An improved manufacturing process for Xyntha/ReFacto AF

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Summary. ReFacto® Antihemophilic Factor is a second-generation antihaemophilia A product manufactured using a process that includes therapeutic grade human serum albumin (HSA) in the cell culture medium, but is formulated without HSA as a stabilizer. Even though this second-generation antihaemophilia product has a good safety profile, a programme was implemented to eliminate all animal- and human-derived raw materials from the production process, thus producing a third-generation product. To that end, HSA has been removed from the master and working cell banks and from the culture medium. The hybridoma-derived monoclonal antibody formerly used in the purification process has been replaced by a chemically synthesized affinity peptide, and a virus-retaining filtration step has been added to enhance the clearance of large viruses, such as retroviruses. The purification process has been validated for the removal of a panel of model viruses and provides significant clearance of all viruses tested. Host cell- and process-derived impurity removal validations also were conducted, including host cell DNA and protein, in addition to the affinity peptide. Compared with the product manufactured according to the original process, these changes had no detectable effect on the structural integrity, stability or clinical efficacy of this antihaemophilia A product. The product produced by the improved manufacturing process is named Xyntha™/ReFacto AF.

Keywords: factor VIII, manufacturing, ReFacto, virus removal filtration, Xyntha

Introduction

Several recombinant factor VIII (FVIII) products are approved in the United States and the European Union for the treatment of haemophilia A [1]. ReFacto® Antihemophilic Factor (Recombinant); Wyeth Pharmaceuticals Inc.; Philadelphia, PA, USA; 2007 is the first B-domain-deleted FVIII and was licenced in the European Union in 1998 and in the United States in 2000. It is a second-generation antihaemophilia A factor product, formulated without the use of human serum albumin (HSA) as a stabilizing excipient [2]. The manufacturing process for the original ReFacto included highly purified, therapeutic-grade HSA in the cell culture medium, to support cell growth and product expression. The ReFacto purification process included an immunoaffinity chromatography step, in which a monoclonal antibody (mAb) was used to selectively purify the FVIII molecule from a complex feedstream [2].

The elimination of animal- and human-derived raw materials from production processes precludes the introduction of adventitious agents, such as viruses, from these sources [3]. Until recently, only one recombinant FVIII product – Advate [Antihemophilic Factor (Recombinant), Plasma/Albumin-Free Method]; Baxter Healthcare Corporation; Westlake Village, CA, USA; 2007 – employed a manufacturing method that eliminated all human- and animal-derived raw materials from its cell-culture process. However, a mAb produced by a murine hybridoma cell line is still being used for the purification of Advate [1].

A programme was implemented to eliminate all animal- and human-derived raw materials from both the cell-culture and purification processes used to produce ReFacto, thereby fully aligning the upstream process with the albumin-free product formulation. The new process, yielding Xyntha™ [Antihemophilic Factor (Recombinant), Plasma/Albumin-Free] for
intravenous use, freeze-dried powder; Wyeth Pharmaceuticals Inc.; 2008/ReFacto AF [morococog alfa AF-CC], was developed with the intent to maintain product characteristics and comparability to ReFacto. This study describes the design strategy, details of production and process modifications, validation data and viral safety assessment of the improved Xyntha/ReFacto AF manufacturing process.

Original ReFacto manufacturing process

The original ReFacto manufacturing process has been described previously [2]. The cell culture process uses an inoculum train, which builds sufficient cell mass to initiate a production culture and subsequent product synthesis in a perfusion bioreactor. SP-Sepharose is used to capture and concentrate the product from the cell free conditioned medium. The product-containing SP eluate is stored frozen prior to further downstream processing. Multiple SP eluates are pooled, treated with a solvent/detergent mixture to inactivate enveloped viruses and loaded directly onto a mAb column. The product is further purified using Q-Sepharose anion exchange chromatography and Butyl-Sepharose hydrophobic interaction chromatography. Finally, size exclusion chromatography is used to exchange the product into the formulation buffer. The highly purified ReFacto is then frozen as a drug substance and tested prior to release for additional manufacturing at the fill/finish facility, to produce the lyophilized vial of ReFacto drug product that is distributed to the patient.

