations considered low risk were missense; in-frame (small deletion/insertions); and frameshift mutations (small deletions/insertions) at or near an A run previously documented to have partial correction of the reading frame (details provided in online supplemental material, available online at www.thrombosis-online.com). High-risk HLA type was defined as having any combination of DR15 and/or DQ06 HLA genotypes (11).

Non-genetic risk factor analysis took the following potential risk factors into account at exposures to rAHF-PFM of ≤10 EDs, ≤20 EDs, and ≤30 EDs: surgery, port placement, intensive treatment (at least five consecutive study days of treatment), intensive treatment at high dose (five consecutive study days of a mean infusion dose of rAHF-PFM >50 IU/kg), and age at first exposure to rAHF-PFM. For inhibitor subjects, the risk factor must have been captured prior to development of inhibitor.

Results

The Institutional Review Boards or Ethics Committees representing each investigational site approved the study protocol. The first subject entered the study on April 1, 2004. The last subject exited on September 11, 2009.

Subjects

Of 66 subjects enrolled (consented) from 24 international sites, 55 (18 PUPs and 37 MTPs) received at least one infusion of rAHF-PFM during the study interval, including one screen failure (▶ Fig. 1A), and 44 subjects completed the protocol.

Eleven subjects did not receive investigative product: six subjects were screen failures, one was considered lost to follow-up and terminated by the investigator after missing two site visits, three withdrew from the study, and one had low haemoglobin prior to enrolment and was withdrawn by the physician. There were no screen failures due to inhibitors.

Eleven of the 55 subjects who received at least one infusion of rAHF-PFM on study did not complete the study per protocol: one terminated participation in the study after developing an inhibitor, in order to enroll in the international ITI study (12), one was withdrawn by the investigator for administration of non-study rAHF PFM considerably outside the permitted 28-day window prior to screening, one was lost to follow-up, one was withdrawn during ITI treatment by the investigator, one was a screen failure inadvertently dosed with one infusion of rAHF-PFM; and six withdrew consent for non-product related reasons[1].

For the 55 subjects who received at least one infusion of rAHF-PFM, the median age at enrolment was seven months (range: 14 days-16 months); 21 (38%) subjects were <6 months old, 26 (47%) were 6–12 months old, and 8 (15%) were ≥13 months old. All of the subjects were male. Thirty-seven (67%) subjects were Caucasian, and 18 (33%) were non-Caucasian, including: nine (16%) Hispanic, five (9%) Black, two (4%) Caucasian/Black, one (2%) Indian (East), and one (2%) Asian/Caucasian. The baseline FVIII level at enrolment was <1% in 53 (96%) subjects, 1% to ≤2% in one (2%) subject, and >2% in one (2%) subject (considered a screen failure and subsequently withdrawn). Seventeen (31%) subjects had a family history of FVIII inhibitor and 35 (64%) did not, while three (6%) subjects had unknown family history. The gene mutations for all 55 subjects were analysed; 45 (82%) had FVIII mutations considered high risk for inhibitor development and 10 (18%) had low-risk FVIII mutations (see *Materials and methods* and ▶ Suppl. Table 2, available online at www.thrombosis-online.com).

[1] Inability to comply with study requirements; objection to blood draws; work-schedule conflict; difficulty with venous access; objection by the parents to the amount of blood drawn for study assessments.

