

# Octagen Corporation

## Recombinant Porcine FVIII

### OBI-1

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2004N-0033

TS15

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- Small start-up development company
- Partnership with Ipsen Ltd, a division of Beaufour-Ipsen, to develop OBI-1 and other FVIII products
- Ipsen Ltd manufactures porcine plasma-derived FVIII, Hyate:C
  - supplies severely constrained for several years

# OBI-1: Characterization

- Manufactured in serum-free medium using a well-characterized BHK cell line
- Expressed as a 170 kDa, glycosylated B-domain deleted heterodimer with a 24 – amino acid linker
- Cleaved intracellularly to a metal ion – linked heterodimer
- Specific activity ~ 12,500 U/mg by 1-stage clotting assay calibrated against NIBSC porcine standard

# OBI-1: Characterization

## Domains Mol.wt. Amino acids

Heavy Chain	A1, A2	84 kDa	740
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Light Chain	A3, C1, C2	75 kDa	684
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# OBI-1: Characterization

Glycosylation Sulfat.

N-linked O-linked Tyros. Disulfides

Heavy Chain	3	2	2	4
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Light Chain	2	1	5	5
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## Porcine FVIII Utility

- Porcine plasma-derived FVIII (Hyate:C) is used clinically to stop/prevent bleeding only when patient has anti-human FVIII inhibitor
- May contain different antigenic determinants than human FVIII contains

# OBI-1: Selected Preclinical Studies

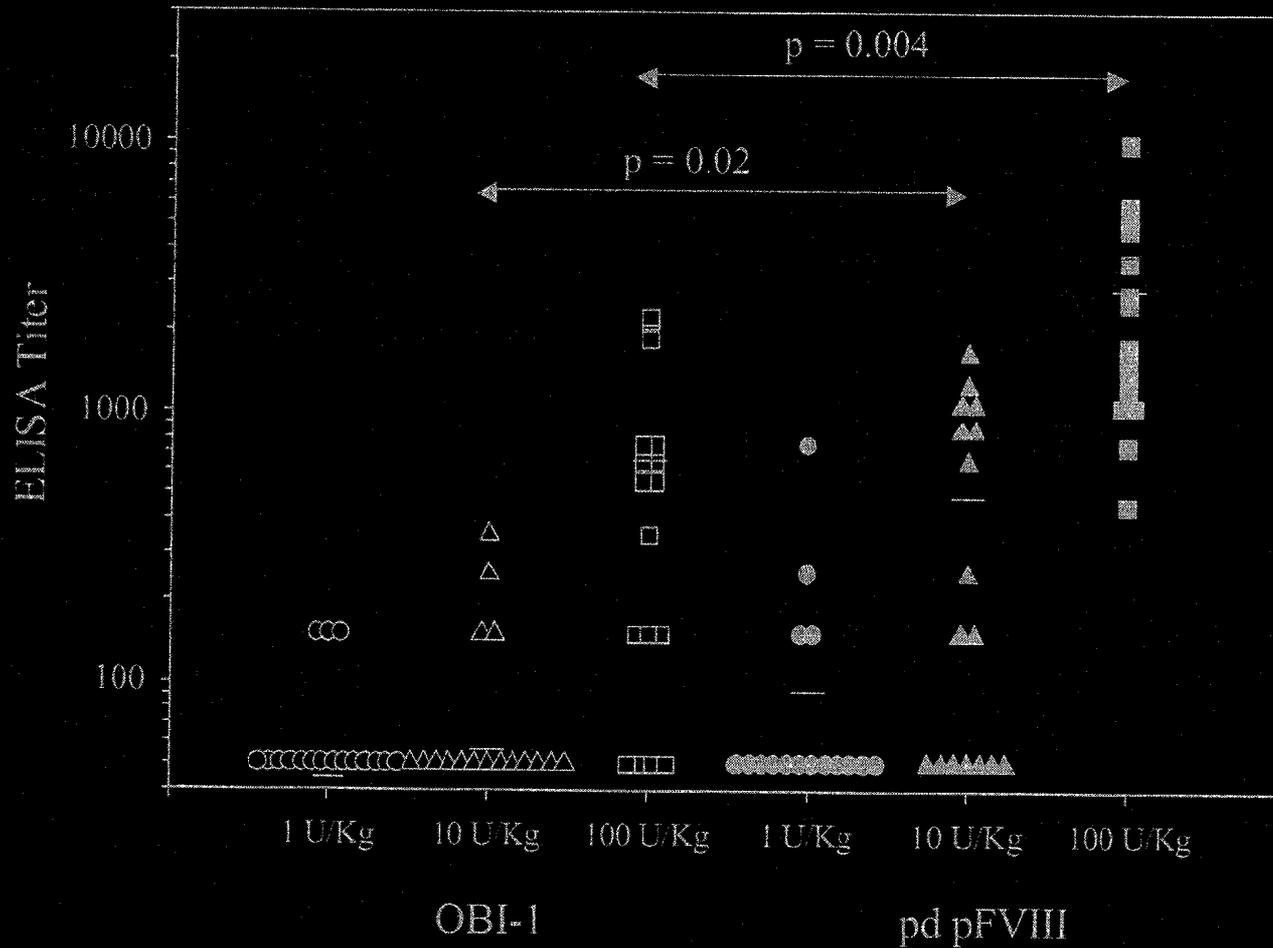
- Comparison of immunogenicity of Hyate:C VS OBI-1 in knock-out hemophilia A mice pre-sensitized to recombinant human
- Comparison of some potential indicators of immunogenicity, as part of a sub-acute toxicology study in cynomolgus monkeys

