Scientific Category: Clinical Trials and Observations

Pegylated, Full-length, Recombinant Factor VIII for Prophylactic and On-demand Treatment of Severe Hemophilia A

Short Title: Pegylated Factor VIII in Severe Hemophilia A

Authors: Barbara A. Konkle, Oleksandra Stasyshyn, Pratima Chowdary, David H. Bevan, Tim Mant, Midori Shima, Werner Engl, Jacqueline Dyck-Jones, Monika Fuerlinger, Lisa Patrone, Bruce Ewenstein, Brigitt Abbuehl

Addresses: 1Puget Sound Blood Center and University of Washington, Seattle, United States, 2Academy of Medical Sciences of Ukraine, Lviv, Ukraine, 3Katharine Dormandy Haemophilia Centre and Thrombosis Unit, Royal Free Hospital, London, United Kingdom, 4St Thomas’ Hospital, London, United Kingdom, 5Quintiles Drug Research Unit at Guy's Hospital, London, United Kingdom, 6Nara Medical University Hospital, Nara, Japan, 7Baxalta Innovations GmbH, Vienna, Austria, 8Baxalta US Inc., Westlake Village, United States

*Correspondence: Brigitt E. Abbuehl, Baxalta Innovations GmbH

Donau-City-Strasse 7, A-1220 Vienna, Austria

Tel +43 1 20 100-2473421; fax +43 1 20 100-2475734; brigitt.abbuehl@baxalta.com

Keywords: Extended half-life, Factor VIII, hemophilia A, pegylated, prophylaxis, BAX 855

Support: The study was funded by Baxter BioScience which has been renamed Baxalta

Text: = 3998 words; Abstract = 248 words; Figures = 5; Tables = 5; References = 23
KEY POINTS

<table>
<thead>
<tr>
<th>BAX 855, a pegylated full-length rFVIII with extended half-life, was highly effective in the prevention and treatment of bleeding events.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No subjects receiving BAX 855 developed FVIII inhibitory antibodies nor experienced unexpected adverse events.</td>
</tr>
</tbody>
</table>
ABSTRACT

Current management of hemophilia A includes prophylaxis with factor VIII (FVIII) replacement every 2-3 days. BAX 855, Baxalta’s pegylated full-length recombinant FVIII (rFVIII), was designed to increase half-life and thus, reduce the frequency of prophylactic infusions while maintaining hemostatic efficacy. BAX 855 was evaluated in previously treated patients (PTPs) with severe hemophilia A, aged 12-65 years. A phase 1 study compared the pharmacokinetic (PK) profile of BAX 855 to licensed rFVIII (ADVATE®). In a pivotal study, the annualized bleeding rate (ABR), PK parameters and the efficacy of bleeding treatment were assessed. In the phase 1 study, the mean half-life ($T_{1/2}$) and the mean residence time (MRT) of BAX 855 compared to ADVATE® were 1.4- to 1.5-fold higher. These results were confirmed in the pivotal study.

The pivotal study met its primary endpoint: prophylaxis with BAX 855 resulted in an ABR that was significantly lower than half the ABR of on-demand treatment ($p<0.0001$). The median ABR was 1.9, and 39.6% of compliant subjects had no bleeding episodes during prophylaxis, while subjects treated on-demand had a median ABR of 41.5. BAX 855 was also efficacious for the treatment of bleeding episodes with 95.9% of bleeding episodes treated with 1-2 infusions and 96.1% having efficacy ratings of excellent/good.

No FVIII inhibitory antibodies or safety signals were identified. These studies provide evidence that BAX 855 was safe and efficacious for on-demand treatment and prophylaxis administered twice weekly in patients with hemophilia A. The studies were registered at ClinicalTrials.gov as NCT01736475 and NCT01599819.
INTRODUCTION

Hemophilia A is an X chromosome-linked recessive, congenital bleeding disorder characterized by a deficiency of functional coagulation factor VIII (FVIII), resulting in a prolonged clotting time that leads to frequent bleeding in joints and soft tissue. Patients with hemophilia A who adopt a prophylactic regimen can reduce their bleeding rate, and thus, reduce the probability of developing chronic arthropathy which leads to disability. Current management of severe hemophilia A (FVIII <1% of normal), includes on-demand treatment for bleeding episodes and prophylaxis. The average half-life (T$_{1/2}$) of FVIII products is in the range of 10-14 hours, thus current prophylactic regimens require infusion of FVIII every other day, or every 2-3 days when based on each patient’s individual pharmacokinetic (PK) profile. An extended half-life rFVIII product would offer additional therapeutic options for patients permitting a reduction in the frequency of prophylactic infusions. Several strategies for creating longer acting replacement factors are in development, including modifications to the FVIII molecule, such as pegylation, glycopegulation, recombinant fusion to Ig Fc, modification of the amino acid sequence to create sites for site-directed pegylation, and disulfide linkage between its light and heavy chains. Chemical modification with pegylation is a well-established method to improve the PK profile by extending T$_{1/2}$ and circulation of therapeutic proteins. With the goal of reducing the number of infusions required per week for prophylaxis, a pegylated recombinant FVIII- rurioctacog alfa pegol (BAX 855) was built to extend FVIII half-life on the manufacturing platform of ADVATE®, a full-length, unmodified rFVIII (Baxalta US Inc., Westlake Village, CA, USA). BAX 855 is manufactured by covalently binding a branched polyethylene glycol (PEG) reagent with a molecular weight of 20 kDa to ADVATE using proprietary technology from Nektar
Therapeutics (San Francisco, CA, USA). BAX 855 is created through controlled pegylation, where approximately 60% of the PEG chains are localized to the B-domain.14

Two clinical studies of BAX 855 were conducted in male patients with severe hemophilia A. A phase 1 study assessed the safety and PK of BAX 855 compared to ADVATE at 2 doses (30 ±3 and 60 ±6 IU/kg) (all doses are IU/kg bodyweight). This was followed by a pivotal study undertaken to evaluate the efficacy, PK (45 ±5 IU/kg), and safety of BAX 855 administered as twice weekly prophylactic or as on-demand therapy in severe hemophilia A. This report describes the efficacy and safety results of these two studies.