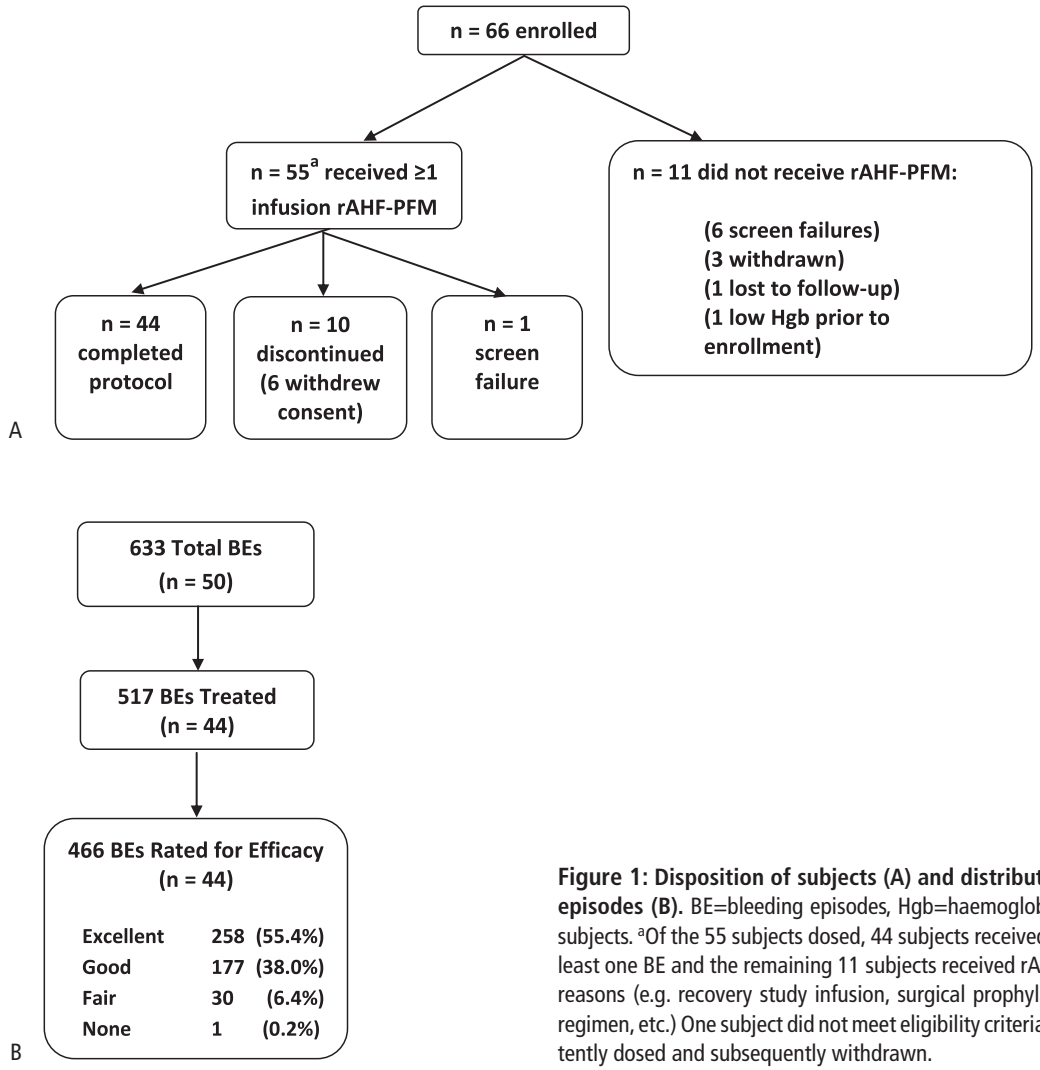

Figure 1: Disposition of subjects (A) and distribution of bleeding episodes (B). BE=bleeding episodes, Hgb=haemoglobin, n=number of subjects. [a]Of the 55 subjects dosed, 44 subjects received treatment for at least one BE and the remaining 11 subjects received rAHF-PFM for other reasons (e.g. recovery study infusion, surgical prophylaxis, prophylactic regimen, etc.) One subject did not meet eligibility criteria but was inadvertently dosed and subsequently withdrawn.

Treatment

The MTPs received a median of 1 (range: 1–4) infusions of non-study rAHF-PFM prior to the start of the study which were factored into the calculation of EDs (administered for haemostatic control of traumatic head BEs, joint BEs, soft tissue BEs, and surgical prophylaxis for circumcision). While these subjects are termed MTPs, they only received a single FVIII replacement product, rAHF-PFM, had extensive records of medical histories, and were infused within a well defined timeframe prior to enrollment according to protocol.

Of the 55 subjects treated with rAHF-PFM, the initial regimen prescribed was on-demand in 47 (85.5%), standard prophylaxis in three (5.5%), and modified prophylaxis in five (9.1%) subjects. An analysis of the treatment regimens of 52/55 subjects (three subjects exited the study too early to be included in this analysis) revealed that the predominant treatment regimen, defined as at least 80% of the time on study, was mixed in 29 (56%) subjects, on-demand in 15 (29%), modified prophylaxis in three (6%), standard prophylaxis in three (6%), and ITI in two (4%). Subjects initially prescribed on-demand treatment were frequently switched to prophylactic treatment. An examination of prescribed regimens in the same 52 subjects demonstrated a statistically significant association between the choice of treatment modality and geographic region of the prescribing physician. Although the majority of subjects were either on an on-demand or a mixed regimen for at least 80% of the time, a significantly higher proportion of subjects in Western Europe (100% [15/15]) than in North America (54% [20/37]) were prescribed prophylaxis at least once (Fisher exact p-value=0.001). An examination of the age at enrolment between geographies in the same 52 subjects described above, showed that although the median age at enrolment was somewhat higher in the EU than in North America (1.02 vs. 0.78 year), this difference was not statistically significant.

During the study, 27 subjects underwent surgical procedures, including 22 port placements combined with circumcision in six

Table 1: Haemostatic efficacy of rAHF-PFM. A) Haemostatic efficacy in bleeding episodes. B) Number of infusions administered to manage bleeding episodes. C) Haemostatic efficacy in surgical procedures.