## Immunogenicity of OBI-1 and Hyate:C in Hemophilia A Mice

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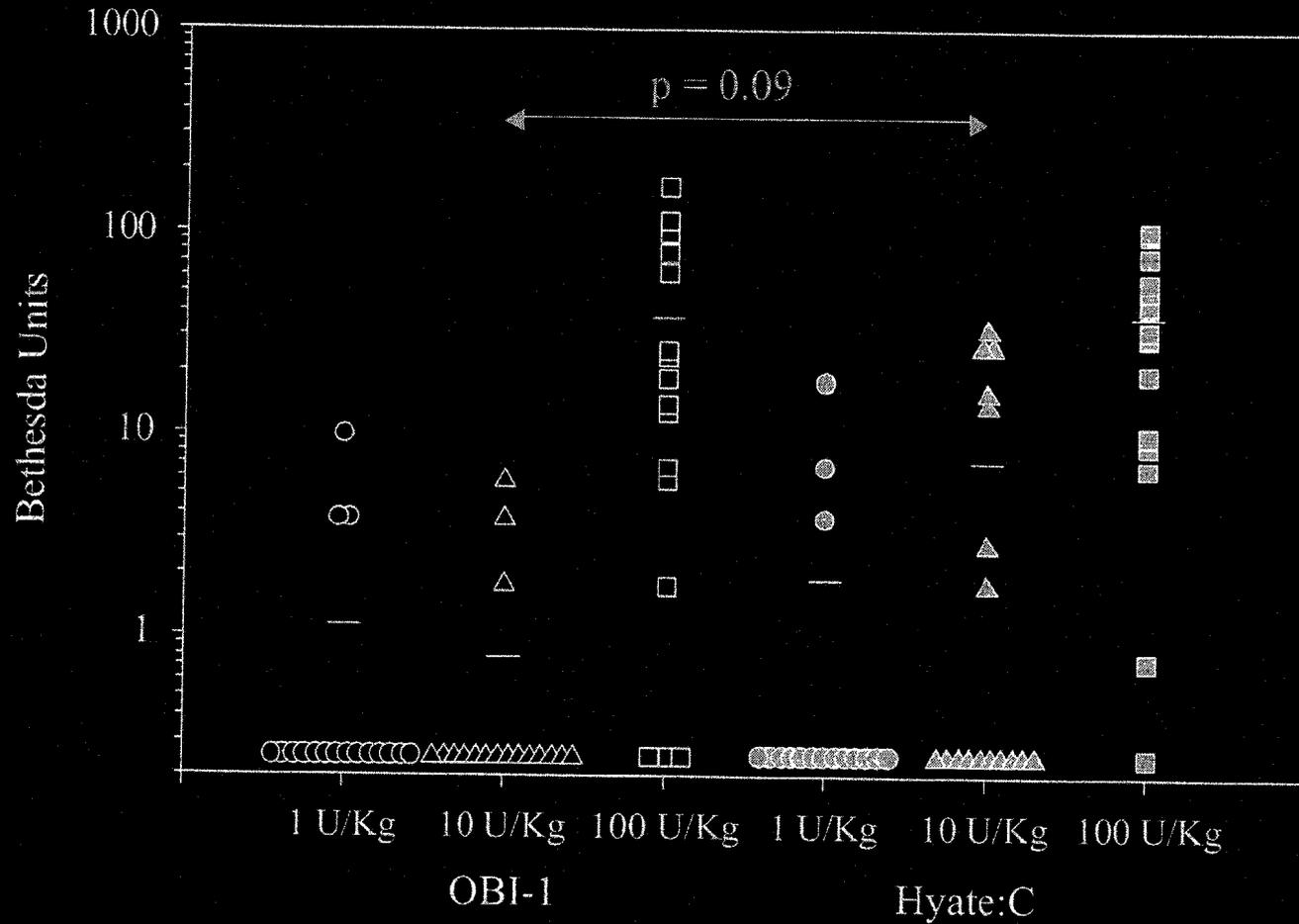
- Immune reactivity tested in Kazazian E16 fVIII knockout mice
- Mice were pre-sensitized with 100 U/kg recombinant human fVIII weekly x 5 to simulate clinical setting A (n=101)
- 6 groups (n = 16-17) received 1, 10, or 100 U/kg of Hyate:C or OBI-1 weekly x 4; test 2 weeks later
- Antibodies tested by ELISA and Bethesda assay