METHODS

The studies received ethical approval by institutional review boards according to ICH GCP guidelines which are based upon principles that have their origin in the Declaration of Helsinki.15 Written informed consent was obtained from all subjects at the time of enrollment. Independent Data Monitoring Committees (DMCs) monitored the subjects’ safety throughout the studies. Study flow and subject disposition are presented in Figure 1.

Study Designs

A phase 1, prospective, open label, cross-over, dose-escalation study evaluated safety and PK of single doses of BAX 855 compared to single doses of ADVATE at 2 doses. In the 30 ±3 IU/kg arm, subjects were infused with ADVATE with collection of 7 post-infusion blood samples for FVIII measurement over a 48 h period. After a 72 h washout period, the same dose of BAX 855 was administered with collection of 14 post-infusion blood samples for FVIII measurement over a 168 h period (refer to Supplemental Data). After review of the data and approval by the DMC, the 60 ±6 IU/kg arm underwent the same evaluations.
A pivotal phase 2/3, multicenter, open-label study evaluated efficacy and safety of prophylactic and on-demand treatment, as well as PK of BAX 855. Subjects were assigned to treatments based upon their pre-study FVIII treatment regimen; however, once 17 subjects were assigned to on-demand treatment, subsequent subjects were assigned to the prophylactic arm, regardless of their previous treatment regimen. The prophylactic treatment regimen of 45 ±5 IU/kg twice weekly dose was designed to assure that a majority of subjects maintain FVIII levels above 1% at all times. This dose was selected based on calculations that took into account the 1.4 to 1.5 fold extended half-life of BAX 855 as compared to ADVATE observed in the phase 1 study. The prophylactic dose was administered for ≥50 EDs or 6 months ±2 weeks. On-demand therapy with BAX 855 at a dose of 10 to 60 ±5 IU/kg occurred for 6 months ±2 weeks. A subgroup of 25 subjects who were to receive prophylactic treatment was included in a PK evaluation. ADVATE was used as a PK comparator; subjects first received 45 ±5 IU/kg ADVATE with collection of 10 post-infusion blood samples for FVIII measurement over a 56 h period followed by a minimum washout period of 72 h and then BAX 855 at 45 ±5 IU/kg with collection of 12 post-infusion blood samples over a 96 h period. A repeat PK followed after subjects completed at least 50 EDs following a minimum washout period of 84 h washout period with collection of 12 post-infusion blood samples over a 96 h period (refer to Supplemental Data). At baseline, the number of target joints, (a joint with ≥3 spontaneous bleeding episodes in any consecutive 6-month period), was recorded. Arthropathy was considered present if reported in the subject’s medical history or if he had underwent joint surgery.

**Patient Population**

The key criteria for inclusion were similar in both studies: a diagnosis of severe hemophilia A (untreated FVIII activity <1%), age 12-65 years, and previous treatment with plasma-derived
FVIII or rFVIII concentrates for \( \geq 150 \) documented exposure days (EDs). Subjects must also have
had no history of FVIII inhibitory antibodies \( (\geq 0.4 \text{ BU using the Nijmegen modification of the}
Bethesda assay or \( \geq 0.6 \text{ BU using the Bethesda assay}) \) at any time prior to screening and no
detectable FVIII inhibitory antibodies as confirmed by the central laboratory at screening. A
complete list of inclusion and exclusion criteria is provided in Supplemental Data.

**Outcome Measures**
The primary outcome measure of the phase 1 study was the number and proportion of subjects
experiencing serious and non-serious adverse events (SAEs/AEs). PK parameters as assessed by
the one-stage clotting assay and the chromogenic assay were secondary outcome measures in
both studies. The pivotal study’s primary efficacy outcome measure was to compare annualized
bleeding rates (ABRs) between the prophylactic and on-demand treatment groups. Secondary
efficacy outcome measures in the pivotal study included evaluating BAX 855 for treatment of
bleeding episodes as “excellent or good” according to a 4-point rating scale (described in
Supplemental Data), the number of infusions to treat bleeding episodes, the time interval
between bleeding episodes, the weight-adjusted consumption of BAX 855, and patient reported
outcomes (PROs) including the SF-36 validated questionnaire to assess quality of life (QoL) and
the Haemo-SYM questionnaire, a hemophilia-specific validated tool.\(^{16}\)

Safety outcome measures in both studies were tolerability and immunogenicity, including the
development of inhibitory antibodies to FVIII and binding antibodies (IgG and IgM) to FVIII,
PEG-FVIII, and PEG analyzed using validated ELISA assays. Antibodies to Chinese Hamster
Ovary (CHO) proteins (considered potential impurities from the manufacturing process) were
assessed only in the pivotal study. Safety outcome measures for both studies included the
occurrence of AEs and SAEs, and changes in vital signs and clinical laboratory parameters.
**Statistical analysis**

In the phase 1 study, the number and proportion of subjects experiencing SAEs and non-serious AEs from the first treatment up to 4 weeks ±4 days after infusion of BAX 855 were summarized. All non-serious AEs that occurred during or after treatment were presented descriptively. In a post-hoc analysis, Spearman's rank correlation test was performed to evaluate the correlation between the T1/2 and clearance of FVIII and VWF.

In both studies, PK parameters were calculated by the non-compartmental analysis module of Phoenix WinNonlin V6.2.1. (Pharsight Corporation, Cary, NC, USA).