A) Overall efficacy of rated treatments			
Rating	**Number of unique subjects**	**Number of BEs**	**BEs (%)**
Excellent	42	258	55.4
Good	36	177	38.0
Fair	11	30	6.4
None	1[a]	1	0.2
All rated	44	466	100.0

BEs=bleeding episodes. [a]The BE for this rating of "none" had three evaluations for three infusions (spontaneous BE of the buttocks). The first and second of the three infusions was rated "none" and the third infusion was rated "good." Haemostatic efficacy in 93.4% of rated BEs was considered excellent or good. Of 517 BEs treated with rAHF-PFM, 466 BEs were rated and 51 had an unknown efficacy rating.

B) Number of infusions administered to manage bleeding episodes			
Infusions	**Number of unique subjects**	**Number of BEs**	**BEs (%)**
1	42	356	68.9
2	34	107	20.7
3	23	35	6.8
≥4	14	19	3.7
All	44	517	100.0

BEs=bleeding episodes. Of 517 treated BEs, 89.6% were controlled with one or two infusions.

C) Haemostatic efficacy in surgical procedures	Intraoperative (Operating Surgeon)		Postoperative (Study Site Investigator)	
	N	**%**	**N**	**%**
Excellent	18	81.8	23	92.0
Good	4	18.2	2	8.0
Total	22	100.0	25	100.0

N=number of surgical procedures. There were a total of 27 surgeries in 27 subjects. Intraoperative: 22 subjects were rated, two did not receive product, and in three subjects, assessment was not done by the surgeon. Postoperative: 25 subjects were rated, and two were not.

subjects and a herniotomy in one subject, four circumcisions, and one venous fistula. Not included in the analysis were two circumcisions, which were conducted prior to enrolment and recorded in medical history, for which one and two infusions of non-study rAHF-PFM were administered. Neither of these subjects subsequently developed inhibitors.

Efficacy

Haemostatic efficacy in bleeding episodes

Fifty subjects experienced a total of 633 BEs, of which 517 in 44 subjects were treated. In some cases it was the opinion of the treating physician and/or the legal representative that a BE did not require an infusion of rAHF-PFM. Children may often experience superficial BEs, such as bruises, which are not treated. Of the 517 treated BEs, 466 received efficacy ratings and 51 had an unknown efficacy rating. All but one (50/51) of these unrated treatments were recorded at a single study site and were not included in the efficacy rating analysis. Some of the infusions rated as "unknown" efficacy were performed pre-emptively for head trauma, in accordance with NHF MASAC recommendations (13). Subsequent head MRI or CT assessments did not reveal a BE, and therefore, an efficacy analysis of these BEs was not possible of these BEs. A flowchart of the distribution of BEs is presented in ▶Figure 1B.

Haemostatic efficacy in 93% of rated BEs was considered excellent (258 [55%]) or good (177 [38%]) (▶Table 1A). To achieve adequate haemostasis, 90% of BEs were treated with one or two infusions; one infusion was administered in 356/517(69%) BEs in 42 subjects and two infusions were administered in 107 (21%) BEs in 34 subjects (▶Table 1B). Fifty-four BEs were treated with three or more infusions; of these, haemostatic efficacy was rated excellent in 22 BEs and good in 22 BEs. Those BEs requiring three or more infusions were: a tongue BE, forehead bruise, mouth/frenulum BE, psoas BE, chest wall BE, cut, antecubital BE, and joint BEs. Of 517 BEs, 42/96 (44%) joint BEs were treated with one infusion compared to 314/421 (75%) of non-joint BEs.

There were a total of 96 joint BEs and 421 non-joint BEs during the study requiring treatment. While BEs were not rated as major or minor, none of the BEs that were reported as SAEs were considered life threatening. The median annualised bleeding rate was 4.83 (range: 0.00–33.71) BEs/year/subject for all 55 subjects. The median annualised bleeding rate tended to increase with the age of enrolment; 4.22 (range: 0.00–17.90) BEs/year/subject for 21 subjects enrolled at <6 months old, 5.90 (range: 0.00–33.71) BEs/year/subject for 26 subjects enrolled at 6–12 months old, and 10.16 (range: 2.40–15.98) BEs/year/subject for eight subjects enrolled at ≥13 months old. Although these differences were not statistically significant they highlight the possibility that bleed rates in very young children may not be constant over time. No conclusions could be drawn from analysis of annualised bleeding rate by regimen in the PUP/MTP population as the study was not designed to make this comparison. In addition, the low number of subjects (three each) receiving modified or standard prophylaxis as a predominant treatment as compared to 15 subjects treated predominantly on-demand does not provide the statistical power to make a comparison between treatments, and the majority of subjects switched treatment regimen during the course of the study. Furthermore, there was great variety in dosing and frequency of prophylactic infusions.

Table 2: Characteristics of inhibitor subjects.