# Hemophilia A Mouse Model: ELISA



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# Hemophilia A Mouse Model: Bethesda assay



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# Hemophilia A mouse model: Summary

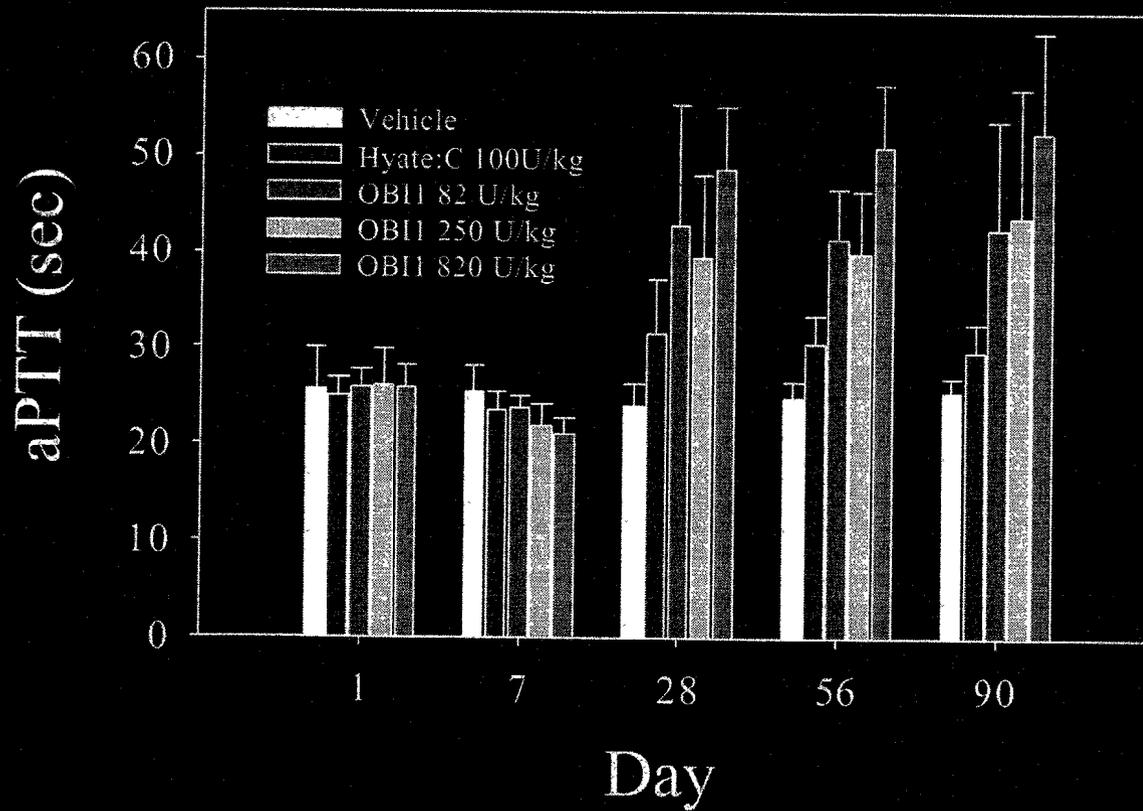
In knockout hemophilia A mice sensitized to human FVIII:

- Hyate:C generated greater non-specific and FVIII-specific IgG when tested by ELISA
- OBI-1 and Hyate:C show no difference in immunogenicity when tested by Bethesda assay testing for inhibitor formation

