The Hemophilia Pipeline

IMPROVING CONVENIENCE, SAFETY AND EFFICACY: DESIGN AND DEVELOPMENT OF NOVEL AGENTS

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Aims of the Hemophilia Pipeline

- Further improve on patient outcomes
  - Better bleeding control
  - Better preservation of joint function
- Reduce the burden of administration
  - Reduce dosing frequency
  - Reduce cost of therapy
- Individualize treatment regimens
  - Adapt to individual pharmacokinetics
  - Individualize treatment regimens
    - Active vs sedentary lifestyles
    - Variability in clinical phenotypes
Hemophilia Clinical Trial Pipeline

**Hemophilia A**
- Octagenate – rFVIII (Octapharma)
- Kogenate PF – rFVIII (Bayer)
- GreenGene F – rFVIII (Green Cross)
- rFVIII:Fc (Biogen Idec)
- BAY94-9027 – PEGylated rFVIII (Bayer)
- BAY855 – PEGylated rFVIII (Bayer)
- CSL627 – SingleChain rFVIII (CSL)
- IB1001 – rFIX (CanGene)
- BAX326 – rFIX (Baxter)
- rFIX:Fc (Biogen Idec)
- CSL654 – rFIX:albumin fusion (CSL)

**Hemophilia B**
- BAX326 – rFIX (Baxter)
- rFIX:Fc (Biogen Idec)
- CSL654 – rFIX:albumin fusion (CSL)

**Cross-Segment**
- MC710 – pdFVIIa + pdFX (Kaketsuken)
- ACE910 – SC bispecific Ab (Chugai)
- siRNA vs Antithrombin (Alnylam)

**Hemophilia With Inhib**
- BAX817 – rVIIa (Baxter)
- Transgenic rhFVIIa (LFB)
- OBI-1 – rpFVIII (Baxter)
- CB813d – rVIIa analogue (Pfizer)
- CSL689 – rVIIa:albumin fusion (CSL)
- rVIIa:CTP (Prolor Biotech)
- CSL669 – rVIIa:albumin fusion (CSL)

- “me too”
- Half life extension
Whatever happened to...

- Fucoidans
- Anti-TFPI aptamer
- rVIIa analogue “fast acting”
- rVIIa-glycoPEGylated “longer lasting”
- Bayer/Maxygen rVIIa
- Inspiration Biopharmaceuticals
BAX499 (Baxter) Aptamer inhibitor of TFPI

- Phase I clinical trial prematurely stopped due to increased bleeding tendency in hemophilia patients
- Associated with an unexpected increased (>25-fold) in plasma TFPI levels
  - BAX499 releases intracellularly-stored TFPI, impacts its metabolism and prolongs the circulatory half-life based on where BAX499 binds to TFPI
- BAX499 unable to compensate for the increased TFPI levels
rVIIa analogue (Vatreptocog alfa)

- 3 point mutation introduced to stabilize the active form of FVIIa
- similar to the structural transition induced by tissue factor
- 30-fold more effective activation of FX
rVIIa analogue (Vatreptocog alfa)

Table 3 Treatment efficacy as evaluated by number of doses of trial product administered to control bleeds within 9 h from initial dose

<table>
<thead>
<tr>
<th>Treatment effect</th>
<th>Vatreptocog alfa, N (%)</th>
<th>rFVIIa, N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 µg kg⁻¹</td>
<td>10 µg kg⁻¹</td>
</tr>
<tr>
<td>Total bleeds</td>
<td>15 (80.0)</td>
<td>19</td>
</tr>
<tr>
<td>Treatment successes*</td>
<td>12 (80.0)</td>
<td>16 (84.2)</td>
</tr>
<tr>
<td>Bleeds controlled with a single dose</td>
<td>3 (20.0)</td>
<td>3 (15.8)</td>
</tr>
<tr>
<td>Bleeds controlled with two doses</td>
<td>5 (33.3)</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td>Bleeds controlled with three doses</td>
<td>4 (26.7)</td>
<td>4 (21.1)</td>
</tr>
</tbody>
</table>

rFVIIa, recombinant FVIIa. *Bleeds successfully controlled with one to three doses of trial product.
Novo Nordisk discontinues development of vatreptacog alfa following analysis of phase 3 results

Novo Nordisk today announced the decision to discontinue the development of vatreptacog alfa, a fast-acting recombinant factor VIIa analogue for haemophilia patients with inhibitors. The decision follows analysis of the data from the phase 3a trial adept™ 2. On 9 August, Novo Nordisk announced that a few patients in the trial had developed anti-drug antibodies to vatreptacog alfa, one patient with a potentially neutralising effect.

