BAY 81-8973, a full-length recombinant factor VIII: manufacturing processes and product characteristics

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BAY 81-8973 (Kovaltry®, Bayer, Berkeley, CA, USA) is an unmodified, full-length recombinant human factor VIII (FVIII) approved for prophylaxis and on-demand treatment of bleeding episodes in patients with haemophilia A. The BAY 81-8973 manufacturing process is based on the process used for sucrose-formulated recombinant FVIII (rFVIII-FS), with changes and enhancements made to improve production efficiency, further augment pathogen safety, and eliminate animal- and human-derived raw materials from the production processes. The baby hamster kidney cell line used for BAY 81-8973 was developed by introducing the gene for human heat shock protein 70 into the rFVIII-FS cell line, a change that improved cell line robustness and productivity. Pathogen safety was enhanced by including a 20-nm filtration step, which can remove viruses, transmissible spongiform encephalopathy agents and potential protein aggregates. No human- or animal-derived proteins are added to the cell culture process, purification or final formulation. The BAY 81-8973 manufacturing process results in a product of enhanced purity with a consistently high degree of sialylation of N-linked glycans on the molecular surface. The innovative manufacturing techniques used for BAY 81-8973 yield an effective rFVIII product with a favourable safety profile for treatment of haemophilia A.

Keywords: BAY 81-8973, factor VIII, glycosylation, haemophilia A, HSP70 heat shock protein

Introduction

Haemophilia A is a congenital blood coagulation disorder resulting from mutations in the F8 gene [1,2]. Patients with severe haemophilia A have levels of clotting factor VIII (FVIII) that are <1% of normal, placing them at risk for spontaneous bleeding episodes [1,3]. These bleeding episodes can be controlled when they occur, with on-demand infusion of a FVIII product, or they can be prevented with FVIII infusions given prophylactically [1].

Commercial production of recombinant FVIII (rFVIII) for treatment of haemophilia A is a challenging process, given the complex nature of the FVIII protein [4]. The first rFVIII products were introduced in the early 1990s; these products used human serum albumin and animal-derived materials in the manufacturing process [4]. Refinements in rFVIII manufacturing have reduced or eliminated use of human- or animal-derived materials, which decreased the potential for pathogen transmission [4].

BAY 81-8973 (Kovaltry®, Bayer, Berkeley, CA, USA) is an unmodified, full-length recombinant human FVIII that has the same FVIII amino acid sequence as the currently marketed product sucrose-formulated rFVIII (rFVIII-FS; Kogenate® FS; Bayer) and is manufactured using innovative techniques [5]. The principal reasons for the development of a new rFVIII manufacturing process were to eliminate human- and animal-derived raw materials from the manufacturing processes, optimize the production process by reducing the number of manufacturing steps, further augment pathogen safety, and develop a new manufacturing platform using the latest technologies.

BAY 81-8973 manufacturing process

The BAY 81-8973 manufacturing process is a result of modifications and enhancements to the manufacturing process used for rFVIII-FS (Table 1, Fig. 1) [6], which itself is based on the manufacturing process used for...
Bayer’s first marketed rFVIII (Kogenate®, Bayer) [7]. A comparison of the principal manufacturing steps for BAY 81-8973 and those for rFVIII-FS and other marketed rFVIII products, including prolonged half-life products, is shown in Table 2.

### Development of the protein-free media for cell culture

Recombinant FVIII products are produced in mammalian cell cultures because of the large size of the FVIII protein and the high degree of posttranslational modifications [7]. Development of a cell culture medium free of human- or animal-derived raw materials is critical to minimizing the risk of pathogen contamination from these sources [7,8].

The cell culture medium used for rFVIII-FS contains human plasma protein solution (HPPS) and recombinant insulin but no proteins derived from animal sources [9]. The cell culture medium used for BAY 81-8973 was modified to eliminate HPPS, resulting in a medium free of human- and animal-derived raw materials [5]. Ethylenediaminetetraacetic acid (EDTA)/FeSO_4_ and a trace metals solution are added to replace the iron and trace metals supplied by HPPS [8]. To replace the shear-protectant function of HPPS, Pluronic® F-68 (BASF, Florham Park, NJ, USA) is added. Pluronic® F-68 is a polylcopolymer that acts as a surfactant, preventing cell damage by allowing cells to drain away from bubbles formed in bioreactors during stirring or sparging of cell cultures [8]. In addition, the BAY 81-8973 manufacturing process uses a 3-(N-morpholino)propanesulphonic acid (MOPS)-histidine buffer instead of sodium bicarbonate to reduce pCO_2_ levels in the cell culture [10, 11]. The final composition of the BAY 81-8973 protein-free cell culture medium was shown to enhance the specific rFVIII productivity of the baby hamster kidney (BHK) cells used to produce BAY 81-8973 [8].