# Monkey Toxicology Study

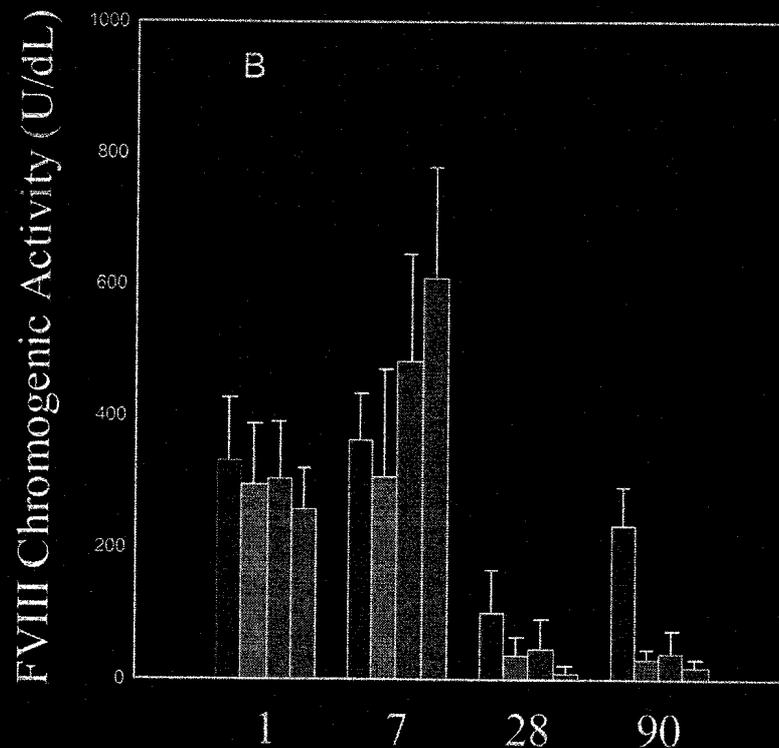
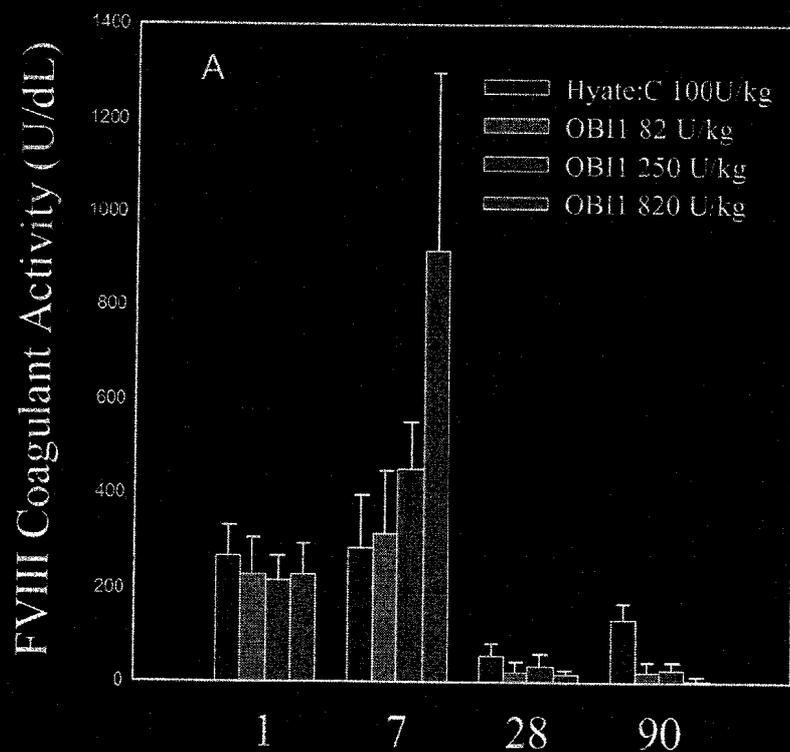
- Groups of cynomolgus monkeys were given daily doses of Hyate:C 100 U/kg or OBI-1 (82, 250 or 825 U/kg)
- Necropsy performed on Days 7, 28 and 90
- Blood samples at Days 7, 28, 56, and 90:
  - Pre-infusion aPTT values, FVIII levels
  - FVIII recovery values: 1 and 6 hr post-infusion
  - Inhibitors: qualitative and inhibitor titers