Subject ID	Peak inhibitor titre (BU/ml)	EDs prior to inhibitor	ITI	Race/ Ethnicity	Family history of inhibitor	Intensive treatment at high dose[a] (Y/N) ≤10, 20,or 30 EDs	Surgery (Y/N) ≤10, 20, or 30 EDs	High risk HLA[b]	Gene mutation classification (high/low risk)
1	9.3	6	N	Caucasian	Unknown	N:N:N	N:N:N	N	frameshift resulting in stop codon[c] (High)
4	1.8	20	Y	Hispanic	N	N:N:N	N:N:N	N	Nonsense (High)
5	24.0	8	Y	Black	N	N:N:N	N:N:N	N	frameshift resulting in stop codon[c] (High)
9	1.96	13	N	Caucasian	Y	N:N:N	Y:Y:Y	Y	inversion; intron 22 (High)
11	21.6	12	Y	Hispanic	Y	Y:Y:Y	Y:Y:Y	Y	inversion; intron 22 (High)
12	1.4	15	Y	Caucasian	Y	Y:Y:Y	Y:Y:Y	N	inversion; intron 22 (High)
17	183.9	7	Y	Hispanic	N	N:N:N	N:N:N	N	inversion; intron 22 (High)
19	3.6	15	Y	Black	Y	Y:Y:Y	Y:Y:Y	Y	inversion; intron 1 (High)
27	1.5	9	N	Caucasian	Y	N:N:N	Y:Y:Y	Y	inversion; intron 22 (High)
28	12.8	18	Y	Caucasian	N	Y:Y:Y	Y:Y:Y	N	inversion; intron 22 (High)
29	1.0	26	N	Caucasian	N	Y:Y:Y	Y:Y:Y	Y	frameshift at or near A run[d] (Low)
35	3.6	11	N	Indian	N	N:N:N	N:N:N	Y	inversion; intron 22 (High)
38	44.8	17	Y	Caucasian/ Asian	N	N:N:N	N:N:N	Y	inversion; intron 22 (High)
39	21.6	16	Y	Hispanic	Y	N:Y:Y	N:N:N	Y	inversion; intron 22 (High)
40	38.4	13	Y	Hispanic	Y	N:N:N	Y:Y:Y	N	splice site (High)
47	4.8	10	Y	Caucasian	Y	N:N:N	N:N:N	Y	inversion; intron 22 (High)

ID= identification; ITI=immune tolerance induction; Y=yes; N=no; BU/ml= Bethesda Units; EDs= exposure days. [a]Intensive treatment at high dose is defined as five consecutive study days of treatment at a mean infusion dose of rAHF-PFM >50 IU/kg within ≤10 EDs, ≤20 EDs, or ≤30 EDs. [b]High risk HLA type: defined as DR15 and/or DQ06. [c]With no prior documentation of partial correction of reading frame. [d]Previously documented to have partial correction of reading frame.

Perioperative management

Intraoperative haemostatic efficacy, as judged by the operating surgeon in 22/27 subjects, was rated excellent in 18 (82%) subjects and good in four (18%) subjects (efficacy was unrated or not applicable in 5/27 subjects) (▶Table 1C). Post-operative haemostatic efficacy, as judged by the investigator in 25/27 subjects, was rated excellent in 23 (92%) subjects and good in two (8%) subjects (efficacy was not rated in 2/27 subjects). The actual blood loss as a percentage of predicted maximum blood loss was equal to or less than 100% for all procedures (median; 20%; range: 0.01–100%).

Safety[2]

Throughout the entire study, 3,877,140 IU of rAHF-PFM were administered to 55 subjects at a median dose of 49.2 (range: 22.6–112.5) IU/kg/subject and a median exposure of 76 (range: 1–414) EDs. This includes rAHF-PFM consumed during ITI treatment. Excluding the 11 subjects who received ITI treatment,

[2] Exposure to product and AEs take into account the time that subjects spent on the entire study, including ITI treatment, although the results of the ITI part of the study will be described in another report.