### Purification process

Imunoaffinity, metal affinity and ion exchange chromatography are used to purify BAY 81-8973 and

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**Table 1. Comparison of BAY 81-8973 and rFVIII-FS manufacturing processes.**

|               | BAY 81-8973 | rFVIII-FS |
|---------------|-------------|-----------|
| Cell line/bank| rBHK-21-HSP70 | rBHK-21   |
| FVIII         | Full-length, unmodified human FVIII | Full-length, unmodified human FVIII |
| Glycosylation | N- and O-linked glycans | N- and O-linked glycans |
|              | High level of highly branched sialylated glycans | Lower level of highly branched sialylated glycans |
|              | Higher level of sialylated glycans | High level of sialylated glycans |
|              | No α-gal-linked glycans detected | No α-gal-linked glycans detected |
| Cell culture  | Perfusion-based bioreactor | Perfusion-based bioreactor |
| Purification  | Three chromatography columns | Six chromatography columns |
|              | Addition of 2 membrane-based chromatography steps | Same process, presentation, excipients, and fill sizes |
|              | Removal of gelatin-sepharose chromatography | Same process, presentation, excipients, and fill sizes |
|              | Addition of 20-nm viral filter | Same process, presentation, excipients, and fill sizes |
| Drug product  | Concentrate rFVIII from the cell culture harvest | Same process, presentation, excipients, and fill sizes |

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BHK, baby hamster kidney; FVIII, factor VIII; HPPS, human plasma protein solution; rBHK-21-HSP70, baby hamster kidney-21 clone expressing recombinant FVIII and transfected with human heat shock protein 70; rFVIII-FS, recombinant factor VIII formulated with sucrose.

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BAY 81-8973 MANUFACTURING  e69

Fig. 1. BAY 81-8973 and sucrose-formulated recombinant factor VIII (rFVIII-FS) manufacturing steps.

remove process- and product-related impurities. The BAY 81-8973 purification process was optimized from the rFVIII-FS process through the elimination of gelatin sepharose chromatography and replacement of a polishing chromatography step with a more efficient disposable membrane adsorber capsule for residual DNA removal [5, 6]. The purification process also includes a 20-nm viral filtration step, which provides robust and orthogonal virus clearance. No human- or animal-derived raw materials are added in the purification process [5].

Viral inactivation and removal

The BAY 81-8973 manufacturing process includes a highly effective and robust enveloped virus inactivation step using a detergent solution in the unpurified intermediate FVIII product [16]. This efficient, state-of-the-art manufacturing process is integrated into the elution and bagging process of the FVIII intermediate product, ensuring a physical process segregation that further protects the product from potential viral contamination [6]. Viral clearance is enhanced using a 20-nm pore size viral filter (pore size is limited by the size of the full-length rFVIII protein) capable of removing viruses and transmissible spongiform encephalopathy (TSE) agents as well as potential protein aggregates [5, 6, 16].

The downstream BAY 81-8973 manufacturing process was validated for viral clearance capability using four model viruses and scaled-down systems representative of current Good Manufacturing Practice (cGMP) steps. The model viruses were xenotropic murine leukaemia virus (X-MuLV), pseudorabies virus (PRV), porcine parvovirus (PPV) and reovirus type 3 (Reo3). The viral clearance capability of the manufacturing process was determined by evaluating the ability of each process step to reduce viral infectivity, as measured using a log_{10} reduction factor (LRF), in which the amount of model virus introduced before the process step was compared with the amount of virus remaining after the process step. Virus infectivity was determined using cytopathic-effect–based 50% tissue culture infective dose (TCID_{50}) assays.

Virus clearance studies demonstrated clearance of all four model viruses using the BAY 81-8973 manufacturing process, including parvovirus, a very small, chemically resistant nonenveloped virus (Table 3) [6]. Studies using naive capture membrane adsorber and naive column resins yielded a total LRF of ≥17.12, ≥17.99, ≥6.13, and ≥8.73 for X-MuLV, PRV, PPV, and Reo3, respectively. Studies using reused capture membrane adsorber and column resins achieved a total LRF of ≥13.72, ≥20.61, ≥7.22, and ≥8.94 for X-MuLV, PRV, PPV, and Reo3, respectively.

Under the worst-case scenario, the total number of endogenous retrovirus-like particles in the clarified tissue-culture-fluid harvest material used to produce a 4000-IU dose is approximately 10^{7.52} particles per
## Table 2. Comparison of principal manufacturing steps for BAY 81-8973 and selected marketed rFVIII products.

|                  | BAY 81-8973 (Kovaltry®) [17] | Kogenate® FS [9,26] | Advate® [27] | Xyntha® [28,29] | Novoeight® [30,31] | Nuwiq® [32,33] | Afstyla® [34–36] | Eloctate® [37,38] | Adynovate® [39] |
|------------------|------------------------------|---------------------|---------------|-----------------|-------------------|----------------|-----------------|----------------|----------------|
| Host cell        | BHK                          | BHK                 | CHO           | CHO             | HEK               | CHO            | HEK             | CHO            | CHO            |
| FVIII molecule   | Full length                  | Full length         | Full length   | B-domain deleted| B-domain deleted  | B-domain deleted| B-domain truncated| Fc fusion protein| Full length; PEGylated|
| Human- or animal-derived additives in cell culture, purification, or formulation processes | None | HPPS in cell culture medium | None | None | None | None | None | None | None |
| Purification     | Rapid membrane adsorber capture | Ion exchange chromatography using monoclonal antibody | Series of chromatography steps | Series of chromatography steps, including affinity chromatography using synthetic peptide affinity ligand | Ion exchange chromatography using monoclonal antibody | Gel filtration | Multimodal cation chromatography | Multistep chromatography process | Affinity chromatography |
|                  | Immunoaffinity chromatography using monoclonal antibody | Metal chelate affinity | Ion exchange chromatography | Metal chelate affinity | Ion exchange chromatography using monochloroethylamine | Size exclusion chromatography | Ion exchange chromatography | Ion exchange chromatography | Ion exchange chromatography |
|                  | Ultrafiltration              | Ultrafiltration/ultrafiltration | Ultrafiltration/ultrafiltration | Ultrafiltration/ultrafiltration | Diafiltration | Ultrafiltration/ultrafiltration | Ultrafiltration/ultrafiltration | Ultrafiltration/ultrafiltration | Ultrafiltration/ultrafiltration |
|                  | 20 nm Detergent             | 35 nm Solvent/detergent | 20 nm Detergent | 20 nm Solvent/detergent | 15 nm Detergent | 20 nm Solvent/detergent | 20 nm Solvent/detergent | 15 nm Detergent | None |
| Viral inactivation | Detergent                  | Solvent/detergent | Detergent | Solvent/detergent | None | Detergent | Detergent | Solvent/detergent | None |