In the pivotal study, approximately 132 subjects were planned for treatment, with the aim of achieving approximately 104 subjects evaluable from the prophylactic arm and 15 subjects from the on-demand arm. For the primary outcome measure, prophylaxis was considered successful if the upper limit of the 95% confidence interval (CI) for the ratio between treatment means didn’t exceed 0.5 (corresponding to a 50% reduction of the mean ABR compared to on-demand treatment). A negative binomial model accounting for prophylaxis vs on-demand, presence or absence of target joints at screening, and age at screening as fixed effects and the duration of the observation period as an offset was used. The primary outcome analysis was performed on the full analysis set (FAS) which included all subjects assigned to treatment, and the per-protocol analysis set (PPAS) (Table 1). Success of bleeding episode treatment was defined as a rating of excellent or good. The 95% CI of mean success rates was calculated within a generalized estimating equation (GEE) model, pooling both treatment regimens, and the lower limit of the CI was compared to a 70% threshold (the lowest clinically acceptable success rate). Power calculations for the analyses of the primary outcome and key secondary outcome measures were performed by Monte Carlo simulations (details are provided in Supplementary Data).
Descriptive statistical analyses were presented for bleeding episodes, PK parameters, and safety. PK parameters were also analyzed for correlations with ABR and VWF:Ag concentrations using Spearman’s rank correlation coefficients. Statistical analysis of the proportion of subjects developing inhibitory antibodies to FVIII was conducted using the Clopper Pearson technique. Changes from baseline to end of treatment in the QoL and PRO assessments were estimated using the Hodges-Lehmann estimator and were compared between prophylaxis and on-demand treatment in a hierarchical testing scheme.

RESULTS

Patients
Demographics are presented in Table 2. In both studies, distributions of characteristics were comparable for each treatment group. In the phase 1 study, 19 subjects received single infusions of BAX 855 and ADVATE for PK analysis at 5 investigative sites. In the pivotal study, 138 patients at 72 investigative sites were assigned to treatment arms, and 137 subjects received at least one dose of BAX 855 (1 was treated only with ADVATE); including 120 subjects who received prophylactic treatment (99 of whom had received prior prophylactic FVIII treatment and 21 who were previously treated on-demand) and 17 subjects who received on-demand treatment (previously treated on-demand). Twenty-seven subjects from the prophylactic arm were evaluated for PK. The median age for both studies was 29 years. All subjects were male and most were White (75.2%) or Asian (24.1%); 1 was Black/African American. One hundred twenty six (126) subjects completed the pivotal study.

Efficacy of Prophylactic Treatment
The efficacy of prophylaxis was assessed in the pivotal study only. The study met its primary endpoint for prophylactic treatment compared to on-demand treatment using a negative binomial model analysis, which demonstrated a >50% reduction in ABR for the prophylactic arm (p<0.0001). A 90% reduction in ABR was observed in the prophylactic arm compared with the
on-demand arm. Descriptive statistics also showed a much lower median (Quartile 1; Quartile 3 [Q1; Q3]) ABR for the prophylactic arm (1.9 [0.0; 5.8]) compared to the on-demand treatment arm (41.5; [31.7; 51.1]). A lower ABR was observed in the prophylactic treatment arm regardless of bleeding type or etiology: joint ABRs were 0.0 (0.0; 2.0) during prophylaxis compared to 38.1 (24.5; 44.6) for on-demand treatment and spontaneous/unknown ABRs were 0.0 (0.0; 2.2) during prophylaxis compared to 21.6 (11.2; 33.2) for the on-demand treatment (Figure 2).

Subjects received a median (Q1; Q3) of 44.6 (42.6; 46.8) IU/kg of BAX 855 per prophylactic infusion. The mean (SD) reduction in dosing frequency from pre-study prophylaxis was 26.7% (27.9), with a reduction of 33.7% (2.5; 36.7). Furthermore, 70.4% of subjects treated prophylactically were able to reduce the frequency of dosing from their pre-study prophylactic treatment regimens by 30% or more which is approximately equivalent to at least one less prophylactic infusion per week when using BAX 855 for prophylaxis. There was a median of 1.96 prophylactic infusions per week, indicating that BAX 855 prophylaxis was administered twice weekly. There was a negative correlation between ABR and T1/2 (-0.49 at the initial PK and -0.42 at the repeat PK), suggesting that subjects with a shorter circulation time had a higher ABR.

The mean (±SD) joint ABR in subjects in the prophylactic arm presenting with target joints at the time of screening was higher (2.2 ±3.2) than in those without target joints (1.2 ±2.4) (Figure 3). As there were only 2 subjects in the on-demand arm without target joints, a comparison of ABR between those with and without target joints is limited. During prophylaxis, 1 subject developed a new target joint and 1 subject had a target joint revert to a non-target joint, while 5 subjects treated on-demand developed new target joints.
In the prophylactic arm there were approximately twice as many subjects with arthropathy (67 subjects) at screening as compared to those without (34 subjects), and there was a higher mean (SD) joint ABR in the subjects with arthropathy (2.1 [3.2]) than in subjects without (1.4 [2.6]).

Approximately 60% of patients on prophylaxis achieved ≥5 months between any hemorrhages, including 39.6% with zero bleeding events. For joint bleeds, approximately 80% of patients achieved ≥6 months between episodes, including 57.4% with zero joint bleeding events. The observed frequency of bleeding was higher at >96 hours after prophylactic infusions than observed at earlier times after infusions, however this represents a time period that is out of compliance with the prophylactic dosing regimen (Table 3).

