# Overview of the structure and manufacturing process for OBI-1, a recombinant porcine sequence, factor VIII for the treatment of acquired hemophilia A

Poster # PT228

**Peter W. Wojciechowski, Chee Kong Lai*, Yi Xiong, Frank Deer**

Baxter BioScience, Technical Service Group, Milford, Massachusetts, USA

## Introduction

**OBI-1 is a purified, B-domain deleted, recombinant porcine sequence FVIII for treatment of acquired hemophilia A patients with autoantibodies to endogenous FVIII. OBI-1 is a ~170 kDa glycoprotein expressed in a baby hamster kidney (BHK) cell line as a 1448 amino acid heterodimer with a heavy (~90 kDa, A1+A2 domain) and light (~80 kDa, A3+C1+C2 domain) chain. The B-domain normally present in the native porcine FVIII is replaced in OBI-1 by a twenty-four amino acid linker that remains attached to the heavy chain. While OBI-1 retains its procoagulant activity, its amino acid sequence is sufficiently different from human FVIII with 86% pairwise sequence homology, to minimize cross-reactivity with inhibitors[1].**

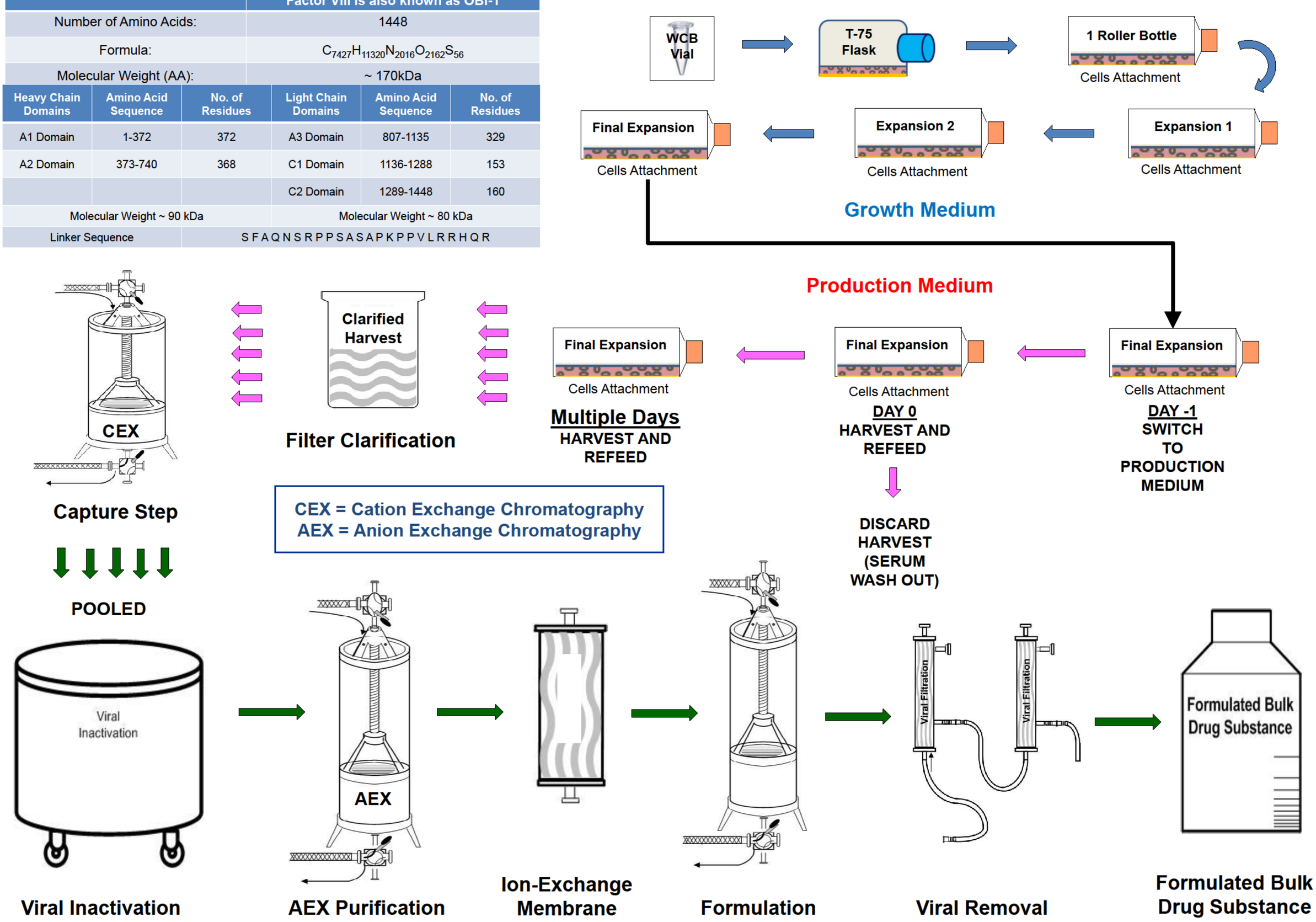
## Methods

In the manufacturing process for OBI-1, BHK cells are expanded from a working cell bank vial into the roller bottles used for OBI-1 production. The growth medium is washed out over two days using a production medium that is free of animal and human derived components. During production the cell culture medium containing OBI-1 is harvested over multiple days. On each day, the harvest is clarified to remove suspended cells and cell debris followed by capture of the product by a cation exchange chromatography step. The daily capture product is frozen until the end of the multiple harvest days, by which all are thawed and pooled together for further processing. The pooled cation exchange eluates undergo incubation with solvent and detergent (S/D) to inactivate any enveloped viruses that may be hypothetically present. A subsequent anion exchange chromatography step is performed to further purify OBI-1 including the removal of cell culture impurities and S/D reagents. The purified material undergoes a pass through ion-exchange membrane and then formulated on another ion-exchange