BHK, baby hamster kidney; CHO, Chinese hamster ovary; FVIII, factor VIII; HEK, human embryonic kidney; HPPS, human plasma protein solution; rFVIII, recombinant factor VIII.
dose ($7.32 \log_{10}$). Thus, the significant retrovirus clearance by the BAY 81-8973 manufacturing process provides a high safety margin of $\geq 9.80 \log_{10}$ with a naive capture membrane adsorber and column resins and $\geq 6.40 \log_{10}$ for reused capture membrane adsorber and resins.

The BAY 81-8973 manufacturing process has also been demonstrated to remove potential contamination from TSE agents. Results from in vivo studies using hamster-adapted Scrapie strain 263K as the TSE agent have shown that the viral filtration step significantly reduced TSE infectivity in hamsters (LRF of 3.25 and 4.29, respectively, for duplicate filtration experiments).

**Final formulation**

BAY 81-8973 is available in the same vial sizes (250, 500, 1000, 2000, 3000 IU) as rFVIII-FS [17]. BAY 81-8973 contains the same excipients in the same quantities and has the same temperature stability and shelf-life as rFVIII-FS. The recommended storage conditions for BAY 81-8973 are $+2^\circ C$ to $+8^\circ C$ ($36^\circ F$–$46^\circ F$) for up to 30 months from the date of manufacture [17]. Within this period, BAY 81-8973 can be stored for up to 12 months at temperatures up to $+25^\circ C$ ($77^\circ F$) [17].

**BAY 81-8973 characteristics**

The manufacturing process used for BAY 81-8973 results in an rFVIII product of enhanced purity with a consistently high degree of sialylation of N-linked glycans on the molecular surface, a posttranslational modification step that is important to the half-life of some mammalian proteins [5, 18, 19]. The BAY 81-8973 molecule has been characterized in detail. Assays for monitoring critical quality attributes are routinely used for release of product lots against specifications. Extended characterization has also been performed to support the body of knowledge and licensing submission.

**Protein backbone profile**

The BAY 81-8973 protein is synthesized as a single-chain 330-kDa precursor with a domain structure of A1-A2-B-A3-C1-C2 subunits. Proteolytic processing yields an A1-A2-B heavy chain and A3-C1-C2 light chain to form a large heterodimeric structure linked by a disulfide bond (Fig. 2). The dimer of heavy and light chains has a combined average molecular mass of 264,723 Da, composed of 2332 amino acids. Glycosylation of the molecule increases the molecular mass to $\geq 330,000$ Da. Further processing leads to a degree of cleavage within the nonactive B domain, as is observed for all full-length FVIII molecules. The FVIII molecule has eight disulphide bonds [4], and these have been confirmed to be as expected using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of tryptic digests. The two assays routinely used for monitoring lot-to-lot production protein profile consistency are size exclusion chromatography (SEC) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

**Size exclusion chromatography**

The principal components of rFVIII products are separated into peaks by SEC, and the peaks are quantified based on their fluorescence emission. The SEC procedure separates the components according to their hydrodynamic volume, or molecular size. To identify

### Table 3. BAY 81-8973 viral clearance validation results.

| Virion size, nm | X-MuLV | PRV | PPV | Reo3 |
|----------------|--------|-----|-----|------|
| Envelope present | Age of resin or device | LRF for model virus (±95% CI)† | LRF for model virus (±95% CI)† | LRF for model virus (±95% CI)† |
| BAY 81-8973 manufacturing process step | Membrane adsorber | Detergent inactivation | Immunofinity column chromatography | Metal affinity column chromatography | 20-nm viral filtration | Total LRF |
| | | | 61 cycles⁴ | 104 cycles⁴ | New (single use) | Cycled |
| | Naive | 2.02 ± 0.21 | 1.36 ± 0.35 | 4.89 ± 0.44 | 4.02 ± 0.23 | 1.07 ± 0.20 |
| | 61 cycles⁴ | 1.25 ± 0.23 | ND | ≥ 5.60 ± 0.10 | ≥ 4.76 ± 0.16 | ND |
| | 104 cycles⁴ | ND | ND | ND | 2.87 ± 0.28 | ND |
| | New (single use) | 1.09 ± 0.40 | 2.97 ± 0.47 | 1.09 ± 0.40 | 2.87 ± 0.28 | 2.97 ± 0.47 |
| | Cycled | 0.37 ± 0.22 | ND | ND | ND | ND |

95% CI, 95% confidence limits; LRF, log$_{10}$ reduction factor; NA, not applicable; ND, not done; PPV, porcine parvovirus; PRV, pseudorabies virus; Reo3, reovirus type 3; X-MuLV, xenotropic murine leukemia virus.