**Efficacy of Bleeding Treatment**

The efficacy of treatment was assessed in the pivotal study in all patients treated for bleeding (Table 4). Subjects were treated with a median (Q1; Q3) dose of BAX 855 of 30.87 (21.2; 45.2) IU/kg per episode and 29.19 (22.3; 44.0) IU/kg for the maintenance of hemostasis, which were additional infusions administered within 48 hours after a treatment. The study met its secondary efficacy endpoint. The rate of success of bleeding treatment (rated excellent or good) was 97%, with a corresponding 95% CI ranging from 94% to 98% and thus significantly larger than 70% (p <0.0001). Of 518 bleeding episodes reported during the study, treatment was rated excellent or good for 91.6%, and 95.9% were treated with 1 or 2 infusions. The dose per infusion used to treat bleeding episodes increased with bleeding severity (Table 4). Compared to the results for all bleeding episodes, the efficacy of treatment was similar for joint and non-joint bleeding episodes (Supplemental Data).
Pharmacokinetics

In the phase 1 study, subjects received a single dose of BAX 855 for the PK assessment. The range of total dose per PK infusion was: 29.4-31.3 IU/kg in the 30 IU/kg arm and 54.0-60.0 IU/kg in the 60 IU/kg arm. In the pivotal study, the range of BAX 855 PK was 40.2-54.8 IU/kg for the initial assessment, and 38.9-48.5 IU/kg for the repeat.

In both studies, mean T$_{1/2}$ and MRT were longer, mean CL was lower, mean AUC$_{0-\infty}$ was greater, and mean IR was similar for BAX 855 compared to ADVATE at the same dose level. At the initial PK assessment for BAX 855 in the pivotal study, the mean T$_{1/2}$ increased by 1.4-fold (Table 5). Pharmacokinetic curves demonstrate an extended PK profile for BAX 855 compared to ADVATE in Figure 4. The MRT increased by 1.5-fold, and AUC$_{0-\infty}$ by 1.9-fold compared to ADVATE using the one-stage clotting assay. As expected, the ratios between a repeat PK (after $\geq$50 EDs) and the initial PK confirmed that individual PK parameters for BAX 855 remained consistent over time, indicating that repeated exposure didn’t affect the PK profile of BAX 855. In the phase 1 study, mean fold increases in MRT with BAX 855 compared to ADVATE were 1.4-fold in the 30 IU/kg arm and 1.5-fold in the 60 IU/kg arm and the mean fold increases in T$_{1/2}$ were 1.4-fold in the 30 IU/kg arm and 1.5-fold in the 60 IU/kg arm. PK results from the chromogenic assay were similar (data not shown).

Pre-infusion VWF:Ag concentration was positively correlated with T$_{1/2}$ for both ADVATE and for BAX 855 at the initial PK and the repeat PK (Spearman’s rank correlation coefficients of +0.63, +0.72 and +0.35, respectively) (Figure 5), which was similar in the phase 1 study (Spearman’s rank correlation coefficients were +0.62 and +0.68 for ADVATE and the combined BAX 855 doses, respectively).
Patient Reported Outcomes (PROs) and Quality of Life (QoL)
In the hierarchical test of PROs, assessed at baseline and at study completion, there were no statistically significant differences in QoL or symptoms over time identified in the prophylactic arm relative to the on-demand arm. However, the change in the Physical Component Score (2.67), Role Physical (4.90), Physical Functioning Score (4.21), and Social Functioning Score of the SF-36 (5.45) in the subjects receiving prophylaxis relative to subjects treated on-demand was larger than established minimally important differences for the SF-36, and therefore, may be considered clinically meaningful changes.

Safety
In the phase 1 study, subjects received a single infusion of BAX 855. No subject experienced an SAE or an AE that was temporally associated with the infusion, and no subject discontinued the study after exposure due to an AE. Eight subjects experienced a total of 11 non-serious AEs, none of which were considered related to BAX 855, and all of which were consistent with the known safety profile of ADVATE.

In the pivotal study, a total of 171 AEs were reported in 73 (53.3%) subjects following the administration of BAX 855 for approximately 6 months. Of these, 5 were SAEs (all considered not related to BAX 855 treatment) in 5 (3.6%) subjects including osteoarthritis, herpes zoster infection, humerus fracture, and neuroendocrine carcinoma and muscle hemorrhage. There were 7 adverse reactions in 6 subjects which were considered to have a reasonable possibility of a causal relationship with the use of BAX 855, including diarrhea, nausea, headache, and flushing – all consistent with the known safety profile of ADVATE.

In the phase 1 study, no subjects had detectable binding antibodies with confirmed specificity to FVIII, PEG-FVIII or PEG at any time point. In the pivotal study no subjects developed
inhibitory antibodies to FVIII of ≥0.6 BU/mL, antibodies to CHO proteins, or persistent binding antibodies to FVIII, PEG-FVIII or PEG. Nine subjects had pre-existing binding antibodies prior to exposure with BAX 855; 1 subject with anti-FVIII IgG, 6 subjects with anti-PEG-FVIII IgG, and 2 subjects with anti-PEG-FVIII IgM and anti-PEG IgM antibodies. Seven subjects who tested negative at screening developed transient IgG antibodies against FVIII (4 subjects) or PEG-FVIII (3 subjects) at 1 or 2 consecutive visits after exposure to BAX 855. Binding antibodies that were detected prior to exposure to BAX 855 or that transiently developed during the study could not be correlated to an impaired treatment efficacy or related AEs. No subjects who developed transient antibodies had PK analysis, and only 3 of the 9 subjects with pre-existing antibodies participated in the PK analysis, so no meaningful conclusion can be drawn, however, the currently limited available data don’t indicate an impact of binding antibodies on PK parameters.

Analysis of clinical chemistry, hematology, and lipid panel laboratory assessments suggest that there were no definite trends towards abnormality over time and clinically significant values could be attributed to pre-existing conditions.

**DISCUSSION**

This is a report of the phase 1 and pivotal clinical studies for BAX 855, a pegylated full-length rFVIII product with extended half-life designed to reduce the frequency of prophylactic infusions while maintaining hemostatic efficacy in patients with hemophilia A. These studies evaluated the safety, efficacy, PK, and immunogenicity of BAX 855, administered as prophylactic and on-demand treatments. The PK results from both studies confirmed an extended half-life of BAX 855 compared to ADVATE, demonstrating that $T_{1/2}$ and MRT of BAX 855 were extended
by approximately 1.4-fold and 1.5-fold, respectively. A repeat PK assessment with BAX 855 conducted after at least 50 EDs showed consistent PK parameters.