column to remove all upstream buffer and replaced with the formulation buffer. The resulting formulated product goes through a nanofiltration step to remove any hypothetical enveloped and non-enveloped viruses and the potency adjusted to produce a formulated bulk drug substance (FBDS) essentially free of viruses and contaminants associated with the manufacturing process. The FBDS is lyophilized to produce final drug product (FDP) in 500 U vials for clinical use.

### *Table 1: Amino Acid & Domain Sequence of OBI-1[1]*

| Name | | | B-Domain Deleted Recombinant Porcine Factor VIII is also known as OBI-1 | | |
|---|---|---|---|---|---|
| Number of Amino Acids: | | | 1448 | | |
| Formula: | | | $C_{7427}H_{11320}N_{2016}O_{2162}S_{56}$ | | |
| Molecular Weight (AA): | | | ~ 170kDa | | |
| **Heavy Chain Domains** | **Amino Acid Sequence** | **No. of Residues** | **Light Chain Domains** | **Amino Acid Sequence** | **No. of Residues** |
| A1 Domain | 1-372 | 372 | A3 Domain | 807-1135 | 329 |
| A2 Domain | 373-740 | 368 | C1 Domain | 1136-1288 | 153 |
| | | | C2 Domain | 1289-1448 | 160 |
| Molecular Weight ~ 90 kDa | | | Molecular Weight ~ 80 kDa | | |
| Linker Sequence | | | S F A Q N S R P P S A S A P K P P V L R R H Q R | | |

### *Figure 1: Manufacturing Process of OBI-1*




## Conclusions

**OBI-1 manufacturing experience at the commercial scale has demonstrated lot-to-lot consistency with respect to purity, potency and structural product quality attributes. The resulting OBI-1 final drug product has consistent procoagulant activity, low cross-reactivity with human FVIII inhibitors and is essentially free of animal/human components or potential pathogens/viruses.**

## References

1. WHF 2014 World Congress Poster #PT223: Elucidation of structure and functional characteristics of OBI-1, a recombinant, porcine sequence FVIII, C.K. Lai, A. Tse, S. Sivakollundu, M. Patel, S. Vangala, L. Zhang, P. Wojciechowski.

Presented at the WFH 2014 World Congress in Melbourne, Australia • May 11-15, 2014

If you have any additional questions, please feel free to contact Baxter Bioscience Medical Information at medinfo@baxter.com.

Conflicts of interest:

Baxter