†The ± sign before the LRF value indicates that it is the minimum LRF validated for the process based on the evaluated process steps and virus assay sensitivity.

‡Not included in calculation of total LRF.

§1 cycle is added to the cycle number before use for virus clearance studies.

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the components present across the BAY 81-8973 SEC profile, fraction collection was performed across the separations. Collected fractions were subjected to potency testing, SDS-PAGE silver staining, Western blot analysis, and mass spectrometry to confirm the identity of the components within each reported SEC peak area (data not presented).

A typical SEC profile for BAY 81-8973 compared with that for rFVIII-FS is shown in Fig. 3. The high-molecular-weight region 1 peak observed in the rFVIII-FS molecule predominantly contains plasma proteins derived from the HPPS added to the cell culture media for that product. This peak is minimized in the BAY 81-8973 molecule owing to the absence of plasma proteins and the presence of the viral filter, which is capable of removing particles >20 nm in size, at the final stage of the purification process. The main SEC peak (region 2) contains the heterogeneous glycoforms of the intact BAY 81-8973 molecule. Region 2 also contains active rFVIII that has been partially cleaved in the C-terminal region of the heavy chain B domain. Proteolytic processing of the inactive B domain is known to occur in all FVIII products, including plasma-derived products (primarily at Arg1313) [20]. All cleaved fragments of the molecule that contain the highly glycosylated B domain elute within this main peak. The region 3 peak contains active BAY 81-8973, primarily identified as the fully B-domain–deleted heavy chain at 90 kDa. The light chain of the molecule is observed at 80 kDa. The C-terminus of the B domain elutes as a diffuse band between 50 and 60 kDa. This region, including any other impurity bands that could theoretically appear, is quantified and subtracted from the reported purity of the molecule. This step is performed for historical tracking purposes rather than as a result of the C-terminus of the B domain being considered an impurity. Haptoglobin bands are identified in the rFVIII-FS product.

**Post-translational modifications**

Multiple N-linked and O-linked glycosylation sites are present on the BAY 81-8973 structure, predominantly within the B domain of the molecule (Fig. 2). As part of routine release testing, glycan consistency and sialylation are recorded for each lot of BAY 81-8973 using an oligosaccharide map.

Released glycans are labelled with a fluorescent label and separated into peaks using high-performance liquid chromatography in normal phase/ion exchange mixed mode, with fluorescence detection. This procedure separates the labelled components primarily according to their ionic charge, which is dependent on the number of sialic acid residues covalently bound to the glycans, and also by their size and structure.

The peptide-N-glycosidase F (PNGase F)–released oligosaccharide map obtained for the N-linked glycans of BAY 81-8973 product reference standard, with the profile observed for the rFVIII-FS lot presented for comparison, is shown in Fig. 5. BAY 81-8973 shows a relative increase in the highly sialylated branched structures. This high level of terminal sialylation is quantified and measured against a
specification. The relative peak areas calculated for BAY 81-8973 and rFVIII-FS are shown in Table 4. In general, >96% of all BAY 81-8973 terminal galactose residues are sialylated, potentially improving the pharmacokinetic profile of the molecule relative to other marketed rFVIII products. As a comparison, in general, >90% of all rFVIII-FS terminal galactose residues are sialylated.
Further characterization to confirm the specific glycan structures and sites was performed using LC-MS analysis of both tryptic- and thrombin-digested samples. Additionally, characterization of the terminal sialylation of the BAY 81-8973 glycans was performed, following sialidase digestion release, to confirm that N-glycolylneuraminic acid (NGNA) and α-galactose–linked sialic acid were consistently below the quantitation limit (<1%).

Tyrosine sulphation is a post-translational modification common to blood coagulation proteins. BAY 81-8973, as expected, has six highly occupied tyrosine sulphation sites at residue 346 in the A1 domain; residues 718, 719, and 723 in the A2 domain; and residues 1664 and 1680 in the light chain, as well as a very low occupancy sulphation site at tyrosine 395 (Fig. 2). These specific sites were confirmed through analysis of tryptic peptide maps and can also be seen on LC-MS profiles of BAY 81-8973 digested with thrombin.

**Pharmacokinetics, efficacy and tolerability in clinical trials**

In clinical trials, the pharmacokinetic profile of BAY 81-8973 was noninferior to that of rFVIII-FS, and for some variables, BAY 81-8973 showed more favourable pharmacokinetics [18]. Compared with rFVIII-FS, BAY 81-8973 had a longer half-life, higher area under the curve, longer mean residence time and slower clearance [18].

In studies of previously treated children, adolescents, and adults with severe haemophilia A, BAY 81-8973 was efficacious when administered as prophylaxis, either 2 or 3 times weekly (or up to every other day in patients ≤12 years old), and as on-demand treatment [21–23]. The incidence of treatment-related adverse events in each individual study was ≤7%, and no FVIII inhibitors developed [21–23].