VWF:Ag levels were positively correlated with $T_{1/2}$ of both ADVATE and BAX 855, which can be explained by the role VWF plays in protecting FVIII from premature degradation in plasma.\textsuperscript{17} Such a positive correlation between $T_{1/2}$ of rFVIII and VWF levels has been previously reported.\textsuperscript{18, 19} This finding suggests that the clinical management of prophylaxis in subjects with low VWF:Ag levels be monitored more closely, as these subjects might benefit from a personalized dosing regimen.\textsuperscript{20}

Subjects in the prophylaxis arm had a significantly reduced (90%) ABR compared to those in the on-demand arm. This reduction in ABR, with a twice weekly infusion frequency with BAX 855, is comparable to reductions in ABR reported by Valentino et al. with the use of ADVATE for prophylaxis administered every 2-3 days.\textsuperscript{9} In that study, the goal of the PK-directed arm was to attain a 1% FVIII trough level for ADVATE, which was similar to the approach taken in this study with BAX 855. This result demonstrates that BAX 855 can achieve the same degree of bleeding prevention as ADVATE with one fewer infusion per week, providing an additional treatment option for hemophilia A patients. The majority of subjects in the prophylactic arm reduced the frequency of dosing from their pre-study prophylactic treatment regimens by at least one less prophylactic infusion per week when using BAX 855 for prophylaxis, also supporting the use of twice-weekly infusions. The time between bleeding episodes was longer for subjects treated prophylactically with BAX 855 compared to subjects treated on-demand. Approximately 60% of patients on prophylaxis achieved $\geq$5 months between any hemorrhages, including 39.6% with zero bleeding events during the treatment period.
ABR showed a negative correlation with T$_{1/2}$ of BAX 855, suggesting a shorter T$_{1/2}$ is associated with a higher rate of bleeding. This finding further argues for performing PK evaluations on hemophilia A subjects to determine if they might benefit from a personalized prophylactic dosing regimen.

Subjects in the prophylactic arm reported clinically meaningful although not statistically significant improvements from baseline to follow-up, relative to subjects in the on-demand arm and according to established minimally important differences in the Physical Component, Role Physical, Physical Functioning, and Social Functioning Scores of the SF-36 questionnaire. However, the study wasn’t powered for this comparison. In addition to the imbalance between the sample sizes of the prophylaxis (121) and on-demand treatment arms (17), the majority of subjects in the prophylaxis arm (82.6%) were on prophylaxis prior to the study, which made demonstration of an improvement of PROs in this population difficult.

BAX 855 demonstrated efficacy in the treatment of bleeding episodes. Of all bleeding episodes treated with BAX 855, 95.9% were treated with 1 or 2 infusions and 96.1% had a hemostatic efficacy rating of excellent or good. This is comparable to the results with ADVATE where the majority of bleeding episodes were treated with 1 or 2 infusions and the hemostatic efficacy of treatment was rated as excellent or good.$^{9,21-23}$

Chemical modification with PEG is a well-established method to improve the PK profile by extending T$_{1/2}$ and circulation of therapeutic proteins.$^{13}$ Preclinical animal studies of BAX 855 showed extended T$_{1/2}$ and clinically relevant prolonged efficacy compared to ADVATE without signs of toxicity or immunogenicity.$^{14}$ To confirm the safety of pegylated rFVIII, safety and immunogenicity of BAX 855 were assessed in both clinical studies. There were no deaths, SAEs,
or allergic reactions related to the use BAX 855 during the studies. No increased risk for PTPs to develop inhibitory antibodies to FVIII after treatment with BAX 855 was observed. There were no immune responses to BAX 855 with a clinical impact and no evidence of hypersensitivity reactions. No subject developed persistent binding antibodies to FVIII, PEG-FVIII or PEG. No new safety concerns were identified in the evaluation of laboratory values and vital signs over time with the use of BAX 855. The results described in this report reflect a relatively short-term exposure (6 months) to BAX 855; however, the adverse reactions reported were consistent with the safety profile of ADVATE, indicating no effect of FVIII-pegylation on product safety.

In conclusion, the results of these studies provide evidence that BAX 855 is safe and efficacious for treating bleeding episodes and for prophylaxis administered twice weekly in patients with severe hemophilia A.

**ACKNOWLEDGEMENTS**

The studies were funded by Baxter Healthcare Corporation. The authors recognize with gratitude the patients and investigators who participated in the studies (complete list of investigators in Supplemental Data). The authors thank the BAX 855 clinical study teams for operational and administrative support: Erik Bjornson, Bruce Brown, Maureen Conlan, Iraj Daizadeh, Josh Epstein, Sandor Fritsch, Frank Horling, Diane Ito, Manuela Koska, Stephan Lehr, Marzena Murawska, Said Omar, Marielle Parise, Elizabeth Schwartz, Marlies Sharkawy, Dikla Sharon, Julia Singer, Barbara Valenta-Singer, and Suzanne Wilson.

**AUTHORSHIP CONTRIBUTIONS**

Investigators B.A. Konkle, O. Stasyshn, and P. Chowdary, of the pivotal study, and T. Mant, D.H. Bevan, and M. Shima of the phase 1 study participated in the studies and contributed to the
writing and critical review of the manuscript. B. Abbuehl oversaw the conduct of the pivotal study and analyzed and interpreted data. M. Fuerlinger reviewed pivotal study data, L. Patrone interpreted data and critically reviewed and contributed to the writing of the manuscript. B. Ewenstein oversaw the design of both studies and critically reviewed the manuscript. W. Engl performed the sample size simulations and the statistical analyses, interpreted the results, and reviewed the manuscript. J Dyck-Jones interpreted data and prepared the manuscript.

