Poster P-W-001

Non-clinical immunogenicity assessment of a new recombinant porcine sequence FVIII (OBI-1)

P. Allacher, F. M. Horling, C. Piskernik, P. Leidenmühler, B. M. Reipert
Baxter BioScience, Vienna, Austria

Introduction

Acquired hemophilia A is a rare but often severe bleeding disorder caused by circulating auto-antibodies that neutralize factor VIII (FVIII) activity [1].

In the past, plasma derived porcine FVIII (Hyate:C) was successfully used for the treatment of patients with acquired hemophilia A, mainly because of the low cross-reactivity of porcine FVIII with the neutralizing autoantibodies against human FVIII [2].
Hyate:C was an approved commercial product, which was discontinued in 2004 for reasons not related to immunogenicity. Since then, no alternative porcine FVIII has been available for treatment of acquired hemophilia A patients.

A new product, recombinant porcine sequence FVIII (OBI-1) is currently in development.
The objective of this study was to assess the immunogenicity of OBI-1 in comparison to Hyate:C in E16 hemophilia A mice [3] and in Cynomolgus monkeys.

Objective

The objective of this study was to assess the immunogenicity of recombinant porcine sequence FVIII (OBI-1) in comparison to plasma derived porcine FVIII (Hyate:C) in E16 hemophilia A mice and in Cynomolgus monkeys.

Methods

E16 hemophilia A mice were pre-sensitized with 5 intravenous (i.v.) doses of recombinant human FVIII (100 U/kg) at weekly intervals to induce antibodies against human FVIII. 16 to 21 weeks after the last injection of human FVIII, mice tested positive for anti-FVIII antibodies received 4 weekly i.v. doses (1, 10 or 100 U/kg) of either OBI-1 or Hyate:C.

Development of total binding as well as neutralizing anti- porcine FVIII antibodies was analyzed two weeks after the last dose.

Total binding antibodies were detected by ELISA using OBI-1 (for OBI-1 treated mice) or purified plasma-derived porcine FVIII (for Hyate:C treated mice) as coating antigen.
Neutralizing antibodies were measured using a Bethesda assay in which human hemophilia A plasma was reconstituted with OBI-1 or Hyate:C, depending on which item the animals received.

Cynomolgus monkeys received either OBI-1 (40 and 100 U/kg) or Hyate:C (100 IU/kg) twice daily for 4 days, simulating a clinical treatment episode. Neutralizing antibodies against porcine FVIII were analyzed on days 1, 15, 29, 43 and 57.

Analysis was done using a validated Nijmegen modification of the Bethesda assay. Briefly, monkey plasma samples were heat-treated at 56°C for 30 minutes to inactivate endogenous monkey FVIII. Equal volumes of test plasma and FVIII deficient plasma containing approx. 100% activity of either Hyate:C or OBI-1 were mixed. A control prepared with congenital FVIII deficient plasma instead of monkey sample served as a reference.

Conclusions

- **No differences in the induction of neutralizing antibodies between Hyate:C and OBI-1 were observed in any of the non-clinical immunogenicity studies**
- **Total immunoglobulin G antibodies against porcine FVIII, as assessed in hemophilic mice pre-treated with human FVIII, were detected after treatment with both OBI-1 and Hyate:C without significant differences between the two groups.**
- **Non-clinical immunogenicity assessment in animal models indicates that the new recombinant porcine sequence FVIII (OBI-1) expresses a similar immunogenicity profile as the porcine plasma-derived FVIII (Hyate:C)**

Acknowledgements
The non-clinical program for OBI-1 was carried out by Octagen Corporation in 2001/2002; Baxter took over the project in 2013.

Results

Hemophilia A mice
Total binding antibodies against porcine FVIII , as detected with an ELISA based assay in human FVIII pre-sensitized hemophilia A mice , were found in both mice treated with Hyate:C and mice treated with OBI-1.
Titers of antibodies against porcine FVIII correlated with the dose of porcine FVIII used for treatment. Hemophilic mice treated with 10 or 100 U/kg of Hyate:C developed higher titers of binding antibodies against porcine FVIII than mice treated with the same doses of OBI-1. OBI-1 and Hyate:C at a dose of 1 U/kg induced similar antibody titers against porcine FVIII [Figure 1A].

Neutralizing antibodies to porcine FVIII were found in both mice treated with Hyate:C and mice treated with OBI-1. No significant differences in titers of neutralizing anti- porcine FVIII antibodies were found between mice treated with OBI-1 and mice treated with Hyate:C for any dose tested [Figure 1B].

Figure 1A: *Total binding* antibodies against OBI-1 or Hyate:C in mice pre-sensitized with human FVIII

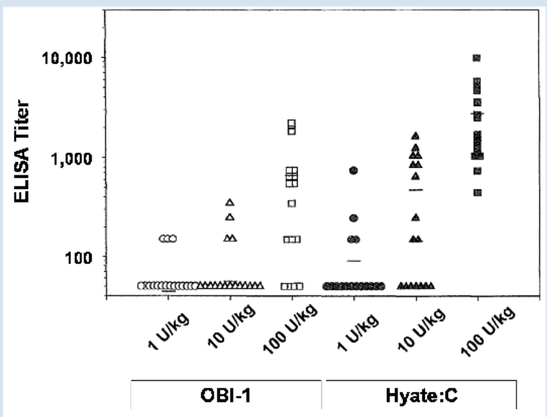


Figure Legend:
Each data point represents the ELISA titer of an individual mouse.
Open symbols: OBI-1 treated mice
Closed symbols: Hyate:C treated mice

Figure 1B: *Neutralizing* antibodies against OBI-1 or Hyate:C in mice pre-sensitized with human FVIII

