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Structural and Functional Characterization of Preclinical and Clinical Batches of BAX 826, a PSAylated Full-length Recombinant FVIII

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INTRODUCTION

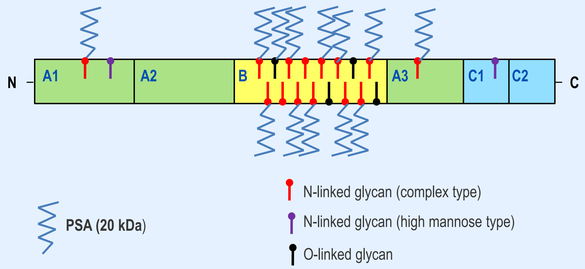
- BAX 826, Baxalta's second investigational extended half-life candidate based on ADVATE [antihemophilic factor (recombinant)], a full length recombinant FVIII molecule with an established extensive safety and efficacy profile, uses another hydrophilic polymer for modification, polysialic acid (PSA), that also aims to extend dosing intervals
- 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

OBJECTIVE

- The aim of the presented studies was to describe the structural and functional characterization of preclinical and clinical phase 1 batches of BAX 826

BAX 826

- **Base Protein**: Full-length rFVIII (ADVATE), albumin, plasma free
- **PSA**: α-2,8-linked polysialic acid, negatively charged hydrophilic polymer, natural constituent of human tissue and biodegradable (to sialic acid)
- **Coupling Technology**: Stable linker, PSA is preferably coupled to the B-domain of rFVIII



METHODS

- **Study material:** Three batches of BAX 826 preclinical bulk drug substance (BDS), three batches of BAX 826 clinical phase 1 BDS, three lots of BAX 826 final drug product (FDP), and one lot of clinical phase 1 FDP. Six batches of unmodified rFVIII as starting material for modification were used for comparative analysis.
- **Thrombin generation assay**: The assay was performed using the reagents of the Technothrombin TGA kit (Technoclone, Vienna, Austria). Human FVIII-deficient plasma containing < 1% of FVIII was supplemented with increasing amounts of samples (0.01 to 1 IU/mL) and the reaction was started by adding a small amount of recombinant human tissue factor complexed with phospholipid micelles.
- **Activation/inactivation by thrombin:** Samples were diluted to 1.0 IU/mL FVIII activity and incubated with 0.5 nM thrombin. Subsamples were withdrawn at intervals up to 40 min. The reaction was stopped by adding thrombin inhibitor, and the cofactor activity of FVIII/VIIIa evaluated by measuring the amount of FXa after 3 min of incubation with FIXa, FX, PL, and $CaCl_2$.
- **Binding to VWF and low-density lipoprotein-receptor-related protein (LRP1)**: Measured using surface plasmon resonance technology (Biacore) where a highly purified plasma-derived VWF or LRP1 was immobilized on the sensor chip surface, the FVIII samples were applied to the chip and binding response units (RU) were evaluated.
- **Peptide Mapping**: FVIII samples were reduced, alkylated, and digested with trypsin. Tryptic peptides were separated by reversed phase chromatography with UV detection at 214 nm.
- **PSA Modification Site Analysis**: FVIII samples were reduced, alkylated, and digested with trypsin. In the first dimension, the tryptic peptides were separated by size exclusion chromatography and fractionated. In the second dimension, the PSA modified peptides were separated by reversed phase chromatography and detected by mass spectrometry.

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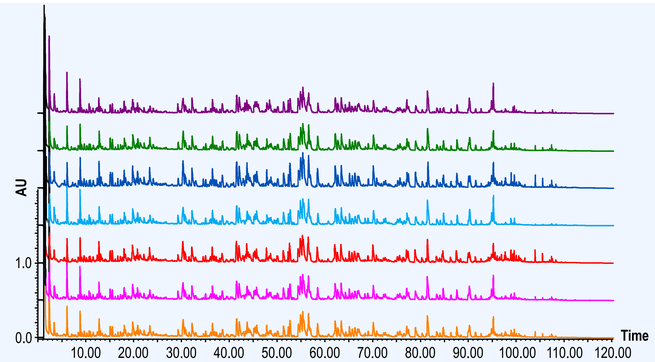
RESULTS