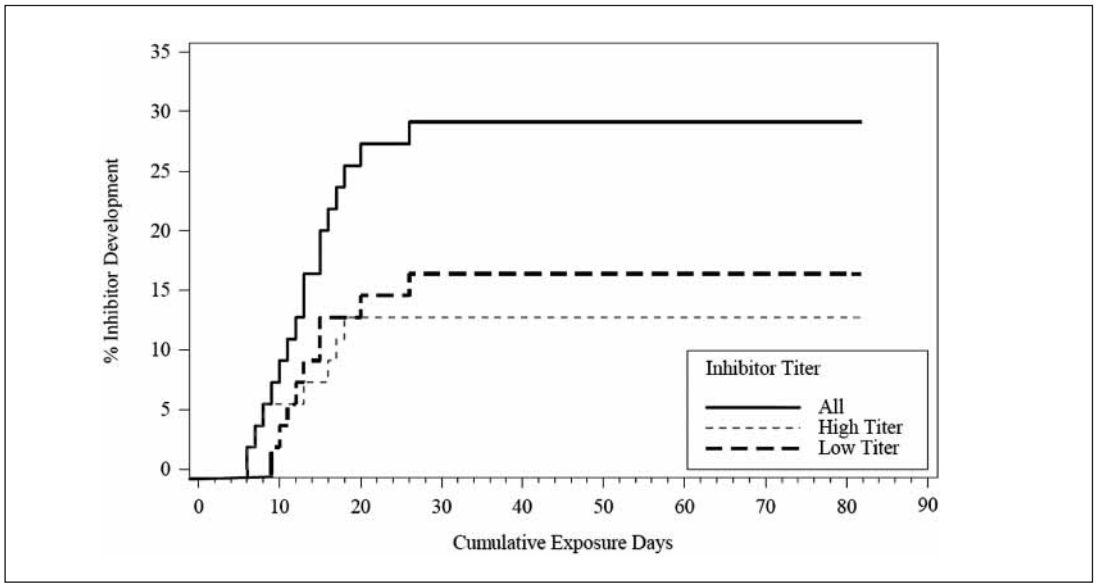

Figure 2: Cumulative incidence of inhibitor development.

1,403,473 IU were administered at a median dose of 45.7 (range: 22.6–110.1) IU/kg/subject and a median exposure of 75 (range-1–87) EDs. Throughout the entire study, the median duration of a subject's participation was 498 (range: 82–1360) days including ITI, and 549 (range: 82–1,360) days excluding subjects who received ITI.

Adverse events

During the study period, 53 subjects experienced 931 AEs, the majority of which were non-serious. Of 885 non-serious AEs, 14 events in eight subjects were considered product-related, none of which were rated severe: the following AEs were rated as moderate (one case of each): diarrhoea, vomiting, peripheral oedema, infection, and urticaria. Five cases of rash occurred in four subjects: one rated as moderate, and four in three subjects rated as mild. Four cases of pyrexia in four subjects were moderate and mild in two subjects each. Two of the cases of pyrexia (one moderate and one mild) occurred shortly after port placement surgery, and were of short duration.

The most commonly reported non-serious non-related AEs in this study are typically seen in the age group investigated: pyrexia, nasopharyngitis, cough, rhinorrhea, diarrhoea, ear infection, vomiting, upper respiratory tract infections, nasal congestion, rash, anaemia, conjunctivitis, procedural pain, diaper dermatitis, otitis media, and wheezing. All other non-serious AEs occurred in less than 10% of subjects.

There were 46 serious adverse events (SAEs) in 28 subjects. Sixteen product-related SAEs in 16 subjects were all cases of inhibitor development. Further, there were 11 complications/infections in six subjects potentially associated with the placement of a port/venous access device. Six of these complications were SAEs, (five catheter-related infections and one catheter site haematoma).

Inhibitor development

The primary safety evaluation in this study addressed inhibitor development. Inhibitory antibodies to FVIII developed in 16/55 (29.1%; 95% CI: 17.1%-41.1%) subjects. Calculations of the incidence of inhibitor development for MTPs (11/37 [29.7%; 95% CI: 15.0%-44.5%]) and PUPs (5/18 [27.8%; 95% CI; 7.1%-48.5%]) were post-hoc analyses. All the subjects who developed inhibitors had severe haemophilia (FVIII <1%). At the time of inhibitor diagnosis, seven subjects were categorised with high-titre inhibitors (>5 BU/ml confirmed with a new blood sample) and nine subjects were categorised with low-titre inhibitors (≤5 BU/ml). There was a similar incidence of inhibitor development for confirmed high-titre inhibitors (12.7%; 95% CI: 3.9%, 21.5%) and for confirmed low-titre inhibitors (16.4%; 95% CI: 6.6%, 26.1%). Eight of 16 inhibitor subjects experienced a peak high-titre inhibitor (>5 BU/ml) at least once during the course of the study (▶Table 2). One peak high titre was an unconfirmed first high titre which occurred prior to ITI treatment without an anamnestic response during ITI, and the confirmation of low titre occurred during ITI. Of these eight peak high-titre subjects, seven underwent ITI on study, four of whom were successfully tolerised, and one exited the study to join the International ITI Study (12). Four subjects with low-titre inhibitors also underwent ITI and were successfully tolerised. Of those four subjects who did not undergo ITI, one fulfilled the criteria for a transient inhibitor while remaining primarily on an on-demand regimen, and the other three subjects were switched from on-demand to prophylactic infusions ranging from 50–100 IU/kg 1–3 times weekly. No inhibitors were detected at study completion in these three subjects, and in two of these three subjects the last two inhibitor determinations were negative.

The median time to inhibitor formation was 13 (range: 6–26) EDs. The median time to inhibitor development was also 13 EDs for both low-titre (range: 9–26) and high-titre (range: 6–18) sub-

Table 3: Summary of risk factors for all subjects in the immunogenicity data set (N=50).

