Efficacy of BAX 826, a Polysialylated Full-length rFVIII, in Mouse Models of Hemophilia A

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INTRODUCTION

- Factor VIII (FVIII) is a critical component of the intrinsic coagulation pathway. FVIII concentrates are used to treat and prevent bleeding episodes in patients with hemophilia A. Prophylactic FVIII levels can only be maintained by administering several infusions per week. Extended FVIII circulation times would reduce the frequency of infusions, increase patient compliance, reduce the number of bleeds, and allow higher trough levels of FVIII to be reached.
- Recently, the first extended half-life recombinant (r)FVIII modified with PEG as a hydrophilic polymers was approved under the brand name ADYNOVATE.
- BAX 826, Baxalta's second investigational extended half-life candidate based on ADVATE (Antihemophilic Factor [Recombinant]), a full length recombinant FVIII molecule with an established extensive safety and efficacy profile, uses another hydrophilic polymer for modification, polysialic acid (PSA) to extend dosing intervals.
- Baxalta announced the submission of a Clinical Trial Application (CTA) to the UK Medicines and Healthcare Products Regulatory Agency (MHRA) to initiate a first-in-human clinical trial to evaluate the safety and efficacy of BAX 826, an investigational, extended half-life recombinant Factor VIII (rFVIII) treatment for hemophilia A.

OBJECTIVE

- The aim of the presented studies was to evaluate the efficacy of BAX 826 in hemophilic mice using a tail-tip bleeding model (TTBM) and carotid occlusion model (COM).

METHODS

- Unmodified rFVIII (ADVATE) was used as the reference compound. Test and reference compounds were administered at the same target dose.
- All animal experiments accorded with Austrian laws governing animal experimentation and were approved by the Institutional Animal Care and Use Committee (IACUC).

Tail-tip Bleeding Model

- 16 (8m/8f) FVIII ko mice per group were used to assess blood loss after treatment. The test and reference item was intravenously administered at a dose of 200 U rFVIII/kg prophylactically 18 to 54 h before the tail was clipped. Buffer was used as negative control item and administered 5 min before tail clip.
- Blood loss was assessed [mg] and adjusted for the animals' body weight [g]. The period of effectiveness was defined as the longest period between administration of the test item and the tail cut that provided statistical evidence for a smaller blood loss compared with buffer.

Carotid Occlusion Model

- 12 (6m/6f) FVIII ko mice per group were used to assess time to vessel occlusion after treatment. The test and reference items was intravenously administered at a dose of 200 U rFVIII/kg prophylactically 6 to 40 h before the carotid artery endothelium was denuded by topical application of FeCl3 and carotid blood flow monitored before and after injury using an ultrasound flow probe. Buffer was administered 15 min before denudation.
- Time to vessel occlusion [min] was assessed. Clinically relevant efficacy was defined as a distinct reduction in median time to occlusion compared with buffer-treated animals. The period of effectiveness was defined as the longest period between administration of the test item and the denudation of the endothelium that provided statistical evidence for a shorter time to occlusion compared with buffer.
- Animals were anesthetized using ketamine and xylazine and humanely killed by cervical dislocation immediately after the end of the observation period.

RESULTS

Tail-tip Bleeding Model

- Efficacy was shown by a distinct decrease in blood loss.
- Median total blood loss in buffer-treated animals was 839 mg or 40.0 mg/g BW (Table 1).
- ADVATE was shown to be efficacious in groups treated up to 30 h before the tail cut (median blood loss of 446.5 mg or 22 mg/g BW; p = 0.0018), but not in the group treated 40 h before the tail cut. BAX 826 was shown to be efficacious in groups treated up to 48 h (423.5 mg or 20 mg/kg BW; p = 0.0212), but not in the group treated 54 h before the tail cut (Tables 1 and 3, Figure 1).

Carotid Occlusion Model

- Efficacy was shown by a distinct decrease in time to occlusion.
- No vessel occlusion was observed in buffer-treated animals within the observation period of 30 min (Table 2).
- ADVATE was shown to be efficacious in groups treated up to 18 h before (median time to occlusion 12.0 min; p = 0.0003) but not in the group treated 24 h before the denudation intervention. BAX 826 was shown to be efficacious in groups treated up to 30 h before (6.0 min; p < 0.0001) but not in the group treated 40 h before the denudation intervention (Tables 2 and 4, Figure 2).

Table 1: Summary of Blood Loss

			Blood loss [mg] adjusted for body weight [g]					Blood loss [mg]
Item	TP	N	Median	IQR	Range	Mean	SD	Median
Advate (●)	-18h	16	0.62	0.2 to 30	0 to 42	13	18	14.5
	-24h	16	17	6.2 to 43	0 to 49	22	19	399.5
	-30h	16	**22**	5.3 to 37	0.3 to 50	23	18	**446.5**
	-40h	16	36	4.8 to 45	0 to 48	28	20	746.0
BAX 826 (●)	-24h	16	1.3	0 to 17	0 to 51	12	18	24.5
	-30h	16	2.9	0.2 to 37	0 to 42	15	18	66.0
	-40h	16	28	15 to 41	0.2 to 44	27	16	632.5
	-48h	16	**20**	0.8 to 44	0 to 48	22	20	**423.5**
	-54h	16	37	29 to 41	3.7 to 47	33	13	757.0
Buffer (●)	-5min	15	40	38 to 44	21 to 51	40	7.5	839.0