Rationale for and details of Xyntha/ReFacto AF process changes

The Xyntha/ReFacto AF manufacturing process was designed to leverage the knowledge and experience with the original ReFacto process by keeping the major process design elements intact (Fig. 1). This modified process closely resembles the original ReFacto manufacturing process, with specific modifications introduced to achieve the goal of eliminating all animal- and human-derived raw materials. The viral safety of the modified process is further enhanced by the introduction of a virus removal filtration (VRF) step and the replacement of the immunoaffinity purification column with a synthetic peptide ligand. The details of and rationale for these process improvements are described below.

The primary goal of the cell-bank adaptation programme was to remove HSA from the culture medium formulation and establish the new master cell bank (MCB) and working cell bank (WCB) lacking any human- or animal-derived raw materials. The philosophy of the cell-line adaptation programme was to leverage previous cell-line development to avoid de novo creation of a new production cell line, cell culture medium and cell culture process. This approach minimizes the potential to impact the cell culture process.

Comparative genotypic analysis was performed to confirm that the new MCB represents the same initial cell clone as the original MCB, and to ensure that no unexpected changes occurred during cell-line adaptation that impacted the B-domain-deleted recombinant FVIII genes or transcripts. The analysis included Southern blot and Northern blot analyses, as well as DNA sequencing. Figure 2 shows the results of the Southern blot analysis evaluating the DNA integration pattern for the ReFacto MCB and the Xyntha/ReFacto AF MCB, which show identical hybridization patterns (Data on File, Wyeth Pharmaceuticals, Collegeville, PA, USA). The consistency of patterns between the Xyntha/ReFacto AF WCB and end-of-production samples establishes the stability of the cell line during production. These analyses clearly established that the MCBs are genotypically indistinguishable with respect to the integrated and amplified recombinant FVIII genes and their expressed transcripts.

To maintain cell viability during the production phase, the Xyntha/ReFacto AF manufacturing process uses the same bioreactor scale and perfusion equipment as the original process. As with the licenced process, the production phase is defined by a temperature shift and a switch to production medium. Media formulation changes have been made to accommodate product synthesis in the absence of albumin through rebalancing key compo-
ments and supplementing the media with additional nutrients, but similar bioreactor control strategies are used to maintain the cells in an optimal state for product synthesis.

The major purification process modifications are the replacement of the immunoaffinity chromatography step with an affinity step using a chemically synthesized polypeptide ligand and the introduction of a VRF step.

The immunoaffinity chromatography step used in the original ReFacto manufacturing process provides excellent removal of such process-related impurities as DNA and host cell proteins (HCPs) [2]. By replacing the mAb with a peptide ligand, higher column-loading capacities and improved resin cleaning afforded by a broader sanitization solution compatibility can be achieved [4]. Most notably, the peptide can be chemically synthesized, eliminating the requirement for a mAb derived from murine hybridoma culture and thereby removing the potential for introduction of an adventitious virus, such as an infectious retrovirus (known to be associated with hybridoma cultures) into the FVIII product stream [5–7].

Several peptides were identified by screening a bacteriophage display library expressing approximately 100 million unique peptides as amino-terminal fusions to the P3 coat protein. These peptides were designed to have five to seven residues between the cysteine residues, which oxidize to form a disulfide bond that results in a constrained ring. The peptide that was ultimately selected, designated TN8.2, was one of a family of related peptides derived from the library containing eight residues, including the cysteines, in the ring. The TN8.2 peptide contains 27 amino acids and includes a flexible linker to provide accessibility of the constrained ring for FVIII binding [4]. Figure 3 illustrates the selected residues of the ring and two flanking residues external to the cystine bond, which were also variegated in the peptide library [4]. The peptide has an acetylated N-terminus and no primary amines, with the exception of a C-terminal lysine residue. This allows for efficient, site-directed immobilization of the peptide via the C-terminal lysine by the amine reactive N-Hydroxysucccinimide immobilization chemistry [4].