# Monkey Toxicology Study: Preinfusion aPTTs



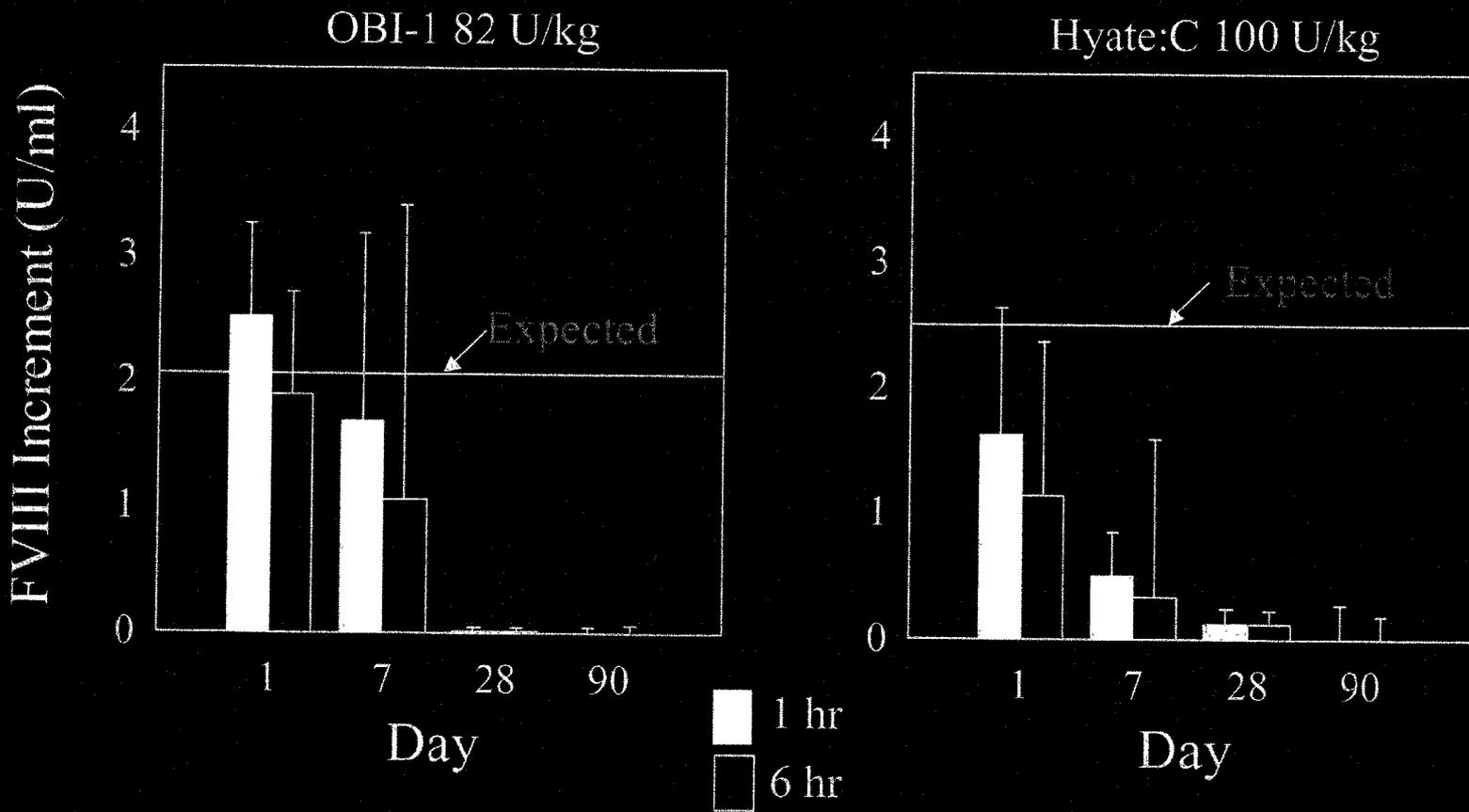
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# Monkey Toxicology Study: Pre-infusion Baseline FVIII Levels



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# Post-Infusion Recovery of OBI-1 and Hyate:C in Cynomolgus Monkeys



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## Immunogenicity of OBI-1 and Hyate:C in Cynomolgus Monkeys