The principal clinical trials for BAY 81-8973 and other marketed rFVIII products are summarized in Table 5. All products have demonstrated efficacy, safety and tolerability in patients with severe haemophilia A. In clinical trials, BAY 81-8973 prophylaxis was efficacious in previously treated patients using either a standard-dose thrice-weekly prophylaxis regimen or a lower-dose twice-weekly regimen [21, 22]. Twice-weekly BAY 81-8973 prophylaxis may be appropriate for patients whose bleeding episodes currently are well-controlled on a twice-weekly regimen and for patients with severe haemophilia A who have a mild bleeding phenotype. In the LEOPOLD clinical trial program, a mild bleeding phenotype and good

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**Table 4.** Relative peak area comparisons for the oligosaccharide map of BAY 81-8973 product reference standard and rFVIII-FS.

| Relative % area            | BAY 81-8973 product reference standard | rFVIII-FS |
|----------------------------|----------------------------------------|-----------|
| Asialo glycans             | 14.4                                   | 16.4      |
| Monosialylated             | 10.0                                   | 22.2      |
| Disialylated               | 32.0                                   | 40.2      |
| Trisialylated              | 30.3                                   | 17.7      |
| Tetrasialylated            | 13.4                                   | 3.4       |

rFVIII-FS, sucrose-formulated recombinant factor VIII.
Table 5. Comparison of key clinical trials in previously treated patients for BAY 81-8973 and selected marketed rFVIII products.

| Adolescents/adults | Study | Duration | Inclusion criteria | Prophylaxis dosing | Number of patients | ABR for prophylaxis, median (Q1; Q3) | Zero bleeds per year (% of prophylaxis group) | Treatment-related AEs | Children Study | Duration |
|-------------------|-------|----------|-------------------|-------------------|-------------------|-------------------------------------|--------------------------------------------|-------------------|--------------|----------|
|                   |       | 12 months| Age 12–65 years  | 20, 25, 30 IU kg⁻¹ 3 times per week (could increase to 30 or 25 IU kg⁻¹ 3 times per week in patients with ≥12 bleeds year⁻¹) | 80 (ITT) 84 (ITT) | 0.0 (0; 1.0) 1.1 (IQR, 4.9) | 27% | 3 treatment-related AEs or SAEs No treatment-related SAEs No inhibitors | LEOPOILD II [21] | ≥50 EDs (≥6–8 months) |
|                   |       | 36 months| Age 12–50 years  | 25 IU kg⁻¹ 3 times per week | 20–40 IU kg⁻¹ every other day (standard) 20–50 IU kg⁻¹ every 3rd day (PK tailored) | 66 (ITT) | 94 | 33% | No treatment-related AEs or SAEs No treatment-related SAEs No inhibitors | Joint Outcome Study [48] | Until patients reached age 6 years |
|                   |       |          | Age 8–64 years  | 30 ± 5 IU kg⁻¹ 3 times per week | 3.7 (IQR, 8.7) | 46% | 19 treatment-related AEs 1 treatment-related SAE (unconfirmed low-titre inhibitor) No confirmed inhibitors | BAY 81-8973 MANUFACTURING |
|                   |       |          | Age ≥12 years | 20–40 IU kg⁻¹ every other day 20–50 IU kg⁻¹ every 3rd day (PK tailored) | 32 | 30% | 4 treatment-related AEs No treatment-related SAEs No confirmed inhibitors | NA | NA |
|                   |       |          | Age 12–65 years| 30–40 IU kg⁻¹ every other day | 0.9 (range, 0–4.2) | 50% | No treatment-related AEs No inhibitors | Guardian 3 [50] | ≥50 EDs and ≥6 months |
|                   |       |          | Age ≥12 years | 20–40 IU kg⁻¹ every other day 20–50 IU kg⁻¹ every 3rd day (PK tailored) | 1.1 (0; 4.2) | 43% | 3 treatment-related AEs No treatment-related SAEs No confirmed inhibitors | Blanchette et al. [49] | ≤50 EDs or 6 months |
|                   |       |          | Age 12–50 years| Individualized prophylaxis | 0.9 (range, 0–4.2) | 45% | 5 treatment-related AEs No inhibitors | NA² | NA² |
|                   |       |          | Age ≥12 years | 45 ± 5 IU kg⁻¹ 2 times per week | 1.9 (0; 4.9) | 43% | Treatment-related AEs 10 patients No treatment-related AEs No SAEs No inhibitors | Kids A-LONG [52] | ≥50 EDs |

(continued)
Table 5. (continued)

| Inclusion criteria | Age ≤12 years | Severe haemophilia A | ≥50 EDs | No inhibitors | BAY 81-8973 (Kovaltry) | Kogenate® FS | Advate® | Xyntha® | Novoeight® | Nuwiq® | Afstyla® | Elocitate® | Adynovate® |
|-------------------|---------------|---------------------|---------|--------------|------------------------|-------------|---------|---------|------------|--------|----------|-----------|------------|
| Prophylaxis dosing | 25–50 IU kg⁻¹ ≥2 times per week | 25 IU kg⁻¹ every other day (Kogenate® or Kogenate® FS) | No inhibitors | 25–50 IU kg⁻¹ every second day | 2 times per week dosing: 25 IU kg⁻¹ on day 1, 50 IU kg⁻¹ on day 4<sup>§</sup> | 4.0 (range, 0–27.1) standard prophylaxis | 30–40 IU kg⁻¹ every other day | 1.9 (0; 6.0) | 1.2 | 1.9 (0; 6.0) | 1.2 | 1.9 (0; 6.0) | 1.2 | 1.9 (0; 6.0) | 1.2 |
| Number of patients | 51 | 65 | 53 | 65 | 59 | 71 |
| ABR for prophylaxis, median (Q1; Q3) | 4.4 (range, 0–37.7) modified prophylaxis | 3.0 (Q1R, 8.5) | 1.9 (range, 0–20.7) | 3.0 (Q1R, 8.5) | 1.9 (range, 0–20.7) | 2.0 (0; 4.0) |
| Zero bleeds per year (% of prophylaxis group) | 45% | NR | 15% | 35% | 34% | 46% |
| Treatment-related AEs | 1 treatment-related AE | 2 high-titre inhibitors | 6 treatment-related AEs | 2 treatment-related AEs | 2 treatment-related AEs | 2 treatment-related AEs |
| No treatment-related SAEs | No inhibitors | No treatment-related SAEs | No treatment-related SAEs | No treatment-related SAEs | No treatment-related SAEs | No treatment-related SAEs |