The peptide binds to the B-domain deleted recombinant FVIII (BDDrFVIII) through the C2 domain of the 80-kD light chain portion of the BDDrFVIII heterodimer. The interaction between BDDrFVIII and the peptide is relatively weak, with a dissociation constant of $\approx 10^{-6}$ M, compared with the mAb that has a substantially lower $k_D$ of $\approx 10^{-10}$ M. This weak binding is overcome by immobilizing the peptide at approximately 125 times the molar concentration of the antibody, thus providing sufficient binding due to mass action. Figure 4 shows the binding isotherm for the immobilized peptide and BDDrFVIII. The data show excellent fit to a Langmuir isotherm ($r^2 = 0.99$), indicating a single mode of interaction, without protein-protein interactions, and a dissociation constant of 0.92 $\mu$m [4].

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The screening conditions for peptide selection closely mimicked the operating conditions for the immunoaffinity chromatography step [8]. This made substitution of the immunoaffinity step with the TN8.2 chromatography resin relatively straightforward. The chromatographic characteristics of the immobilized peptide are very similar to the immunoaffinity resin. Table 1 compares the most relevant performance characteristics of both resins [8]. The resins have very nearly identical performance with respect to product quality attributes, i.e., product capture and impurity removal. The immobilized peptide performs better with respect to the more practical aspects of performance, i.e., recovery, capacity and cleaning conditions. The process pool derived from the TN8.2 Sepharose step is highly pure, based on sodium dodecyl sulphate–polyacryl-

Table 1. Comparison of TN8.2 and immunoaffinity purification step performance [8].

<table>
<thead>
<tr>
<th>Immunoaffinity</th>
<th>TN8.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDDrFVIII recovery (average of &gt;10 cGMP runs)</td>
<td>63</td>
</tr>
<tr>
<td>CHO protein removal (log_{10} reduction)</td>
<td>3.7</td>
</tr>
<tr>
<td>DNA removal (log_{10} reduction)</td>
<td>4.1</td>
</tr>
<tr>
<td>BDDrFVIII informs captured</td>
<td>M, 170 000 fusion protein + multiple heterodimer forms</td>
</tr>
<tr>
<td>Elution peak volume (column volumes)</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Dynamic capacity – (IU mL^{-1})</td>
<td>20 000 (estimate)</td>
</tr>
<tr>
<td>Process control loading limit (IU mL^{-1})</td>
<td>5000</td>
</tr>
<tr>
<td>Sanitization solution</td>
<td>0.1 M acetic acid, 20% ethanol, pH 4.0</td>
</tr>
</tbody>
</table>

The goal of the new VRF step is elimination of noninfectious retroviral-like particles and potential adventitious viruses by means of the optimally sized 35-nm pore-size filter [9]. The single-use VRF device retains viral particles based on sieving properties, while allowing passage of the Xyntha/ReFacto AF protein. The virus filter was selected to provide robust clearance of such large viruses as retroviruses. The product passes freely through the membrane pores into the permeate, whereas viral particles, if present, are retained by the membrane. Filters with a smaller pore size, which have the capacity to retain parvoviruses, gave rise to variable product retention and subsequent yield losses (Data on File, Wyeth Pharmaceuticals). The VRF step was intentionally placed after the Q-Sepharose chromatography step because this process intermediate has no detergent present, which might complicate the operation of a filtration step, thereby enabling consistent performance of the VRF.