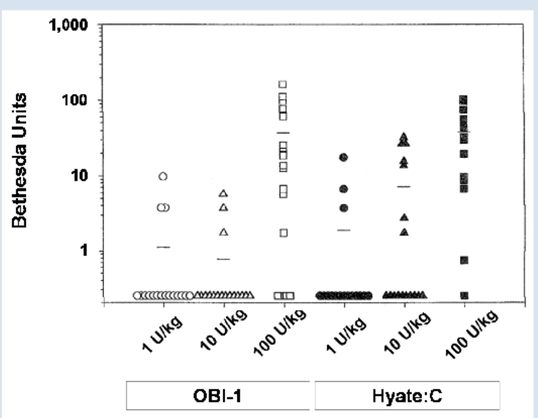


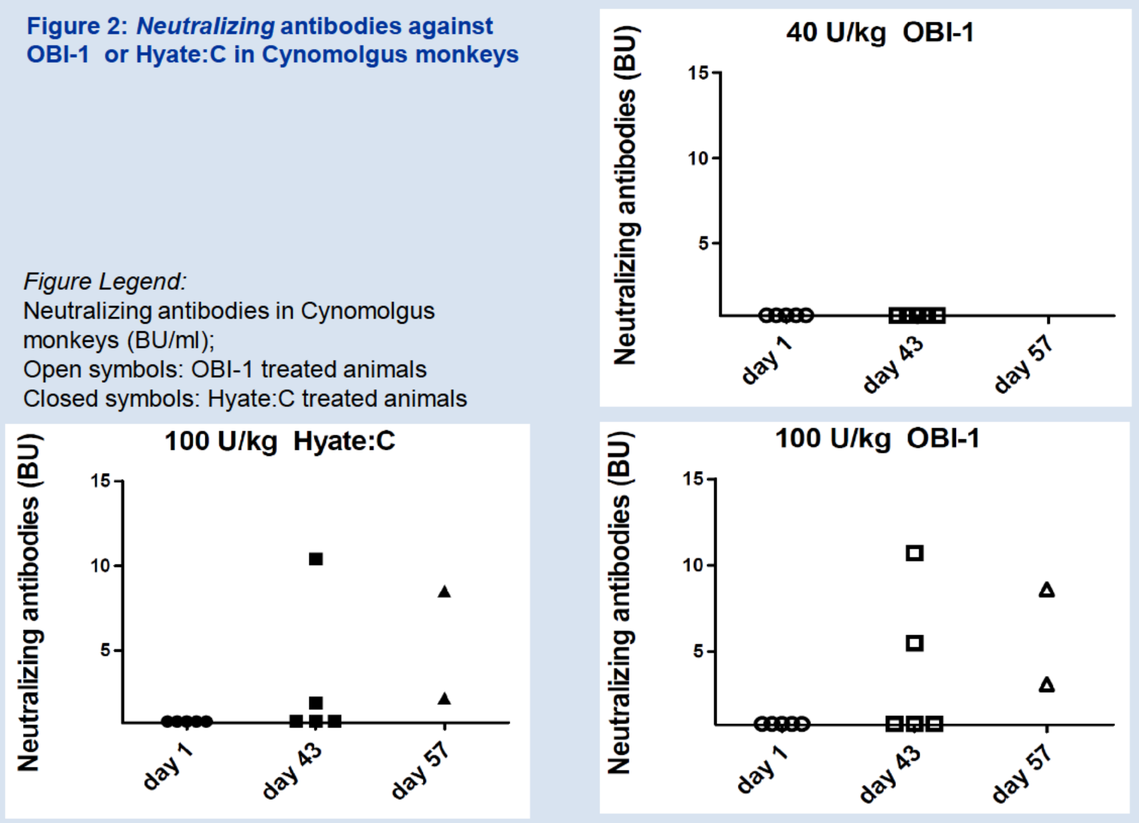
Figure Legend:
Each data point represents the Bethesda titer of an individual mouse.
Open symbols: OBI-1 treated mice
Closed symbols: Hyate:C treated mice

Cynomolgus monkeys
No monkey treated with 40 U/kg OBI-1 developed neutralizing anti- porcine FVIII antibodies. 2 of 5 cynomolgus monkeys treated with 100U/kg OBI-1 and 2 of 6 cynomolgus monkeys treated with 100 U/kg Hyate:C developed neutralizing anti- porcine FVIII antibodies.

At day 57 (end of observation period), titers of neutralizing anti- porcine FVIII antibodies were 8.5 and 2.2 BU/ml in animals treated with Hyate:C, and 8.6 and 3.1 BU/ml in animals treated with OBI-1 (Fig. 2)

Figure 2: *Neutralizing* antibodies against OBI-1 or Hyate:C in Cynomolgus monkeys

Figure Legend:
Neutralizing antibodies in Cynomolgus monkeys (BU/ml);
Open symbols: OBI-1 treated animals
Closed symbols: Hyate:C treated animals



References
1. Franchini M, Mannucci PM. Acquired haemophilia A: A 2013 update. Thromb Haemost 2013; 110:1114-1120
2. Morrison AE, Ludlam CA, Kessler C. Use of porcine factor VIII in the treatment of patients with acquired hemophilia. Blood. 1993 Mar 15;81(6):1513-20.
3. Parker ET, Craddock HN, Barrow RT, Lollar P. Comparative immunogenicity of recombinant B domain –deleted porcine factor VIII and Hyate:C in hemophilia A mice presensitized to human factor VIII. J Thromb Haemost 2004; 2: 605-611

Disclosures
All authors are full-time employees of Baxter Innovations GmbH.

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.

Baxter

DOI: 10.3252/pso.eu.WFH2014.2014