Bispecific IgG Antibody against FIXa and FX to Treat Hemophilia A

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Chugai Pharmaceutical Co., Ltd.
Manabu Wada, Ph.D
ACE910: Humanized anti-FIXa / anti-FX bispecific IgG4 antibody

- Background and concept of ACE910
- Identification of lead bispecific antibody
- Multidimensional optimization of the lead bispecific antibody
- Profile of clinical candidate ACE910
Hemophilia A; bleeding disorder by genetic dysfunction of coagulation factor VIII (FVIII)

<table>
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<th>FVIII:C</th>
<th>Annualized bleeding episodes (without prophylactic treatment)</th>
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<tr>
<td>Severe</td>
<td>&lt;1%</td>
<td>30 - 40</td>
<td>Half of patients are severe with significant decrease of QOL</td>
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<tr>
<td>Moderate</td>
<td>1~5%</td>
<td>5 - 6</td>
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<tr>
<td>Mild</td>
<td>5~40%</td>
<td>1 - 2</td>
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- Current therapy: **routine supplementation of FVIII agent**
- Issues in FVIII supplemental therapy for hemophilia A
  - Intravenous injections (very low bioavailability after sc injection)
  - Frequent injections (half life 12 hours, 2~3 times every week)
  - Development of FVIII inhibitors (anti-FVIII alloantibodies)
Concept of ACE910; FVIII mimicking bispecific antibody

- Coagulation factor VIII (FVIII)

Factor VIII (FVIII)

- Anti-FIXa and FX bispecific IgG antibody

Mimics cofactor function of FVIII

Bispecific antibody
Concept of ACE910; FVIII mimicking bispecific antibody

- Overcoming the issues of FVIII therapy by bispecific antibody
  - Long half life (infrequent dosing)
  - Subcutaneous injection
  - Not affected by FVIII inhibitors
  - Not induce FVIII inhibitors

Anti-FIXa and FX bispecific IgG antibody
Mimics cofactor function of FVIII

Immunizing each antigen independently, to obtain anti-FIXa and anti-FX antibodies. Screening of anti-FIXa/FX bispecific antibodies with FVIII-mimetic function.

Multidimensional optimization from a lead bispecific antibody
- Humanization
- Bispecific IgG antibody enabling engineering (ART-Ig technology)
- FVIII-mimetic activity
- Pharmacokinetic profile
- Immunogenicity
- Physicochemical property (stability, solubility etc)

Clinical candidate
ACE910 (recombinant humanized anti-FIXa/FX bispecific IgG4 antibody)
- Antibody mimicking Coagulation factor VIII (Eight) to factors IXa (9a) & X (10)

ART-Ig enables large scale production of asymmetric human IgG bispecific antibodies

Uncontrolled four chain expression produces 10 random antibody combinations.

ART-Ig; Three protein engineering technologies to facilitate manufacturing of BiAb

- Common light chain by FR/CDR shuffling
- Isoelectric point (pl) engineering: Decrease pl, Increase pl
- Preferential heavy chain heterodimerization by proprietary mutation

Ten H/L combinations

ART-Ig: Asymmetric Re-engineering Technology Immunoglobulin

IEC purification of target BiAb

Improve expression efficiency of BiAb

Antibody charge

-- +/- ++
Screening of lead bispecific antibody from large number of bispecific combinations

Derived from immunized mouse, rat etc

Anti-FIXa antibodies

~200 clones

Fused with human constant κ or λ

Fused with human IgG2 or IgG4

Fc heterodimerization engineering

FIXa binding region

>40,000 clones

Anti-FX antibodies

~200 clones

Fused with human constant κ or λ

Fused with human IgG2 or IgG4

FX binding region

Bispecific antibodies against FIXa and FX

FVIII mimetic activity was screened by chromogenic assay using the mixture of BiAb and other side-products
ART-Ig (1); identification of common light chain by FR/CDR shuffling of two different light chains

Bispecific antibody with two heavy chains and two light chains

Generate CDR shuffled light chain variants

Three CDRs from each light chain

Screen by functional activity

Bispecific antibody with common light chain

Chimeric of CDRs and FRs derived from both light chains
Process of identification of lead bispecific antibody with common light chain in ACE910

Number of evaluated clones

>40,000 clones → 188 clones → 7 clones → 24 clones

Identification of lead common light chain by FR/CDR shuffling in ACE910

Generate 24 FR/CDR shuffled variants

Screening by FVIII-mimetic activity

BS15L

Common light chain of lead antibody BS15

24 FR/CDR shuffled variants

3 CDRs and 4 FRs from each light chain

c1L

c2L

c3L

Sequences of CDR1 and CDR2 of c3L are identical to those of c2L, respectively.
ART-Ig (2); hetero/homodimer separation by isoelectric point (pI) engineering

- Lead antibody
- + variable region optimization

Engineered each heavy chain variable region to have different pI

No separation by ion exchange chromatography (IEC)

Complete separation of bispecific antibody

pI engineering enabled purification of bispecific antibody by IEC.
pI engineering of bispecific antibody and purification by CIE in ACE910

Optimization by pI engineering

Cation exchange chromatography of hBS560

BiAb heterodimer

Low pl Hch homodimer

High pl Hch homodimer

SP Sepharose Fast Flow, Stepwise elution
ART-Ig (3); Fc heterodimerization engineering for increasing production efficiency

Variable region optimized antibody

~25%

~50%

~25%

~90%

~5%

~5%

Only 50% of the whole antibody is bispecific antibody

Engineered CH3/CH3 interface to promote heterodimerization by controlled electrostatic interaction

Highly selective (~90%) bispecific production

+ proprietary CH3/CH3 interface mutation

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Optimization to improve pharmacokinetics by lowering the isoelectric point

Low pl antibody has smaller clearance (Protein Eng Des Sel, 2010, Igawa T. et al.)