CONFLICT OF INTEREST DISCLOSURES

B. Abbuehl, B. Ewenstein, W. Engl, M. Fuerlinger, L. Patrone, and J. Dyck-Jones are employees of Baxalta and own stocks or shares in Baxalta/Baxter, the sponsor of the study.

The authors below disclose Baxter as a funding source, however, Baxter has been renamed Baxalta. B.A. Konkle discloses research support: Baxter, Biogen-Idec, NovoNordisk, Octapharma; and consultancy: Baxter, Biogen-Idec, Bayer, NovoNordisk, Pfizer, CSL Behring. O. Stasyshn received research funding and honoraria from Baxter Healthcare Corporation. P. Chowdary discloses: research support: CSL Behring, Novo Nordisk and Pfizer; Advisory boards: Baxter, Biogen, CSL Behring, Novo Nordisk, Pfizer and Sobi. D.H. Bevan discloses receiving grants from Grifols; personal fees from Pfizer and CSL Behring; grants, personal fees and non-financial support from Baxter; grants and non-financial support from Novo-Nordisk and Biogen-Idec; personal fees and travel support from SOBI; and grants from Alnylam outside the submitted work. D. Bevan has also served on advisory boards for Pfizer, CSL Behring, and SOBI. T. Mant is employed by Quintiles and received support from Baxter to conduct the study. TM is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London. M. Shima
discloses: research support: Chugai, Bayer, Baxter, NovoNordisk, Pfizer, CSL-Behring, KAKETSKEN, Biogen-Idec; consultancy: Chugai Pharmaceutical; honoraria: Chugai, Roche, Bayer, Baxter, NovoNordisk, Biogen-Idec, Pfizer; and member of scientific advisory board: Chugai, Roche, Bayer, Baxter, Biogen-Idec, CSL Behring, NovoNordisk, Pfizer.
REFERENCES


### Table 1 Criteria for Inclusion in the Per-protocol Analysis Set (PPAS) of the Pivotal Study

<table>
<thead>
<tr>
<th>Regimen</th>
<th>Treatment</th>
<th>Criterion</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>Prophylaxis</td>
<td>Infusion interval of 5 or more days did not occur more than 3 times</td>
<td>107 (89.2)</td>
</tr>
<tr>
<td>(N=120)</td>
<td></td>
<td>The daily dose was below 35 IU/kg in no more than 10% of the infusions</td>
<td>116 (96.7)</td>
</tr>
<tr>
<td>Break-through</td>
<td>Blood episode</td>
<td>Dose to treat the bleed was below 5 IU/kg for minor bleed, below 10 IU/kg for moderate bleed, or below 25 IU/kg for a major bleed for no more than 5 bleeds (minor/moderate/major taken together)</td>
<td>120 (100.0)</td>
</tr>
<tr>
<td>On-demand</td>
<td>Bleeding episode</td>
<td>Dose to treat the bleed was below 5 IU/kg for minor bleed, below 10 IU/kg for moderate bleed, or below 25 IU/kg for a major bleed for no more than 5 bleeds (minor/moderate/major taken together)</td>
<td>17 (100.0)</td>
</tr>
</tbody>
</table>

Abbreviations: N= subjects who received treatment; n=subjects who met criterion for inclusion in PPAS

Note: 121 subjects were assigned to the prophylaxis regimen (full analysis set), however, there were only 120 subjects (safety analysis set) who received prophylactic treatment with BAX 855, of which 101 qualified for the PPAS. One subject received ADVATE for PK and then discontinued the study.

Note: For guidelines for treatment of bleeds and definition of minor, moderate, or major bleed, refer to Supplemental Data.
Table 2 Subject Demographics in Subjects who Received BAX 855

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Phase 1 Study&lt;sup&gt;a&lt;/sup&gt; N=19</th>
<th>Pivotal Study Prophylaxis N=120</th>
<th>Pivotal Study On-demand N=17</th>
<th>Pivotal Study All N=137</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age (min-max) [y]</td>
<td>29 (18-60)</td>
<td>28 (12 – 58)</td>
<td>32 (13 – 56)</td>
<td>29 (12 – 58)</td>
</tr>
<tr>
<td>Median Weight (min-max) [kg]</td>
<td>82.6 (52.5-128.0)</td>
<td>73.0 (39.5 – 137.5)</td>
<td>77.0 (48.0 – 91.0)</td>
<td>73.0 (39.5 -137.5)</td>
</tr>
<tr>
<td>Race [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>16 (84.2)</td>
<td>92 (76.7)</td>
<td>11 (64.7)</td>
<td>103 (75.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (10.5)</td>
<td>27 (22.5)</td>
<td>6 (35.3)</td>
<td>33 (24.1)</td>
</tr>
<tr>
<td>Black/African American</td>
<td>0 (0.0)</td>
<td>1 (0.8)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Presence of Target Joints [n (%)]</td>
<td>NA</td>
<td>78 (65.0)</td>
<td>15 (88.2)</td>
<td>93 (67.9)</td>
</tr>
<tr>
<td>Presence of Hemophilic Arthropathy [n (%)]</td>
<td>NA</td>
<td>72 (60.0)</td>
<td>8 (47.1)</td>
<td>80 (58.4)</td>
</tr>
<tr>
<td>Hepatitis C Virus Antibody Positive [n (%)]</td>
<td>NA</td>
<td>64 (53.3)</td>
<td>12 (70.6)</td>
<td>76 (55.5)</td>
</tr>
</tbody>
</table>

Abbreviations: N=number of subjects, NA = not applicable (data not collected)

Note: Two subjects participated in both studies.