Risk factor		Inhibitor negative (N=34)	Inhibitor positive (N=16)	Odds ratio (95% CI)
		N	N	
Family history of inhibitors	Yes	6	8	4.95 (1.29, 19.06)
	Unknown	2	1	
	No (ref.)	26	7	
Race/Ethnicity	Non-Caucasian	8	9	4.18 (1.18, 14.82)
	Caucasian (ref.)	26	7	
FVIII gene mutation[a]	High risk	26	15	4.62 (0.52, 40.58)
	Low risk (ref.)	8	1	
High risk HLA[b]	Yes	15	9	1.63 (0.49, 5.39)
	No (ref.)	19	7	
Intensive treatment at high dose[c] (≤20 EDs +)	Yes	4	6	4.50 (1.05, 19.25)
	No (ref.)	30	10	
Intensive treatment[d] (≤30 EDs +)	Yes	10	7	1.87 (0.54, 6.40)
	No (ref.)	24	9	
Surgery (≤30 EDs)[e]	Yes	13	8	1.62 (0.49, 5.36)
	No (ref.)	21	8	
Port placement (≤30 EDs)[e]	Yes	11	6	1.25 (0.36, 4.34)
	No (ref.)	23	10	
Age at first exposure (months)	6–12 months	16	7	0.44 (0.10, 2.01)
	13–18 months	5	5	0.31 (0.06, 1.64)
	<6 months (ref.)	13	4	

The immunogenicity data set included all those developing an inhibitor and all those who were inhibitor-free with at least 10 EDs. [a] Gene mutation was categorised as follows: large deletion, inversion, nonsense, splice site, frameshift resulting in stop codon, with no prior documentation of partial correction of reading frame were assessed as high risk. Missense, in-frame, frameshift at or near a run previously documented to have partial correction of reading frame were assessed as low risk. [b] High risk HLA type: defined as DR15 and/or DQ06. (DR15 and DQ06 analysed individually were not significant risk factors). [c] Intensive treatment and high dose: defined as five consecutive study days of a mean infusion dose of FVIII >50 IU/kg. (≤20 EDs and ≤30 EDs were significant risk factors, but ≤10 EDs was not). [d] Intensive treatment: defined as at least five consecutive days of FVIII treatment. (≤10 EDs, ≤20 EDs, and ≤30 EDs were not significant risk factors). [e] ≤10 EDs, ≤20 EDs, and ≤30 EDs were not significant risk factors. ref.=reference class, + Refers to Advate: non-study or investigational product

jects. A cumulative inhibitor incidence plot demonstrating time to inhibitor development is presented in ▶Figure 2. The FVIII epitopes targeted by inhibitor subjects' antibodies were identified by affinity selection and clustered mainly in the C2 domain (nine subjects), the A2 domain (five subjects), and the A3 domain (three subjects). Epitope analysis results and discussion are provided in online supplemental material (available online at www.thrombosis-online.com).

Risk factor analysis

An immunogenicity analysis set of 50 subjects was used for the calculation of the odds ratio (OR) of risk factors for inhibitor development, which included all 16 subjects with inhibitors and subjects without inhibitors who had at least 10 EDs (▶Table 3). Univariate analysis identified statistically significant OR results for three risk factors: family history of inhibitor (4.95 [95% CI: 1.29–19.06]), non-Caucasian ethnicity (4.18, [95% CI: 1.18–14.82]), and intensive treatment at high dose within the first 20 EDs (4.50, 95% CI: 1.05–19.25). Intensive treatment at high dose in all but one subject, where it was administered for a BE, consisted of perioperative infusions in the context of port placement. In the 10 subjects who had intensive treatment at high dose, the four subjects who did not develop inhibitors had a median product exposure of 80 (range: 75–82) EDs throughout the study. Intensive treatment at high dose tended to be administered very early in the study, after a median of 3.5 (range: 0–13) EDs. Intensive treatment alone was not associated with an increased risk. Neither surgery nor port placement alone were found to be risk factors for inhibitor development; however, most subjects who received intensive treatment at high dose, which was a significant risk factor, were treated because of surgery. In some risk factor categories, the number of subjects was very small and conse-

quently the analysis lacked statistical power to generate a meaningful result. Other putative risk factors not found to impart a statistically significantly increased risk of inhibitor development were FVIII gene mutations, the DR15 and DQ06 HLA genotypes, and age at first exposure.

Inhibitor incidence in low-risk subpopulations of subjects who lacked the risk factors confirmed in this study was examined. While 16/55 (29.1%) subjects in the entire study population developed inhibitors, 7/37 (18.9%) subjects who were Caucasian and 2/25 (8.0%) subjects who were both Caucasian and had no family history developed inhibitors. In the 20 subjects who were Caucasian, had no family history of inhibitors, and did not receive intensive treatment at high dose (lacked all three risk factors) there was no inhibitor development (0/20 [0.0%]). These 20 subjects had a median of 77 (range: 1–82) EDs: 16/20 subjects had ≥30 EDs, 2/20 had <20 to ≥10 EDs and 2/20 subjects had <10 EDs. Throughout the study, these 20 subjects had a median of 10.0 (range: 0.0 to 29.0) BEs.