The battery of purity tests performed on the ReFacto MCB was designed in accordance with the guidelines provided in several regulatory documents [10–12]. Monitoring for adventitious viruses utilizes in vitro testing on indicator cell lines, which have been shown to be sensitive to a broad range of viruses, especially to those that are known to infect Chinese hamster ovary (CHO) cells. Lot-to-lot coverage for adventitious contamination during production is provided by testing unprocessed bulk (cells plus the conditioned medium) taken on the final harvest day of the manufacturing run. Additional coverage for minute virus of mice (MVM), a reported contaminant of some industrial CHO cell production cultures, is provided by using either an in vitro assay with an indicator cell line susceptible to MVM infection (324K or A9) or an MVM polymerase

Fig. 4. Equilibrium binding measurement for the TN8.2 and B-domain deleted recombinant factor VIII (BDDrFVIII) interaction measured on TN8.2 Sepharose resin [4]. The equilibrium-binding isotherm was determined using repeated BDDrFVIII challenges to a resin sample; 50 µL of a 50% slurry of resin was combined with 450 µL of a BDDrFVIII solution and binding allowed to approach equilibrium (generally 30 min). The resin supernatant was then removed and replaced with another 450 µL of BDDrFVIII solution. This process was repeated until the concentration of BDDrFVIII in the supernatant solution approached that of the challenge solution. Bound protein was determined from the difference between the units offered and the units remaining in the supernatant solution at equilibrium for each BDDrFVIII challenge using a chromogenic FVIII activity assay.

The screening conditions for peptide selection closely mimicked the operating conditions for the immunoaffinity chromatography step [8]. This made substitution of the immunoaffinity step with the TN8.2 chromatography resin relatively straightforward. The chromatographic characteristics of the immobilized peptide are very similar to the immunoaffinity resin. Table 1 compares the most relevant performance characteristics of both resins [8]. The resins have very nearly identical performance with respect to product quality attributes, i.e., product capture and impurity removal. The immobilized peptide performs better with respect to the more practical aspects of performance, i.e., recovery, capacity and cleaning conditions. The process pool derived from the TN8.2 Sepharose step is highly pure, based on sodium dodecyl sulphate–polyacryl-

amide gel electrophoresis (SDS–PAGE) analysis (see Fig. 5), with all of the protein impurities detected by SDS–PAGE in the load to this step removed during processing [4,8].
chain reaction analysis [13]. No adventitious viruses were detected when the MCB samples were tested using any of the in vitro or in vivo methods. Furthermore, no infectious retrovirus activity was detected in the cocultivation assay. Transmission electron microscopy showed the cells to be free of all virus-like particles other than the type A and C particles previously reported to be present at low levels in CHO cells [14–16]. On the basis of these results, it was concluded that no detectable adventitious microbial or viral agents had been introduced into the Xyntha/ReFacto AF MCB and that no infectious retroviruses were present.

The purification steps of the Xyntha/ReFacto AF process were also evaluated for their ability to remove or inactivate model viruses. The Xyntha/ReFacto AF viral clearance and inactivation studies were designed and executed in accordance with regulatory guidelines [12]. At least two orthogonal modes of clearance were identified for each virus evaluated in this study. This study used a panel of model viruses with an emphasis on viruses known to infect CHO cells (see Table 2). These viruses include three enveloped viruses [parainfluenza virus (PI-3), pseudorabies virus (PRV), and xenotropic murine leukaemia virus (X-MuLV)] and two nonenveloped viruses [MVM and Reovirus-3 (Reo-3)] (Data on File, Wyeth Pharmaceuticals). The model viruses used in this evaluation were selected to cover a wide variety of virus families that differ by size, genome type, envelope and resistance to physicochemical inactivation. The panel of viruses used in this assessment differs from those used in the assessment of the original process [3,4], reflecting a shift in focus from viruses representing adventitious agents that could potentially be present in the HSA used in the original cell culture medium, to a panel that is typically used to evaluate recombinant protein production via a process devoid of animal- or human-derived raw materials [17,18].