<sup>a</sup> Data generated from 2 Japanese subjects in the phase 1 study are not included in the report. An analysis of the one-stage clotting assay results for these subjects revealed a number of outliers, and therefore were not considered in the overall interpretation of PK data for BAX 855 and ADVATE.
Table 3 Summary of Bleeding Episodes by Time after Prophylactic BAX 855 Infusions

<table>
<thead>
<tr>
<th>Cause of Bleeding Episode</th>
<th>Parameter</th>
<th>≤24 h</th>
<th>&gt;24 to ≤48 h</th>
<th>&gt;48 to ≤72 h</th>
<th>&gt;72 to ≤96 h</th>
<th>&gt;96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>Number of infusions in analysis</td>
<td>5907</td>
<td>5862</td>
<td>5763</td>
<td>4297</td>
<td>1338</td>
</tr>
<tr>
<td>Spontaneous/Unknown</td>
<td>Number of Bleeding Episodes</td>
<td>26</td>
<td>40</td>
<td>42</td>
<td>25</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Bleeding Episodes per prophylactic infusion</td>
<td>0.0044</td>
<td>0.0068</td>
<td>0.0073</td>
<td>0.0058</td>
<td>0.0179</td>
</tr>
<tr>
<td>Injury</td>
<td>Number of Bleeding Episodes</td>
<td>21</td>
<td>30</td>
<td>30</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Bleeding Episodes per prophylactic infusion</td>
<td>0.0036</td>
<td>0.0051</td>
<td>0.0052</td>
<td>0.0021</td>
<td>0.0112</td>
</tr>
<tr>
<td>All</td>
<td>Number of Bleeding Episodes</td>
<td>47</td>
<td>70</td>
<td>72</td>
<td>34</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Bleeding Episodes per prophylactic infusion</td>
<td>0.0080</td>
<td>0.0119</td>
<td>0.0125</td>
<td>0.0079</td>
<td>0.0291</td>
</tr>
</tbody>
</table>

Note: A bleeding episode at >96 hours could occur only if the subject deviated from the protocol specified infusion interval, since 96 hours was the maximum. The analysis was performed on the FAS, which included subjects with prophylactic infusion intervals ≥5 days.
Table 4 Efficacy of Treatment of Bleeding Episodes in the Pivotal Study

<table>
<thead>
<tr>
<th>Number of Infusions to Treat Bleeding Episodes – n (%)</th>
<th>1 infusion</th>
<th>2 infusions</th>
<th>Total (1 or 2 infusions)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>443 (85.5%)</td>
<td>54 (10.4%)</td>
<td>497 (95.9%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Median (Q1 ; Q3) dose per infusion to treat a bleeding episode – IU/kg</th>
<th>All Bleeds/Severities (N=518)</th>
<th>Minor (N=245)</th>
<th>Moderate (N=238)</th>
<th>Severe/Major (N=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29.0 (20.0; 39.2) IU/kg</td>
<td>25.5 (16.9;37.6) IU/kg</td>
<td>30.9 (23.0; 43.1) IU/kg</td>
<td>36.4 (29.0; 44.5) IU/kg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemostatic Efficacy Rating – n (%)</th>
<th>Excellent or good</th>
<th>Number of infusions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>498 (96.1%)</td>
<td>1.2 (0.7)</td>
</tr>
</tbody>
</table>

*PPAS for patients in prophylaxis and on-demand groups (N = 118). For PPAS criteria, refer to Table 1.
Table 5  BAX 855 vs ADVATE Pharmacokinetics in the Pivotal Study

<table>
<thead>
<tr>
<th></th>
<th>Before prophylactic treatment</th>
<th>After 6 months of prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>ADVATE (N=26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAX 855 Initial (N=26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio: BAX 855 Initial / ADVATE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAX 855 Final (N=22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio BAX 855 Final / Initial</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T1/2 - (hours)</strong></td>
<td>10.4 (2.2)</td>
<td>16.0 (4.9)</td>
</tr>
<tr>
<td></td>
<td>14.3 (3.8)</td>
<td>16.0 (4.9)</td>
</tr>
<tr>
<td></td>
<td>1.4 (0.25)</td>
<td>1.2 (0.47)</td>
</tr>
<tr>
<td><strong>MRT (hours)</strong></td>
<td>12.9 (3.0)</td>
<td>20.7 (4.8)</td>
</tr>
<tr>
<td></td>
<td>19.6 (5.3)</td>
<td>20.7 (4.8)</td>
</tr>
<tr>
<td></td>
<td>1.5 (0.18)</td>
<td>1.1 (0.26)</td>
</tr>
<tr>
<td><strong>AUC0-∞ (IU•h/dL)</strong></td>
<td>1168 (425)</td>
<td>2009 (631.53)</td>
</tr>
<tr>
<td></td>
<td>2073 (778)</td>
<td>2009 (631.53)</td>
</tr>
<tr>
<td></td>
<td>1.9 (0.91)</td>
<td>1.1 (0.5)</td>
</tr>
<tr>
<td><strong>CL (dL/[kg•h])</strong></td>
<td>0.0455 (0.0217)</td>
<td>0.0247 (0.00823)</td>
</tr>
<tr>
<td></td>
<td>0.0276 (0.0203)</td>
<td>0.0247 (0.00823)</td>
</tr>
<tr>
<td></td>
<td>0.613 (0.28)</td>
<td>1.0 (0.27)</td>
</tr>
<tr>
<td><strong>IR ([IU/dL]/[IU/kg])</strong></td>
<td>2.37 (0.536)</td>
<td>2.3 (0.64)</td>
</tr>
<tr>
<td></td>
<td>2.49 (0.694)</td>
<td>2.3 (0.64)</td>
</tr>
<tr>
<td></td>
<td>1.1 (0.36)</td>
<td>1.0 (0.22)</td>
</tr>
</tbody>
</table>

Mean (standard deviation [SD]) results from the one stage clotting assay are shown. Similar results were obtained with the chromogenic assay: T1/2 ratio for BAX 855 Initial / ADVATE was 1.5 (0.4622) and for BAX 855 Final / Initial was 1.1 (0.2906).
FIGURE LEGENDS

Figure 1. Both clinical studies were open label with no randomization. In the phase 1 study (panel A) the sequence of treatment was ADVATE first followed by BAX 855 for each subject in each cohort. Nine subjects in the 30 IU/kg arm were treated first. After review of the data from the first cohort and approval by the DMC, treatment of 10 subjects in the 60 IU/kg arm commenced. Only one treated subject was excluded from the PK analysis set due to a bleeding episode within 4 days of infusion. In the pivotal Study (panel B), treatment assignment depended on the subjects’ prior treatment: Subjects previously on prophylaxis were assigned to the prophylaxis arm. The first 17 subjects who previously received on-demand treatment were assigned to the on-demand arm, then additional subjects were assigned to the prophylaxis arm. The PK subset comprised 26 subjects in the prophylaxis arm. Twelve subjects discontinued during prophylaxis: 1 for a surgical procedure, 1 due to screen failure, 2 due to discontinuation by the subject, 4 due to an adverse event, and 4 for protocol violation.