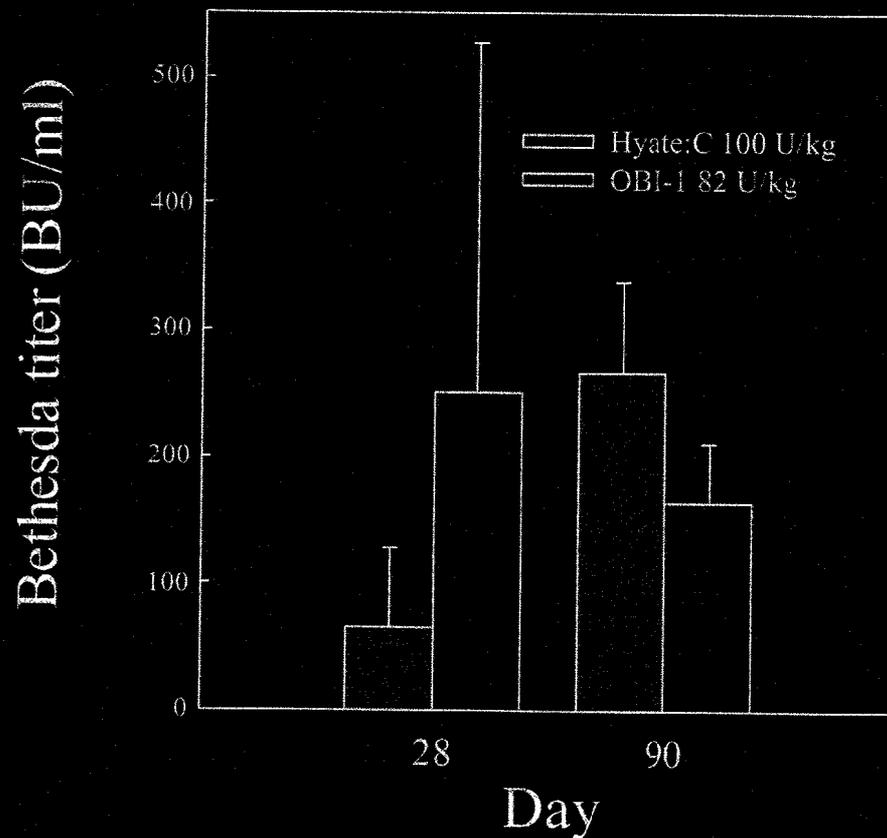
- Baseline FVIII levels and clinical findings consistent with “acquired hemophilia” suggest that cross-reactive anti-monkey FVIII inhibitors developed in BOTH groups
- Anti-monkey FVIII inhibitors at Day 28
  - 0/6 in Hyate:C group
  - 3/6 in OBI-1 group (0.5 – 2.7 BU/ml)

# Immunogenicity of OBI-1 and Hyate:C in Cynomolgus Monkeys

Qualitative screen for anti-porcine FVIII inhibitor

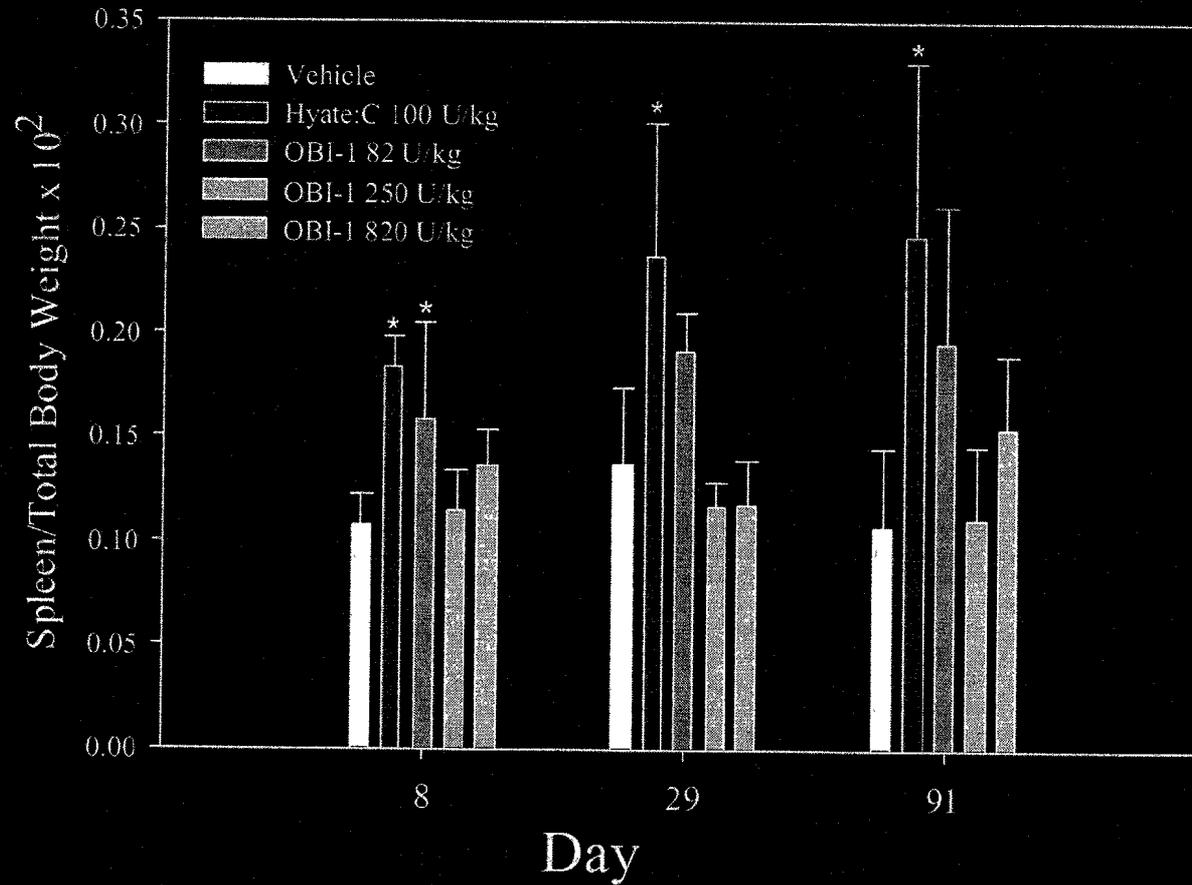
<u>Day</u>	<u>Hyate:C</u> 100U/kg	<u>OBI-1</u> 82U/kg
0	0/12	0/12
7	1/12	0/12
28	8/8	8/8
90	4/4	4/4