As a result of the importance of the TN8.2 Sepharose and Planova 35N VRF steps in ensuring the viral safety of the product, viral clearance studies were performed for these steps for all five viruses in the panel. For the S/D virus inactivation step, runs were conducted for each of the three enveloped viruses. The demonstration of comparable performance of the laboratory scale to the manufacturing scale for each step establishes the laboratory-scale systems as valid models of the manufacturing steps for use in virus validation studies. For the TN8.2 Sepharose step, both unused and used resins were tested because of the importance of the affinity chromatography step in the viral safety package. Each chromatography eluate included the virus present in the prepeak, peak and postpeak fraction, as a worst-case estimate. The ratio and temperature of the S/D chemicals were at the lowest acceptable levels, and the VRF permeate included the virus present in the maximum filter flush volume, representing the worst-case conditions for the relevant step.

Table 2 illustrates the results of the virus validation studies. A significant reduction in the virus titre was observed for all TN8.2 Sepharose runs, with the majority of virus particles removed in the load eluate and washes. These results demonstrate that the mechanism of removal was consistent with affinity chromatography, where, due to the specificity of binding between the ligand (TN8.2) and the desired product (Xyntha/ReFacto AF), impurities do not bind to the column or are weakly retained and removed prior to product elution. The performance of unused and used TN8.2 Sepharose resins was also consistent. The S/D step provided instantaneous and complete inactivation of the three enveloped viruses tested. The VRF step provided significant removal of all viruses tested with the exception of MVM, which would be expected to pass through the 35-nm pore of this filter. The

<table>
<thead>
<tr>
<th>Virus</th>
<th>SP Sepharose</th>
<th>S/D Inactivation</th>
<th>TN8.2 Sepharose</th>
<th>Q Sepharose</th>
<th>Planova 35N Nanofiltration</th>
<th>Butyl Sepharose</th>
<th>Total LRV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuLV</td>
<td>ND</td>
<td>&gt;3.24</td>
<td>&gt;2.99</td>
<td>ND</td>
<td>&gt;5.18</td>
<td>ND</td>
<td>&gt;11.4</td>
</tr>
<tr>
<td>MMV</td>
<td>1.46</td>
<td>2.52</td>
<td>0.52*</td>
<td>0.63*</td>
<td>1.23</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>PI-3</td>
<td>ND</td>
<td>&gt;4.93</td>
<td>1.51</td>
<td>&gt;4.95</td>
<td>ND</td>
<td>&gt;11.4</td>
<td></td>
</tr>
<tr>
<td>Reo-3</td>
<td>ND</td>
<td>4.40</td>
<td>&gt;5.93</td>
<td>ND</td>
<td>&gt;6.00</td>
<td>&gt;10.3</td>
<td></td>
</tr>
<tr>
<td>PRV</td>
<td>ND</td>
<td>&gt;4.90</td>
<td>3.13</td>
<td>&gt;6.00</td>
<td>ND</td>
<td>&gt;14.0</td>
<td></td>
</tr>
</tbody>
</table>

*Log reduction values (LRV) <1.0 are not included in the calculation of total LRV.
ND, not done; Butyl, Butyl-Sepharose; env, enveloped; LRV, log removal value; MVM, minute virus of mice; nonenv, nonenveloped; PI-3, parainfluenza virus; PRV, pseudorabies virus; Reo-3, Reovirus-3; S/D, solvent/detergent; SP, SP-Sepharose; VRF, virus removal filtration; X-MuLV, xenotropic murine leukaemia virus.
total reduction across all the tested steps was between 5.2 and >10.3 log for the nonenveloped viruses, and between >11.4 and >14.0 log for the enveloped viruses. The total log removal values (LRVs) for all the tested viruses are quite high, with consistency between duplicates for all steps, which demonstrates that the purification process provides excellent assurance of removal and inactivation of model viruses. The overall inactivation and removal package compares favourably with the package for the original process; the Xyntha/ReFacto AF process has higher total LRV for MuLV, higher LRVs for nonenveloped virus (i.e. Reo-3) and incorporates the addition of a third robust step for virus clearance (i.e. Planova 35N VRF) (Data on File, Wyeth Pharmaceuticals).