A linear regression analysis of antibody formation to heterologous proteins demonstrated that five subjects had a slight increase over time in antibodies against CHO or murine IgG proteins, but no statistically significant increases could be demonstrated and therefore the percentage of subjects who developed antibodies is 0% for all three heterologous proteins. No clinically relevant correlation could be found, as assessed by an examination of AEs of allergic reaction (including those considered related to rAHF-PFM [i.e. rash, urticaria]) or elevated eosinophil counts in temporal relationship with infusions.

Discussion

In this clinical study of 55 PUPs and MTPs with severe and moderately severe haemophilia A, haemostatic efficacy of rAHF-PFM was confirmed in the treatment of BEs and surgical prophylaxis. One or two infusions of rAHF-PFM were used to manage 90% of BEs, and the efficacy was considered excellent or good in 93% of treated BEs that were rated, consistent with previously reported values for rAHF-PFM (1, 2) and related rFVIII products (14–16). Likewise, intraoperative and postoperative haemostatic efficacies are comparable to previously reported values (3).

In 22 BEs treated with three or more infusions, the haemostatic efficacy was rated as excellent, although the protocol definition for excellent haemostatic efficacy only allowed a single infusion. The most likely explanation for this discrepancy could be the serious nature of the BEs where additional infusions were administered for maintenance of haemostasis.

Although more subjects were prescribed on-demand treatment than prophylaxis as the first treatment modality (47 vs. 8), on-demand subjects tended to be switched to a prophylactic regimen at a later date, when they became more physically active, following increasing numbers of BEs, or at a standard age determined by the investigative site. Patients <2 years of age generally have fewer BEs than children >2 years and thus tend to be initially prescribed an on-demand regimen, but switched to prophylaxis later (17–19). Investigators in the EU prescribed prophylaxis (either as first treatment option or switched from on-demand treatment) in a statistically significantly greater proportion of subjects than did the investigators in North America.

Approximately 30% of PUPs with severe haemophilia A develop inhibitory antibodies that diminish the efficacy of FVIII replacement therapy (4–6). The results obtained in the current study are concordant with these published results: inhibitors developed in 16/55 (29.1%) subjects. Notably, 8/16 (50.0%) inhibitor subjects never experienced a peak high-titre inhibitor (>5 BU/ml). BEs in low-titre subjects can still be managed with FVIII concentrate as demonstrated in the four subjects with low-titre inhibitor not undergoing ITI who were successfully managed with rAHF-PFM. In the ITI part of the study, all four low-titre subjects were successfully tolerised, compared to 4/7 high-titre subjects (unpublished results). Of the 16 subjects who developed inhibitors, 11 went on to ITI treatment in the ITI part of the study, eight of whom were successful[3]. One subject who developed an inhibitor was withdrawn to undergo ITI in the International ITI study (12), from where he was withdrawn again and eventually tolerised with rAHF-PFM[4]. Inhibitor development was similar between MTPs (29.7%) and PUPs (27.8%), indicating that no bias was introduced into the study by using subjects who had limited previous exposure to rAHF-PFM.

Our finding of a median time to FVIII inhibitor development of 13 (range: 6–26) EDs is consistent with values reported in other previously untreated cohorts (14, 20). Epitope analysis in this study agreed with published findings that inhibitory antibodies from patients with haemophilia A are most commonly directed against epitopes in the A2 and C2 domains of FVIII (21).

Subject characteristics were evaluated as risk factors for inhibitor development. The OR of risk for developing inhibitors was significantly higher in subjects with a family history of inhibitor (4.95 [95% CI: 1.29–19.06]). This is in agreement with the findings of the Malmö International Brother Study and an analysis of a subset of 332 subjects from the CANAL cohort which report ORs of 3.2 (95% CI: [2.1–4.9]) and 3.7 (95% CI: [1.5–9.2]), respectively (22, 23). Santagostino et al. reported that family history of inhibitors was more frequent in patients with inhibitors compared with inhibitor-free controls (20% vs. 2%) in an Italian case-control study of children with haemophilia (24).

Non-Caucasian ethnicity (4.18, [95% CI: 1.18–14.82]) was also found to be a significant risk factor for inhibitor development in the current study. Maclean et al. report non-Caucasian ethnicity to be a significant risk factor (OR: 4.7 [95% CI: 1.5–14.7]) (25). In a retrospective cohort study, Gouw et al. demonstrated that patients of African and Hispanic descent had a relative risk of inhibitor formation of 2.4 and 2.5, respectively, compared to Caucasians (26). Aledort also reported that the prevalence of inhibitors in African-Americans and Latinos is greater than that of Caucasians

[3] Successful ITI was defined by having attained 2 successive negative inhibitor titres <0.6 BU/ml (Nijmegen method), supported by a IR ratio of =0.66, calculated using the initial IR and the test IR determined at the same central laboratory.