ABR, annualized bleeding rate; AE, adverse event; ED, exposure day; ITT, intent to treat; IQR, interquartile range; NA, not available; NR, not reported; PK, pharmacokinetic; rFVIII, recombinant factor VIII; Q1, Q3, quartile 1; quartile 3; SAE, serious adverse event.

<sup>†</sup>A maximum of 10% of patients with FVIII:C of 1%–2% could be enrolled if they showed clinical severity and met all other inclusion criteria.

<sup>‡</sup>Paediatric data have not yet been reported.

<sup>§</sup>25 IU kg⁻¹ on day 1 and 50 IU kg⁻¹ on day 4 to start, adjusted to 25–65 IU kg⁻¹ every 3–5 days as necessary.

<sup>¶</sup>Adjustments possible based on available PK data and bleeding pattern up to 80 IU kg⁻¹ and minimum interval of every 2 days.

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Inclusion criteria Age ≤12 years Severe haemophilia A ≥50 EDs No inhibitors Age <30 months FVIII:C ≤2 joint bleeds No inhibitors Age <6 years Severe or moderately severe haemophilia A FVIII:C ≤2% ≥50 EDs No inhibitors Age <12 years Severe haemophilia A ≥50 EDs No inhibitors Age ≤11 years Severe haemophilia A ≥50 EDs No inhibitors

Prophylaxis dosing 25–50 IU kg⁻¹ ≥2 times per week 25 IU kg⁻¹ every other day (Kogenate® or Kogenate® FS) No inhibitors 25–50 IU kg⁻¹ every second day 25–60 IU kg⁻¹ every day or 3 times per week

Dose and frequency could be modified as needed (modified prophylaxis)

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Number of patients 51 65 53 65 59 71

ABR for prophylaxis, median (Q1; Q3) 4.4 (range, 0–37.7) modified prophylaxis 30–40 IU kg⁻¹ every other day 1.9 (range, 0–20.7) 2.0 (0; 4.0)

Zero bleeds per year (% of prophylaxis group) 45% NR 15% 35% 34% 46%

Treatment-related AEs 1 treatment-related AE 2 high-titre inhibitors 6 treatment-related AEs 2 treatment-related AEs 2 treatment-related AEs 2 treatment-related AEs

No treatment-related SAEs No inhibitors No treatment-related SAEs No treatment-related SAEs No treatment-related SAEs No treatment-related SAEs

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ABR, annualized bleeding rate; AE, adverse event; ED, exposure day; ITT, intent to treat; IQR, interquartile range; NA, not available; NR, not reported; PK, pharmacokinetic; rFVIII, recombinant factor VIII; Q1, Q3, quartile 1; quartile 3; SAE, serious adverse event.

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response to twice-weekly prophylaxis was associated with older age (≥30 years), few target joints, low number of joint bleeds, high von Willebrand factor levels (≥120%) and high FVIII trough levels at 72 hours postinfusion [16, 24, 25]. Other considerations in determining the appropriateness of a twice-weekly regimen include the patient’s activity level, level of adherence to prophylaxis and FVIII pharmacokinetic profile. Patients currently receiving prophylaxis three times weekly may be candidates for twice-weekly dosing if they have difficulty adhering to the more frequent regimen, depending on their history of bleeding frequency.

Conclusions

BAY 81-8973 is an unmodified, full-length recombinant human FVIII approved for treatment of haemophilia A. BAY 81-8973 has the same FVIII amino acid sequence as rFVIII-FS and is manufactured using innovative manufacturing techniques. Characterization data show that the molecule has the expected profiles for protein size distribution and sequence, including the expected disulphide bonds. Aggregate levels are lower in the BAY 81-8973 product relative to those in rFVIII-FS, primarily owing to the removal of plasma proteins. The posttranslational modification sites and structures are confirmed, and high levels of sialylation of the branched N-glycans have been shown. This profile could potentially translate to benefits for the patient. The BAY 81-8973 manufacturing process results in a product of enhanced purity that has been shown to be efficacious and well-tolerated for prophylaxis and on-demand treatment of bleeding episodes in patients with severe haemophilia A.

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Disclosures

All authors are employees of Bayer.