Process validation

The Xyntha/ReFacto AF manufacturing process was validated to ensure effective and consistent performance within the established limits of the operating parameters. Although the albumin-free cell culture (AFCC) manufacturing process is quite similar to the original ReFacto process, a significant duplication of the process validation package was undertaken to provide a clear comparison of performance and consistency between the two processes.

Compared with those of the original ReFacto process, the cell culture growth and production phases of the Xyntha/ReFacto AF manufacturing process demonstrate consistent and adequate rates and densities of cell growth, cell removal and harvest operations. Cell culture robustness has been established using laboratory studies that test critical control parameter limits and show acceptable performance and product quality.

In-process, cell culture limits are unchanged relative to the original ReFacto manufacturing process for all critical parameters, except for minor adjustments in the temperature of the seed bioreactor, the dissolved oxygen of the production bioreactor, the inoculum densities of the seed bioreactor and production bioreactors and the cumulative cell age during production. Cell culture robustness studies were conducted in either small shake flasks or 2-L laboratory bioreactors, which have both been qualified as appropriate scale-down models of the full-scale bioreactor.

Stability studies were performed to ensure that the cell culture medium could be stored for a sufficient period to allow routine manufacturing prior to use. To assess the stability of an extended lineage of Xyntha/ReFacto AF WCB cells at the end of their validated cell age, the WCB cells at this age limit were used to inoculate a production bioreactor for the synthesis of the clinical drug substance. All in-process control parameters were achieved during the course of this cell-culture production run. Characterization of the drug-substance batches manufactured from this run showed that the drug substance that was produced met all release specifications. Together with additional cell-line characterization data from Southern and Northern blot hybridizations and DNA sequencing analyses, these studies support the in vitro age limit of the Xyntha/ReFacto AF WCB cells.

The purification process has been validated for removal of host cell-derived, media-derived and purification process-derived impurities [2] (Data on File, Wyeth Pharmaceuticals). The pattern of CHO protein removal by the Xyntha/ReFacto AF purification process is very similar to that of the original process. The most efficient step for removing host cell-derived impurities is the affinity chromatography step (AFCC uses the TN8.2 Sepharose resin) [2]. During the AFCC process, 3.4-log removal of CHO protein was consistently demonstrated. The subsequent Q-Sepharose step provides an additional 1.2-log removal, and the remaining Butyl-Sepharose and Superdex 200 steps contribute a combined reduction of 0.8 log (see Table 3). The pattern of DNA removal by the Xyntha/ReFacto AF purification process is also very similar to that of the original manufacturing process. A 3.9-log removal of DNA has been consistently demonstrated for the TN8.2 Sepharose step (Data on File, Wyeth Pharmaceuticals). The Q-Sepharose step provides an additional >1.9 log removal. The removal across the Butyl-Sepharose and Superdex 200-μg chromatography steps could not be shown to contribute to the reduction, as the

<table>
<thead>
<tr>
<th>Batch</th>
<th>TN8.2 eluate (LRV)</th>
<th>Q eluate (LRV)</th>
<th>HIC eluate (LRV)</th>
<th>GF eluate (LRV)</th>
<th>Total (LRV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.3</td>
<td>1.1</td>
<td>0.4</td>
<td>0.5</td>
<td>5.4</td>
</tr>
<tr>
<td>2</td>
<td>3.3</td>
<td>1.2</td>
<td>0.5</td>
<td>0.4</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>1.4</td>
<td>0.6</td>
<td>0.3</td>
<td>5.8</td>
</tr>
<tr>
<td>4</td>
<td>3.6</td>
<td>1.2</td>
<td>0.6</td>
<td>0.3</td>
<td>5.7</td>
</tr>
<tr>
<td>5</td>
<td>3.3</td>
<td>1.1</td>
<td>0.3</td>
<td>0.4</td>
<td>5.1</td>
</tr>
<tr>
<td>6</td>
<td>3.3</td>
<td>1.3</td>
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<td>0.3</td>
<td>5.2</td>
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<tr>
<td>7</td>
<td>3.5</td>
<td>1.1</td>
<td>0.4</td>
<td>0.3</td>
<td>5.3</td>
</tr>
<tr>
<td>8</td>
<td>3.3</td>
<td>1.0</td>
<td>0.4</td>
<td>0.3</td>
<td>5.0</td>
</tr>
<tr>
<td>Mean</td>
<td>3.4</td>
<td>1.2</td>
<td>0.4</td>
<td>0.4</td>
<td>5.4</td>
</tr>
</tbody>
</table>