IQR, inter-quartile range (middle 50% of data); SD, standard deviation; TP, time point of administration before tail cut

Table 2: Summary of Time to Occlusion

			Time to occlusion [min]		
Item	TP	N	Median	IQR	Range
Advate (●)	-6h	12	5.4	4.4 to 6.4	2.1 to > 30
	-12h	12	8.5	5.8 to > 30	0.0 to > 30
	-18h	12	**12.0**	6.9 to > 30	4.3 to > 30
	-24h	12	> 30	> 30 to > 30	12.0 to > 30
BAX 826 (●)	-18h	12	6.1	5.5 to 12	2.7 to > 30
	-24h	12	13.0	4.9 to > 30	3.4 to > 30
	-30h	12	**6.0**	3.8 to 13	3.2 to 30
	-40h	12	> 30	25.0 to > 30	6.6 to > 30
Buffer (●)	-15min	12	> 30	> 30 to > 30	> 30 to > 30

Figure 1: Summary of Blood Loss [mg] Adjusted for Body Weight [g]

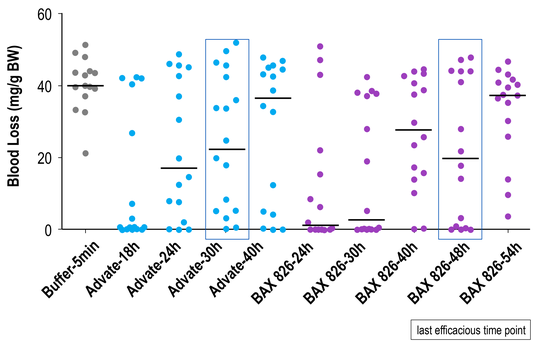
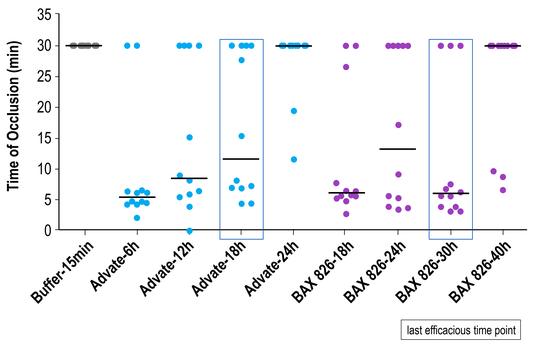


Figure 2: Summary of Time to Occlusion [min]



In summary, the period of effectiveness assessed in this study was 30 h for ADVATE and 48 h for BAX 826, indicating a prolongation of efficacy of the polysialylated rFVIII product compared with the unmodified rFVIII concentrate. Furthermore, intravenous injection of BAX 826 was well tolerated in all animals across all treatment groups without any signs of acute toxicity.

In summary, the period of effectiveness assessed in this study was 18 h for ADVATE and 30 h for BAX 826, indicating a prolongation of efficacy of the polysialylated rFVIII product compared with the unmodified rFVIII concentrate. Furthermore, intravenous injection of BAX 826 was well tolerated in all animals across all treatment groups without any signs of acute toxicity.

Table 3: Estimation of Period of Effectiveness (Tail-tip Model)

Comparison	Relative effect	One-sided 97.5% CI for the relative effect	One-sided p-value
Advate -18h vs. buffer	0.862	0.728 to 1	< 0.0001
Advate -24h vs. buffer	0.746	0.556 to 1	0.0055
Advate -30h vs. buffer	0.767	0.587 to 1	**0.0018**
Advate -40h vs. buffer	0.646	0.441 to 1	0.0817
BAX 826 -24h vs. buffer	0.850	0.691 to 1	< 0.0001
BAX 826 -30h vs. buffer	0.896	0.788 to 1	< 0.0001
BAX 826 -40h vs. buffer	0.754	0.580 to 1	0.0021
BAX 826 -48h vs. buffer	0.708	0.507 to 1	**0.0212**
BAX 826 -54h vs. buffer	0.675	0.482 to 1	0.0377

CI ... confidence interval. One-sided p-value for the alternative hypothesis of a smaller blood loss than with buffer.

Table 4: Estimation of Period of Effectiveness (Carotid Occlusion Model)

Comparison	One-sided p-value
Advate -6h vs. buffer	< 0.0001
Advate -12h vs. buffer	0.0003
Advate -18h vs. buffer	**0.0003**
Advate -24h vs. buffer	0.0741
BAX 826 -18h vs. buffer	< 0.0001
BAX 826 -24h vs. buffer	0.0009
BAX 826 -30h vs. buffer	**< 0.0001**
BAX 826 -40h vs. buffer	0.0349

CONCLUSION

- BAX 826 was shown to be safe and efficacious in two mouse models of hemophilia A.
- The effect of BAX 826 is prolonged compared with an unmodified rFVIII (ADVATE).

DISCLOSURES

*All authors are employees of Baxalta (Baxalta Innovations GmbH), now part of Shire.
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