[4] Personal communication, Charles Hay and Michael Tarantino, 2010.

(27), and Carpenter et al. report an increased inhibitor incidence in Mexican Hispanic patients (OR: 1.5 [95% CI: 1.1–1.9]) (28).

The third significant risk factor identified in the study was exposure to intensive treatment at high dose, defined as five consecutive days of a mean infusion dose >50 IU/kg/day, within ≤20 EDs, with an OR of 4.50 [95% CI 1.05–19.25]. In the CANAL cohort study of 366 PUP subjects, it was found that intensive treatment at first exposure was associated with a 3.3-fold higher incidence of inhibitor development, and intensive treatment during the first 50 EDs was associated with a two fold higher incidence of inhibitor formation (20). Ter Avest et al. reported an OR of 7.7 (95% CI: 3.8,15.2) for five consecutive days of treatment at first treatment in a PUP population (23).

To examine the impact of risk factors on inhibitor development, inhibitor incidence in subjects who lacked the high risk factors confirmed in this study were examined. As subpopulations were defined with successively lower risk (Caucasian, no family history of inhibitors, and no intensive treatment at high dose), inhibitor incidence progressively decreased from 29.1%, to 18.9%, to 8.0% and ultimately to 0.0%. This progressive decrease and the absence of inhibitor development in the lowest risk population confirm the influence of these risk factors on inhibitor development in the present study.

In several analyses of data from PUPs, high-risk mutations of the FVIII gene were associated with increased inhibitor risk: Maclean et al. (25) report an OR of 5.1 (95% CI: 1.9–13.7) in 143 subjects with genetic analysis, and Gouw et al. (20) report an OR of 2.8 (95% CI: 1.5–5.0) in 312 patients with genetic analysis. However, in the current study, which had a considerably smaller sample size, high-risk mutations of the FVIII gene were not found to be statistically significant risk factors, although the majority of subjects (15/16) who developed inhibitors had a high-risk mutation such as inversion, nonsense, or splice site mutation. In this study, intensive treatment at high dose was employed almost exclusively in the context of port-placement. Therefore, any surgical intervention requiring intensive treatment at high dose including port placement should only be considered after careful evaluation of the potential risks and benefits.

In order to confirm the increasing importance of avoiding immunologic danger signals during prophylactic treatment of PUPs, as described by Kurnik et al. (29), a prospective, historically controlled clinical study has been initiated by Baxter to evaluate an early low-dose prophylactic regimen. The study is planned to avoid immunological danger signals, such as tissue and cell damage (e.g. surgical procedures, BEs), which activate inflammatory responses, at the time of rAHF-PFM infusion.

The incidence of inhibitor formation observed in the current study is aligned with previous reports as are the findings of significant associations of inhibitor development with non-Caucasian ethnicity, family history of inhibitors, and intensive treatment at high dose within ≤20 EDs. In summary, the results of this study confirm an already established overall record of safety and haemostatic efficacy of rAHF-PFM for the routine clinical management and perioperative coverage of patients with severe to moderately severe haemophilia A in PUPs and MTPs.

What is known about this topic?

- Studies of previously untreated patients (PUPs) and minimally treated patients (MTPs) with haemophilia A are rare because of the small number of subjects available. These studies can provide valuable information on the immunogenicity, efficacy, and safety of factor (F)VIII replacement treatment in a vulnerable population.
- Development of FVIII inhibitory antibodies is one of the most serious adverse events that can occur in PUPs and MTPs treated with FVIII. Inhibitor incidence has been demonstrated in other studies to be approximately 30%.
- Genetic and non-genetic risk factors contribute to the development of inhibitors.

What does this paper add?

- FVIII replacement treatment with rAHF-PFM (Advate®) was found to be safe and effective for the management and perioperative coverage of PUPs and MTPs with severe to moderately severe haemophilia A.
- The incidence of FVIII inhibitor development in this study was 29.1%, confirming previous findings in other studies.
- In this study, family history of inhibitors, non-Caucasian ethnicity, and intensive treatment at high dose (mean dose of ≥50 IU/kg/day for at least five consecutive days) were significant risk factors for inhibitor development.

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Conflicts of interest

The work presented is a Baxter-sponsored clinical study. Several authors (N. Guzmán-Becerra, J. A. Dyck-Jones, B. M. Ewenstein,

[5] Currently in Portland, OR, USA.

and B. E. Abbuehl) are employed by Baxter Healthcare, Inc. G. Auerswald, A. A. Thompson, M. Recht, D. Brown, and R. Liesner were investigators in the study and therefore their institutions received research support from Baxter Healthcare, Inc. In addition, G. Auerswald received grants for symposia or clinical studies and congresses from Baxter, Bayer, CSL-Behring, Novo Nordisk and Biotest. M. Recht also received institutional research support from Novo Nordisk and Biogen Idec, and R. Liesner previously received payment from Baxter for consultancies and honoraria.

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