GF, gel filtration; HCP, host cell protein; HIC, hydrophobic interaction chromatography; LRV, log removal value; Q, Q-Sepharose.
DNA levels at this stage are below the limit of detection of the assay [2] (Data on File, Wyeth Pharmaceuticals). Overall, the purification process removes DNA to levels below the limit of <14 pg 1000 IU\(^{-1}\) of drug substance (Data on File, Wyeth Pharmaceuticals). This level is significantly lower than current World Health Organization recommended levels of <100 pg per dose of cellular DNA [10].

Process-derived impurities are introduced into the product stream by purification process buffers and resins. The removal of a representative set of these components was validated, including ethylenediaminetetraacetic acid (EDTA), tri-n-butyl-phosphate (TNBP), Octoxynol 9, TN8.2 ligand, ethylene glycol and ammonium acetate (Data on File, Wyeth Pharmaceuticals). The safety of the Xyntha/ReFacto AF drug substance with respect to the TN8.2 ligand residuals has been established by a combination of acute toxicology studies performed on the TN8.2 ligand, along with a comprehensive validation package addressing the consistency of TN8.2 ligand leaching and removal. The validation package combines concurrent removal studies for cGMP batches and small-scale spike and removal studies. The small-scale spike/removal studies demonstrated a minimum of a 2.0-log reduction for the Q-Sepharose step and a 0.8-log reduction for the Butyl-Sepharose step [4]. All batches of the drug substance had no detectable TN8.2 (calculated to be <5.7 ng mL\(^{-1}\)) (Data on File, Wyeth Pharmaceuticals). To put this level into perspective, in an acute toxicology study conducted in Sprague–Dawley rats and used to support both the IND and the licensure application, the levels of TN8.2 peptide tested were 300 million-fold higher than the maximum TN8.2 residual level that could be contained in a 2000-IU dose (corresponding to a ≥ 8.3-log safety margin). Even at the level tested in the rats, there were no effects observed in the toxicity study [4].

**Stability, comparability overview and clinical evaluation of Xyntha/ReFacto AF**

The formulations for both the drug substance and the drug product were not changed for the Xyntha/ReFacto AF process. This minimizes the likelihood of any changes in the drug substance or product stability profiles, as the improved manufacturing process provides material that is comparable in purity and quality to that of the original process. To confirm these expectations, stability studies were conducted on three batches of commercial final drug product and three batches of intermediate drug substance. The commercial material was monitored through 36 months, which is the current shelf-life for Xyntha/ReFacto AF (Data on File, Wyeth Pharmaceuticals). Accelerated studies at higher temperatures were also performed for both the final drug product and the intermediate drug substance and were monitored through 6 months. Several parameters were assessed in both the long- and short-term studies, including identity, potency, purity and quality. The assays and specifications used in the stability programme for the Xyntha/ReFacto AF drug substance are a subset of the analytic procedures performed at release because they are indicative of stability. Results of the assays used to monitor the stability of final drug product and intermediate drug substance during storage for all validation batches demonstrate no significant changes in terms of potency, quality or purity for Xyntha/ReFacto AF (Data on File, Wyeth Pharmaceuticals).
A complete and comprehensive programme was designed to compare ReFacto and Xyntha/ReFacto AF on both structural and functional bases. Comparisons of the various sources of Xyntha/ReFacto AF were also conducted: nonclinical, clinical and commercial materials (Data on File, Wyeth Pharmaceuticals; Personal communication, M. Jankowski). The overall assessment evaluated the following complementary elements: process comparability (cell culture and purification), drug-substance release testing data, detailed structural characterization and analysis, in vitro functional analysis and forced decomposition analysis. Shown in Fig. 6 is a comparative analysis using SDS–PAGE of representative ReFacto and Xyntha/ReFacto AF, demonstrating the high degree of similarity between the two materials (Data on File, Wyeth BioPharma).