# Immunogenicity of OBI-1 and Hyate:C in Cynomolgus Monkeys: Anti-Porcine FVIII Inhibitor Titers



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# Splenomegaly in Hyate:C-Treated Monkeys



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## Immunogenicity of OBI-1 and Hyate:C in Cynomolgus Monkeys

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- Day 1 incremental recovery value was almost three-fold greater for OBI-1 compared to Hyate:C (2.94 vs 1.04)
  - Was immunogenic dose comparable?
- Hyate:C Monkeys developed splenic lymphoid hyperplasia
  - Non-specific immune responses due to contaminating porcine plasma proteins?
- Cross-reactive anti-monkey FVIII inhibitors apparently developed in BOTH groups
  - Joint and soft tissue bleeding, venepuncture bruising
  - Acquired hemophilia-like findings

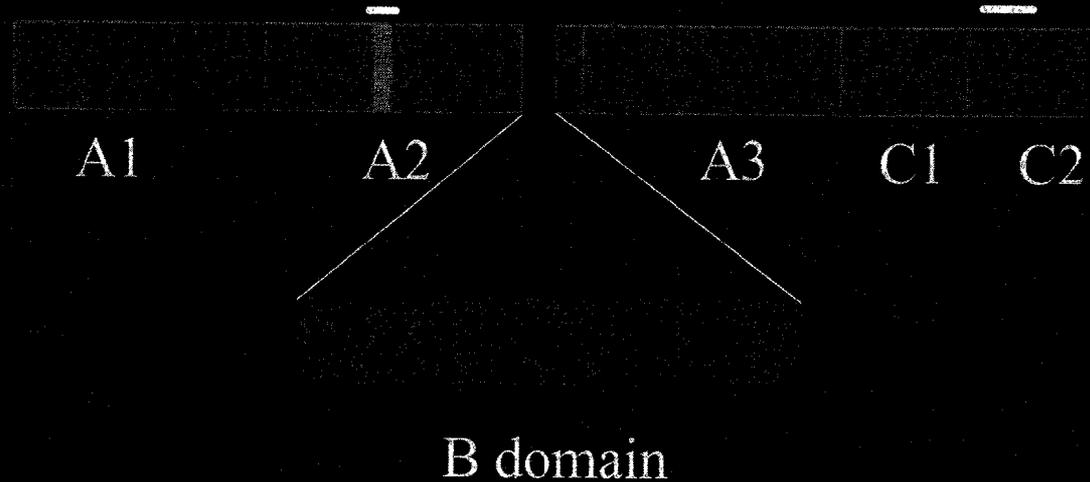
# Summary

- The immune response in cynomolgus monkeys to OBI-1 appeared greater than to Hyate:C
  - Increased intrinsic immunogenicity
  - Increased bioavailability
  - Decreased immunosuppression
- The immune response to OBI-1 and Hyate:C in hemophilia A mice sensitized to human FVIII
  - Was not significantly different by Bethesda assay
  - Was greater in Hyate:C mice by ELISA