PK improving variable region engineering
- Removing positive charge cluster
- Lowering pl

Pharmacokinetics of bispecific antibodies in mice

Positive charge cluster of activity enhanced variant hBS106
Optimization to increase solubility to improve manufacturability of bispecific antibody

- Removing hydrophobic residues from the surface
- Changing the surface charge distribution

hBS376 showed precipitation or liquid-liquid phase separation at below 100mM NaCl

Issue for purification process development and high concentration formulation

ACE910 (5 mg/mL)
Optimization to eliminate deamidation site in CDR to enable liquid formulation

Cation exchange chromatography analysis (initial, 40°C for 2 weeks)

hBS560

HN

HCDR3 -HN----- (deamidation)

hBS660

RQ

HCDR3 -HQ----- (no deamidation but loss of bioactivity)

HCDR3 -RQ----- (no deamidation and maintain bioactivity)
Evaluation of potential immunogenicity of clinical candidate ACE910

- During the optimization process, each mutation was checked by *in silico* immunogenicity prediction system not to increase T cell epitope (deimmunization)

- *In silico* immunogenicity evaluation of ACE910

  ![Graph showing immunogenic risk scores for various proteins](image)

  - Epibase (Lonza)
  - Epimatrix (Epivax)

  - ACE910

- *In vitro* helper T cell proliferation assay showed ACE910 to be non-immunogenic
Lead optimization process from common light chain lead antibody BS15 to clinical candidate ACE910

Multidimensional optimization

Common light chain lead antibody

Clinical candidate

ACE910
In vitro enzymatic activity of ACE910 requires bispecific binding to FIXα and FX

Simultaneous binding to both FIXα and FX is required for FVIII-mimetic activity

Requirement of FIXα and phospholipid for FVIII-mimetic activity (data not shown)
ACE910 has strong *in vivo* hemostatic activity in cynomolgus monkey model

ACE910 (n=4) 0.3, 1, 3 mg/kg IV bolus

rpoFVIII* (n=4) 10 U/kg IV BID

* rpoFVIII: recombinant porcine FVIII (not cross with anti-cFVIII)

Vehicle (n=6) Non-treated

- ACE910 reduced bleeding progression and showed strong hemostatic activity *in vivo*
- The hemostatic effect was comparable to the repeated doses of rpoFVIII 10 U/kg BID

Change of hemoglobin level (hemorrhagic anemia)

Mean ± SE

*: $P < 0.05$ (Day 3)
Pharmacokinetics of clinical candidate ACE910 and sc formulation developability

Pharmacokinetics of ACE910 after iv and sc injection in cynomolgus monkey

- T\(_{1/2}\) of ACE910 was ~3 weeks at any of the doses (linear PK)
- SC bioavailability was calculated as nearly 100%
  (FVIII agent T\(_{1/2}\) is 12 hours and SC bioavailability is very low)

High concentration subcutaneous formulation developability
- Concentration up to 200mg/mL was feasible
- Stable 150 mg/mL liquid formulation with low viscosity was achieved
  (FVIII agents are intravenous injection with lyophilized formulation)
ACE910 has strong *in vitro* coagulation activity even in the presence of anti-FVIII inhibitors

Thrombin generation assay in FVIII-deficient human plasma with or without FVIII inhibitor

- ACE910 showed dose dependent coagulation activity in thrombin generation assay in FVIII-deficient plasma
- ACE910 but not FVIII demonstrated strong coagulation activity even in the presence of FVIII inhibitors
- Anti-ACE910 idiotype antibodies did not inhibit FVIII mimetic activity (data not shown)

ACE910 is expected to be effective for hemophilia A patients with inhibitors
Manufacturing of ACE910 for clinical study

- 2,500 liter scale GMP manufacturing with standard IgG production platform
  - CHO cell fermentation, protein A and CIEX and AIXEX purification
  - Antibody productivity (or titer) similar to conventional monoclonal IgGs
  - Comparable purification process recovery as conventional monoclonal IgGs

- High purity bispecific IgG antibody was recovered
  - Lot-to-lot consistency confirmed between multiple GMP manufacturing lots

- First asymmetric bispecific human IgG4 antibody to enter into clinic (2012)
Clinical development of ACE910

- **Phase 1**
  Phase 1 study of ACE910 in healthy volunteers and hemophilia A

  To evaluate the tolerability, safety, PK and PD profiles in healthy adult male volunteers and severe hemophilia A patients with and without inhibitors

- **Phase 1/2**
  Extension study of the Phase 1 study of ACE910 in hemophilia A patients

  To evaluate the long-term safety and additionally the inhibitory effect on bleeding for exploratory purpose in severe hemophilia A patients with and without inhibitors

* The latest result of clinical study will be presented in coming American Society of Hematology annual meeting (ASH 2014) on this December 6-9 at San Francisco.
Summary
Anti-FIXa/FX bispecific antibody ACE910 for hemophilia A

- Lead antibody screened from more than 40,000 bispecific antibodies
- Asymmetric human bispecific IgG antibody GMP manufacturing enabled by
  - FR/CDR shuffling to identify of common light chain
  - pI engineering for ion exchange chromatography purification
  - CH3 heterodimerization mutation to improve productivity
- Therapeutic potential of the lead antibody enhanced by optimizing
  - FVIII-mimetic activity
  - pharmacokinetics
  - immunogenicity
  - solubility and stability
- Clinical candidate ACE910 demonstrated
  - strong coagulation activity *in vitro* even in the presence of FVIII inhibitors
  - strong hemostatic activity, long half life and high bioavailability in monkey
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ACE910 research project team member

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