References

1 Berntorp E, Shapiro AD. Modern haemophilia care. Lancet 2012; 379: 1447–56.
2 Aledort L, Ljung R, Mann K, Pipe S. Factor VIII therapy for hemophilia A: current and future issues. Expert Rev Hematol 2014; 7: 573–85.
3 Srevastava A, Brewer AK, Mauser-Bunschoten EP et al. Guidelines for the management of hemophilia. Haemophilia 2013; 19: e1–47.
4 Orloff NA, Kovnin SV, Vorobiev II, Gabi-bov AG, Vorobiev AI. Blood clotting factor VIII: from evolution to therapy. Acta Naturae 2013; 5: 19–39.
5 Humphries T, Regan L, Garger S, Amona O, Maas Enriquez M. BAY 81-8973: a new full-length recombinant factor VIII created through state-of-the-art manufacturing, offering dosing flexibility to the hemophilia A community. Presented at: Hemostasis and Thrombosis Research Society, New Orleans, LA, USA, April 16–18, 2015.
6 Vogel JH, McDonald T, Hesslem A et al. BAY 81-8973 – a new full-length recombinant FVIII product using novel manufacturing technologies. Presented at: World Federation of Hemophilia, Buenos Aires, Argentina, July 10–14, 2010.
7 Boedecker BG. Production processes of licensed recombinant factor VIII preparations. Semin Thromb Hemost 2001; 27: 385–94.
8 Chan S-Y, Harris K, inventors. Preparation of recombinant factor VIII in a protein free serum. US Patent 5804420. September 8, 1998.
9 Kogenate® FS (antithrombinic factor [recombinant] formulated with sucrose). Full Prescribing Information. Whippany, NJ: Bayer, 2016.
10 Goudar CT, Matanguihan R, Long E et al. Decreased pCO2 accumulation by eliminating bicarbonate addition to high cell-density cultures. Biotechnol Bioeng 2007; 96: 1107–17.
11 Matanguihan R, Sajan E, Konstantinov K, Zacharius M, Olson C, inventors. Process and medium for mammalian cell culture under low dissolved carbon dioxide concentration. US Patent 6, 338, 964, January 15, 2002.
12 Chan S, Tang Y, Tao Y, Wu Y, Kelly R, inventors. Use of molecular chaperones for the enhanced production of secreted recombinant proteins in mammalian cells. US Patent US 2003/0048608 A1, 2003.
13 Willmund F, del Alamo M, Pechmann S et al. The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. Cell 2013; 152: 196–209.
14 Ishaque A, Thirit J, Murphy JE, Konstantinov K. Over-expression of Hsp70 in BHK-21 cells engineered to produce recombinant factor VIII promotes resistance to apoptotic and enhances secretion. Biotechnol Bioeng 2007; 97: 144–55.
15 Vogel JH, Nguyen H, Giovannini R et al. A new large-scale manufacturing platform for complex biopharmaceuticals. Biotechnol Bioeng 2012; 109: 3049–58.
16 Data on File, Whippany, NJ: Bayer.
17 Kovaltry® (antithrombinic factor [recombinate]). Full Prescribing Information. Whippany, NJ: Bayer, 2016.
18 Shah A, Delesen H, Garger S, Lalezari S. Pharmacokinetic properties of BAY 81-8973, a full-length recombinant factor VIII. Haemophilia 2015; 21: 766–71.
19 Bovenschen N, Rijken DC, Havekes LM, van Vlijmen BJ, Mertens K. The B domain of coagulation factor VIII interacts with the asialoglycoprotein receptor. J Thromb Haemost 2005; 3: 1257–65.
20 Jankowski MA, Patel H, Rouse JC, Mar-zili LA, Weston SR, Sharpe PJ. Defining ‘full-length’ recombinant factor VIII: a comparative structural analysis. Haemophilia 2007; 13: 30–7.
21 Kavakli K, Yang R, Rusen L, Beckmann H, Tserelgidou-Stoeter D, Maas Enriquez M. Prophylaxis versus on-demand treatment with BAY 81-8973, a full-length plasma-protein–free rFVIII product: results from a randomized trial (LEOPOLD II). J Thromb Haemost 2015; 13: 360–9.
22 Ljung R, Kenet G, Mancuso ME et al. BAY 81-8973 safety and efficacy for prophylaxis and treatment of bleeds in previously treated children with severe haemophilia A: results of the LEOPOLD Kids Trial. Haemophilia 2016; 22: 354–60.
23 Saxena K, Lalezari S, Oldenburg J et al. Efficacy and safety of BAY 81-8973, a full-length recombinant factor VIII: results from the LEOPOLD I trial. Haemophilia 2016; 22: 706–12.
24 Mancuso ME, Beckmann H, Maas Enriquez M. BAY 81-8973 prophylaxis efficacy in patients with severe hemophilia A: analyses of annualized bleeding rate outcomes in the LEOPOLD I trial. Presented at: International Society on Thrombosis and Haemostasis, Toronto, ON, Canada, June 20–23, 2015.
25 Church N, Ayagari R, Pocsok J et al. Patterns of prior treatment and bleeds among others.
patients with severe hemophilia A: impact of clinical phenotype in the selection of BAY 81-8973 dosing frequency in the LEOPOLD I trial. Presented at: American Society of Hematology, Orlando, FL, USA, December 5–8, 2015.

26 Lusher JM, Scharrer I. Evolution of recombinant factor VIII safety: KOGENATE and Kogenate FS/Bayer. Int J Hematol 2009; 90: 446–54.

27 Advate® (antihemophilic factor [recombinant] plasma/albumin-free method). Full Prescribing Information. Westlake Village, CA: Baxter Healthcare Corporation, 2015.

28 Xyntha® (antihemophilic factor [recombinant] plasma/albumin-free). Full Prescribing Information. Philadelphia, PA: Wyeth Pharmaceuticals Inc., 2014.