# Reducing the Immunogenicity of Human FVIII

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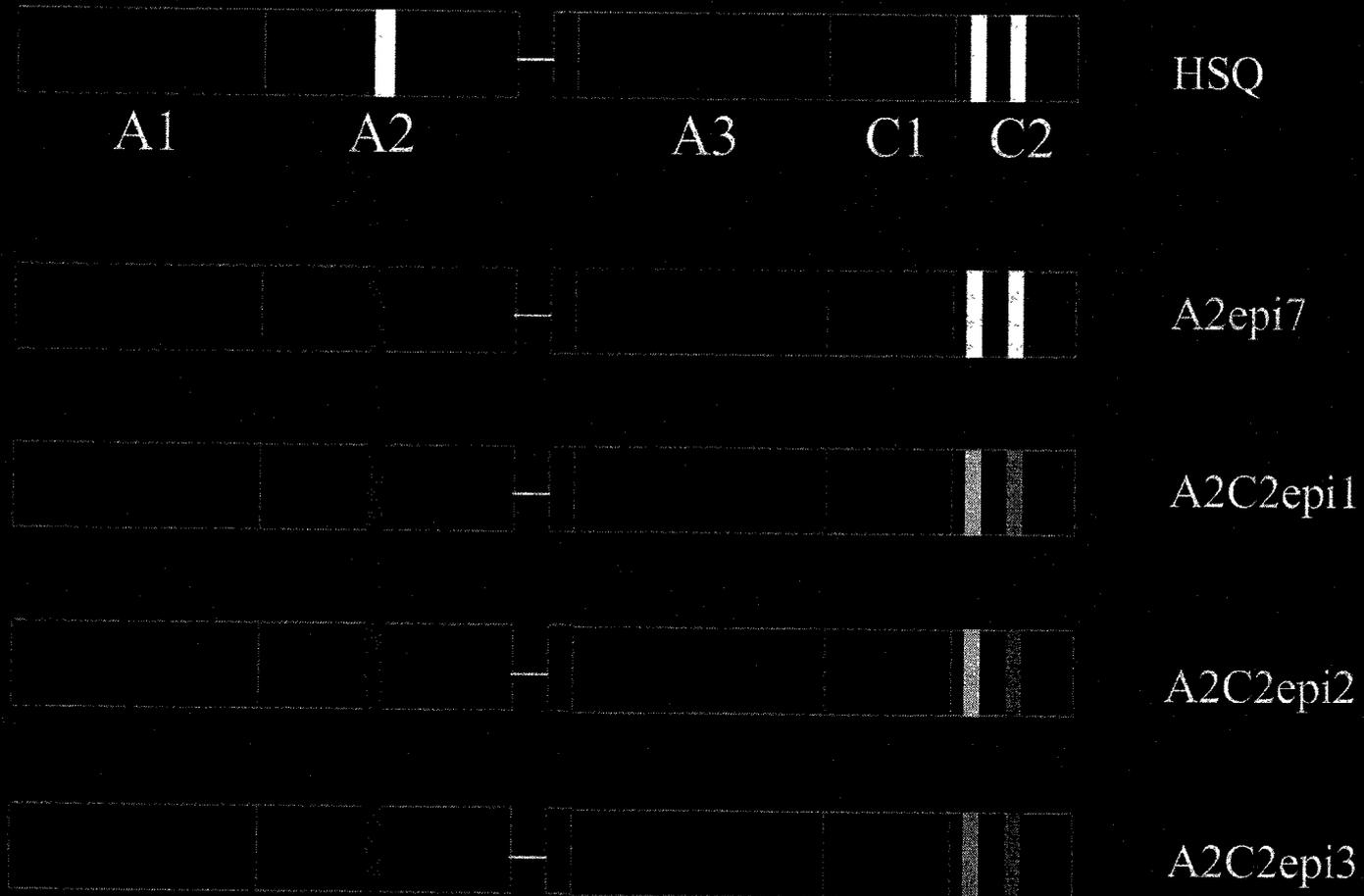
# Major Factor VIII Inhibitory Epitopes



Hypothesis:

Mutagenesis of antibody epitopes can reduce the immunogenicity of human factor VIII

# Candidate Low Immunogenicity FVIII Constructs



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# Immunogenicity of HSQ and FVIII Mutants in Hemophilia A Mice