Figure 2. In a descriptive analysis of 118 subjects in the PPAS, the median (Q1; Q3) and mean (SD) ABRs were computed for the prophylactic arm vs. the on-demand arm, for all, joint, and spontaneous bleeding episodes.

Figure 3. Mean annualized bleeding rates (ABRs) in subjects who received prophylactic treatment with BAX 855 are depicted by target joint status at screening. There were 101 subjects in the PPAS including 69 subjects with no target joints at screening and 32 subjects with target joints at screening. Median ABR values are also presented.

Figure 4. The one-stage clotting assay data shown represent median (Q1; Q3) FVIII plasma levels and the nominal sampling times as indicated on the linear X axis. BAX 855 (dashed gray
line) demonstrated an extended half-life compared to ADVATE (solid black line). The PK assessments shown were conducted in 26 subjects at their first exposure to BAX 855.

**Figure 5.** Scatterplots and Spearman rank correlation analysis of $T_{1/2}$ are displayed. The $T_{1/2}$ of BAX 855 was positively correlated to pre-infusion VWF antigen plasma concentrations (panel A) and negatively correlated with ABR (panel B). The $T_{1/2}$ values for these analyses were derived from PK assessments conducted in 26 subjects at their first exposure to BAX 855 using the one-stage clotting assay. The circles represent individual subject values. An apparent outlier appears in Panel A for a subject with a very low $T_{1/2}$ and a high ABR; it is of note that no inhibitory antibodies to FVIII were detected in this subject.
FIGURES

Figure 1 Disposition of Subjects

A. Phase 1 Study
- Enrolled: N=24
  Screen failures: N=2
  3 subjects discontinued before treatment
- Cohort 1 n=9
  PK at (30 IU/kg)
  ADVATE – BAX 855
  PK analysis set
  n = 8
  (1 subject had bleeding episode within 4 days of investigational product infusion)
- Cohort 2 n=10
  PK at (60 IU/kg)
  ADVATE – BAX 855
  PK analysis set
  n = 10
  (analyses done separately for 2 Japanese subjects)

B. Pivotal Study
- Enrolled: N=159
  Screen failures: N=21
  Prior Prophylaxis: N=111
  Prior On-demand: N=43
  Initial PK at 45 IU/kg:
    ADVATE & BAX 855
    N=26
  Discontinued
  N=12
  Repeat PK at 45 IU/kg
    BAX 855
    N=22
  Completed
  N=109
  PPAS
  N=101
  Prophylaxis
  (45 IU/kg 2x/week for ≥50 EDs or 6 months)
  N=121 (FAS)
  N=17
  On-demand
  (10–60 IU/kg for 6 months)
  N=17
  Completed
  N=17
Figure 2: Annualized Bleeding Rates by Bleeding Site and Cause in the Pivotal Study

<table>
<thead>
<tr>
<th></th>
<th>Median (Q1;Q3) ABR</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>1.9 (0.0; 5.8)</td>
<td>3.7 (4.7)</td>
</tr>
<tr>
<td><strong>Joint</strong></td>
<td>0 (0.0; 2.0)</td>
<td>1.8 (3.0)</td>
</tr>
<tr>
<td><strong>Spontaneous/unknown</strong></td>
<td>0 (0.0; 2.2)</td>
<td>2.1 (3.5)</td>
</tr>
</tbody>
</table>

Figure 3: Annualized Bleed Rates by Target Joint Status in Subjects Receiving Prophylaxis

<table>
<thead>
<tr>
<th></th>
<th>Median (Q1;Q3) ABR</th>
<th>Mean (SD) ABR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All</strong></td>
<td>3.4 (0.0; 5.8)</td>
<td>3.7 (4.4)</td>
</tr>
<tr>
<td><strong>Joint</strong></td>
<td>1.9 (0.0; 6.0)</td>
<td>3.6 (4.9)</td>
</tr>
<tr>
<td><strong>Spontaneous/unknown</strong></td>
<td>0.0 (0.0; 1.8)</td>
<td>2.2 (3.2)</td>
</tr>
</tbody>
</table>

- **Subjects with no target joints at screening (N=69)**
- **Subjects with target joints at screening (N=32)**
Figure 4 Pharmacokinetic curves of FVIII activity vs time for BAX 855 and ADVATE for the Pivotal Study
Figure 5 Correlation of Half-life of BAX 855 at Initial Pharmacokinetic Analysis with VWF antigen Concentrations and ABR in the Pivotal Study

A

BAX 855 Initial PK Correlation coefficient (Spearman): + 0.72

B

BAX 855 Initial PK Correlation coefficient (Spearman): -0.49
Pegylated, full-length, recombinant factor VIII for prophylactic and on-demand treatment of severe hemophilia A

Barbara A. Konkle, Oleksandra Stasyshyn, Pratima Chowdary, David H. Bevan, Tim Mant, Midori Shima, Werner Engl, Jacqueline Dyck-Jones, Monika Fuerlinger, Lisa Patrone, Bruce Ewenstein and Brigitt Abbuehl