29 Kelley B, Jankowski M, Booth J. An improved manufacturing process for Xyntha/ReFacto AF. Haemophilia 2010; 16: 717–25.

30 NovoEight® (antihemophilic factor [recombinant]). Full Prescribing Information. Plainsboro, NJ: Novo Nordisk A/S, 2015.

31 Tiede A, Klamroth R, Oldenburg J. Turoctocog alfa (recombinant factor VIII). Manufacturing, characteristics and clinical trial results. Hamostaseologie 2015; 35: 364–71.

32 Nuwiq® (antihemophilic factor [recombinant]). Full Prescribing Information. Hoboken, NJ: Octapharma USA, Inc., 2015.

33 Winge S, Yderland L, Kannicht C et al. Development, upscaling and validation of the purification process for human-cl rhFVIII (Nuwiq®), a new generation recombinant factor VIII produced in a human cell-line. Protein Expr Purif 2015; 115: 165–75.

34 Afstyla® (antihemophilic factor [recombinant], single chain). Full Prescribing Information. Marburg, Germany: CSL Behring, 2016.

35 Zollner SB, Raquet E, Muller-Cohrs J et al. Preclinical efficacy and safety of rVIII-SingleChain (CSL627), a novel recombinant single-chain factor VIII. Thromb Res 2013; 132: 280–7.

36 Schmidbauer S, Wirtel R, Kreuter J et al. Characterization of recombinant single chain FVIII, rVIII singlechain (CSL627). Haemophilia 2012; 18: 37.

37 Eloctate® (antihemophilic factor [recombinant], Fc fusion protein). Full Prescribing Information. Cambridge, MA: Biogen, 2016.

38 McCue J, Kshirsagar R, Selvetti K et al. Manufacturing process used to produce long-acting recombinant factor VIII Fc fusion protein. Biologicals 2015; 43: 213–9.

39 Adynovate® (antihemophilic factor [recombinant], pegylated). Full Prescribing Information. Westlake Village, CA: Baxter, 2016.

40 Manco-Johnson MJ, Kempton CL, Reding MT et al. Randomized, controlled, parallel-group trial of routine prophylaxis vs. on-demand treatment with sucrose-formulated recombinant factor VIII in adults with severe hemophilia A (SPINART). [published correction appears in J Thromb Haemost 2014;12:119–122.] J Thromb Haemost 2013; 11: 1119–27.

41 Valentino LA, Mamonov V, Hellmann A et al. A randomized comparison of two prophylaxis regimens and a paired comparison of on-demand and prophylaxis treatments in hemophilia A management. J Thromb Haemost 2012; 10: 359–67.

42 Recht M, Nemes L, Matsyiak M et al. Clinical evaluation of moroctocog alfa (AF-CC), a new generation of B-domain deleted recombinant factor VIII (BDDrFVIII) for treatment of haemophilia A: demonstration of safety, efficacy, and pharmacokinetic equivalence to full-length recombinant factor VIII. Haemophilia 2009; 15: 869–80.

43 Lentz SR, Msegav M, Ozelo M et al. Results from a large multinational clinical trial (guardian™ 3) using prophylactic treatment with turoctocog alfa in paediatric patients with severe haemophilia A: safety, efficacy and pharmacokinetics. Haemophilia 2013; 19: 698–705.

44 Lisitchkov T, Hampton K, von Depka M et al. Novel, human cell line-derived recombinant factor VIII (human-cl rhFVIII; Nuwiq®) in adults with severe haemophilia A: efficacy and safety. Haemophilia 2016; 22: 225–31.

45 Mahliangi J, Kulczkowski K, Karim FA et al. Efficacy and safety of rVIII-Single-Chain: results of a phase I/3 multicenter clinical trial in severe hemophilia A. Blood 2016; 128: 630–7.

46 Mahliangi J, Powell JS, Ragni MV et al. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. Blood 2014; 123: 317–23.

47 Konkle BA, Staszyshyn O, Chowdary P et al. Pegylated, full-length, recombinant factor VIII for prophylactic and on-demand treatment of severe hemophilia A. Blood 2015; 126: 1078–85.

48 Manco-Johnson MJ, Abshire TC, Shapiro AD et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. N Engl J Med 2007; 357: 535–44.

49 Blanchette VS, Shapiro AD, Liesner RJ et al. Plasma and albumin-free recombinant factor VIII: pharmacokinetics, efficacy and safety in previously treated pediatric patients. J Thromb Haemost 2008; 6: 1319–26.

50 Kulkarni R, Karim FA, Glamocanin S et al. Results from a large multinational clinical trial (guardian™ 3) using prophylactic treatment with turoctocog alfa in paediatric patients with severe haemophilia A: safety, efficacy and pharmacokinetics. Haemophilia 2013; 19: 698–705.

51 Klukowska A, Szczepanski T, Vadovin V, Knaub S, Jansen M, Liesner R. Novel human cell line-derived recombinant factor VIII (Human-cl rhFVIII, Nuwiq®) in children with severe haemophilia A: efficacy, safety and pharmacokinetics. Haemophilia 2016; 22: 232–9.

52 Young G, Mahliangi J, Kulkarni R et al. Recombinant factor VIII Fc fusion protein for the prevention and treatment of bleeding in children with severe hemophilia A. J Thromb Haemost 2015; 13: 967–77.