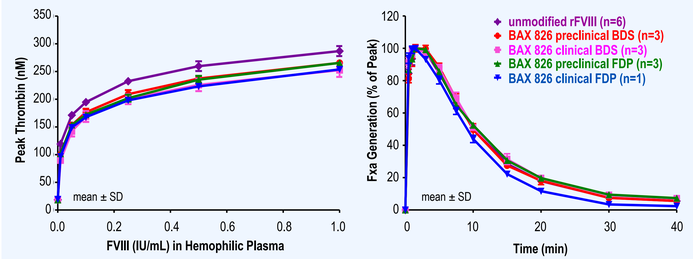
Determination of Primary Structure - Peptide Mapping



- Peptide mapping confirmed consistency of the primary structure of BAX 826 preclinical and clinical phase 1 batches and unmodified rFVIII.

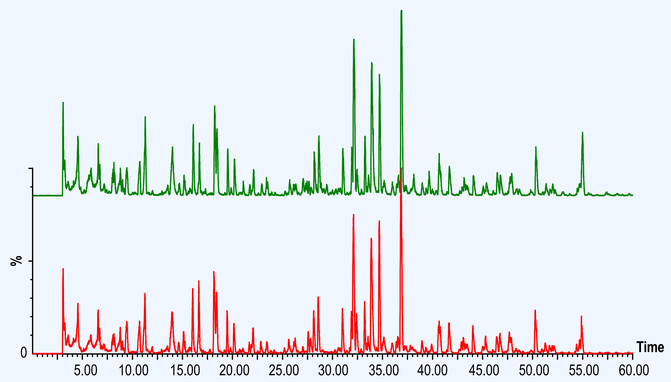
Thrombin Generation

Susceptibility to Thrombin



- BAX 826 corrected the impaired thrombin generation of a human FVIII-deficient plasma in a concentration-dependent manner, similar to unmodified rFVIII and consistently between preclinical and clinical phase 1 batches.
- The susceptibility to thrombin was highly consistent between preclinical and clinical phase 1 batches of BAX 826 and similar to unmodified rFVIII.

Determination of PSA Modification Sites – Two-dimensional HPLC-MS

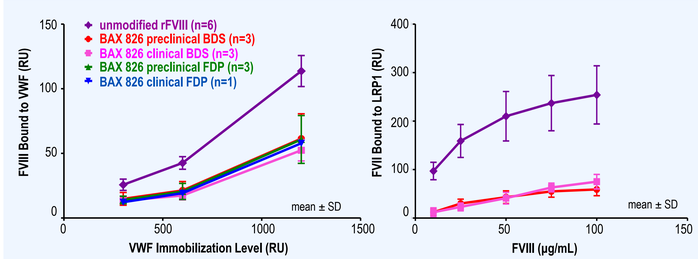


Domain	Possible modification sites	Detected PSA sites
Heavy chain (A1, A2)	1	1
B-domain	20	11
Light chain (A3, C1, C2)	1	1
Total	22	13

- Two-dimensional chromatography of PSAylated peptides confirmed consistency of PSA binding sites of preclinical and clinical phase 1 batches.
- 85% of the PSA-sites are located on the B-domain.

Binding to VWF

Binding to LRP1 Clearance Receptor



- Compared with unmodified rFVIII, the binding of BAX 826 to VWF and LRP1 was reduced.
- BAX 826 batches from preclinical and clinical phase 1 manufacturing showed similar and consistent characteristics.

CONCLUSION

- BAX 826 is a polysialylated rFVIII derivative of well-defined primary structure and fully retained hemostatic activity that can be manufactured reproducibly. The reduced VWF and LRP1 binding properties qualify BAX 826 as an attractive new extended half-life FVIII drug candidate with an alternative mechanism of half-life prolongation.

Disclosures: *Authors are employees of Baxalta (Baxalta Innovations GmbH), now part of Shire.