Pivotal clinical studies were initiated to meet the requirements for the approval of a new FVIII product. These clinical studies were designed to meet FDA requirements for FVIII product, which include (i) a comparative pharmacokinetics (PK) study and (ii) an efficacy/safety study in previously treated patients (PTPs). The PK profile of Xyntha/ReFacto AF vs. FLrFVIII was assessed in a randomized, double-blind, crossover study of 30 PTPs using the one-stage clotting assay. In addition, the safety and efficacy of Xyntha/ReFacto AF were assessed in a total of 94 PTPs during 6 months of prophylaxis supplemented with on-demand treatment. A follow-up PK study was conducted after 6 months [18,19].

Ninety percent (90%) confidence intervals about the ratios of the geometric least square mean values of key PK parameters ($AUC_{\infty}$, $AUC_t$ and $K$ value) were within the bioequivalence window of 80–125%, demonstrating the PK equivalence of Xyntha/ReFacto AF and Advate. Of the 94 patients, two had transient, low-titer, de novo, clinically silent inhibitors, both of which were negative on follow-up testing. This study also demonstrated that the Xyntha/ReFacto AF manufacturing process did not affect PK, safety or efficacy outcomes in a clinical setting [19].

Discussion

The Xyntha/ReFacto AF manufacturing process was designed to maximize the viral safety profile of the product and minimize the probability of any observable changes relative to the product derived from the original process. The key changes to the cell culture steps of the manufacturing process were focused on removing animal- and human-derived raw materials in the MCB and the cell culture medium. The purification process eliminated the use of a hybridoma-derived murine mAb and introduced a VRF step. The Xyntha/ReFacto AF purification process is the first to use a chemically synthesized polypeptide for purification of FVIII. The product formulation was unchanged to ensure that the stability profile of the various dosages would remain at the limits established with the original licenced process.

This conservative set of changes was limited in scope to avoid any unintentional impact on product characteristics that could influence the PK profile, clinical efficacy and/or safety. A strong biochemical and biophysical comparability package supports the unchanged nature of the ReFacto product, despite these process improvements. Multiple clinical trials conducted with ReFacto produced by the AFCC process proved that ReFacto manufactured via this third-generation technique was safe and efficacious. A double-blind, randomized PK crossover study.

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Fig. 6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of purified ReFacto and Xyntha/ReFacto AF. Silver stained reducing SDS–PAGE of representative ReFacto and Xyntha/ReFacto AF drug substance batches. Approximately 1 μg of each sample was reduced with dithiothreitol (DTT) prior to analysis. The 6.5% SDS–PAGE was performed using standard Laemmli conditions. The major ReFacto and Xyntha/ReFacto AF isoforms are represented by the 170 kDa product of the translational fusion expression vector and the 90 kDa heavy chain and the 80 kDa light chain of the fully processed protein.
demonstrated that Xyntha/ReFacto AF was PK-equivalent to a full-length rFVIII, and routine prophylaxis with Xyntha/ReFacto AF was found to be effective in preventing haemorrhages in patients with pre-existing target joint(s) [20].

A comprehensive and complete process validation programme was conducted to ensure product safety and process consistency. Following international guidelines for recombinant protein production, all steps in the cell culture and purification processes were evaluated and appropriate process control ranges were established. The clinical programme used several lots of drug substance and drug product produced at full scale in the commercial manufacturing plant, confirming the consistency of the Xyntha/ReFacto AF process.

The combination of these process design elements, comparability assessments, clinical studies and process validation studies provides assurance of the safety and process consistency of the Xyntha/ReFacto AF process.

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Disclosures

M. Jankowski and J. Booth are employees of Wyeth.

References