Company Announcement, Sept 28, 2012
BAY86-6150 (Bayer)
Bioengineered rVIIa

• Key limitation of rVIIa is low affinity for platelet membranes
• GLA domain binds phospholipid (PL) membrane
• DNA shuffling of VIIa GLA domain with those of other GLA-domain proteins with strong PL binding
  – 5 amino acid substitutions resulted in 150-fold increase in membrane affinity and 40-fold gain in procoagulant activity
• Additional modifications to extend half-life through introduction of novel sugar conjugates
• Clinical safety and efficacy evaluated in the TRUST clinical trial program

Biochem. J. (2011) 435, 1–16
BAY86-6150 (Bayer) Bioengineered rVIIa

Bayer Provides Update on Phase II/III Trial of BAY 86-6150

Leverkusen, Germany, May 3, 2013 – Bayer HealthCare today announced that a Phase II/III trial evaluating the efficacy and safety of BAY 86-6150 in people with hemophilia A and hemophilia B with inhibitors has been discontinued. The hope that BAY 86-6150 might help patients with inhibitors to achieve better control of their disease could not be fulfilled due to the detection of a neutralizing antibody in the trial.
rFIX

Cambridge, Mass. (USA), July 10, 2012 – Inspiration Biopharmaceuticals, Inc. (Inspiration) today announced that the U.S. Food and Drug Administration (FDA) has notified the Company that the agency has placed a clinical hold on clinical trials evaluating the safety and efficacy of IB1001, an intravenous recombinant factor IX (rFIX) being investigated for the treatment and prevention of bleeding episodes in people with hemophilia B.

The clinical hold impacts two ongoing IB1001 clinical trials – a phase 3 study evaluating the safety and efficacy of IB1001 to treat and prevent bleeding episodes in adults with hemophilia B, and a phase 3/3b study evaluating the safety and efficacy of IB1001 to treat and prevent bleeding episodes in previously treated pediatric subjects with hemophilia B. The adult study has completed its primary analysis period.

Inspiration recently reported to the FDA that, during the course of routine laboratory evaluations conducted as part of an ongoing phase 3 clinical trial, the Company discovered that a higher proportion of individuals treated with IB1001 have developed antibodies to proteins from the Chinese hamster ovary, or CHO, host cells used to manufacture the therapy than was expected based on earlier study data. Inspiration has notified clinical sites in the U.S. to hold treatment of patients with IB1001. Inspiration is also sharing the FDA directive with regulators in countries outside of the U.S. where the studies are being conducted.
Strategies to Extend the Half Life of Recombinant Clotting Factors

- Reduce interaction with clearance receptors
  - PEGylation

- Rescue endocytosed proteins from intracellular degradation pathways through interaction with the neonatal Fc receptor (FcRn)
  - Fc fusion proteins
  - Albumin fusion proteins

- Enhanced interaction with Von Willebrand Factor
  - Single chain factor VIII variants
Half-life Extension of Biologics
<table>
<thead>
<tr>
<th>Name</th>
<th>Indication</th>
<th>Administration</th>
<th>Approval Year</th>
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<tbody>
<tr>
<td>Peginesatide</td>
<td>Anemia</td>
<td>Monthly</td>
<td>2012</td>
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<tr>
<td>Pegloticase</td>
<td>Gout</td>
<td>Q2-4 weeks</td>
<td>2010</td>
</tr>
<tr>
<td>Certolizumab pegol</td>
<td>RA/Crohn’s</td>
<td>Q2 weeks</td>
<td>2008</td>
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<tr>
<td>Pegaptanib</td>
<td>Macular degeneration</td>
<td>Q6 weeks, intravitreal</td>
<td>2004</td>
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<td>Pegfilgrastim</td>
<td>Neutropenia</td>
<td>Single dose per course</td>
<td>2002</td>
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<td>Pegvisomant</td>
<td>Acromegaly</td>
<td>Daily</td>
<td>2002</td>
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<tr>
<td>PEGylated Interferon 2a</td>
<td>Chronic hepatitis</td>
<td>Weekly</td>
<td>2001</td>
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<tr>
<td>PEGliposomal doxorubicin</td>
<td>Chemotherapy</td>
<td>Single dose</td>
<td>2001</td>
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<tr>
<td>PEGylated Interferon 2a</td>
<td>Chronic hepatitis</td>
<td>Weekly</td>
<td>2000</td>
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<tr>
<td>Pegaspargase</td>
<td>Acute leukemia</td>
<td>Single dose</td>
<td>1994</td>
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<tr>
<td>PEG ADA</td>
<td>SCID</td>
<td>Weekly</td>
<td>1990</td>
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</table>
FVIII

FVIII-PEG

Extended half-life

Degradation

Excretion?

Association with vWF

Hemostatic challenge

vWF Dissociation + Procoagulant Function

Clearance in Liver

Extended $t_{1/2}$

Fecal

Urinary

PEG Excretion?

Fig. 12
Pharmacokinetics of BAX 855 in macaques – repeated dosing

Turecek, Hamostaseologie (2012) 32(s1):S29-S38
Phase I Study of BAY94-9027

- 14 subjects with severe Hem A 21-58 y.o.
- 25 IU/kg 2x/wk and 60 IU/kg 1x/wk
- 19 h half-life vs 13 h for rFVIII-FS
- No treatment-related serious adverse events including no inhibitors or antibodies directed against PEG or BAY94-9207
Glycopegylated FIX: first human dose trial in Hem B

- 16 men with severe hem B
- Half-life of 93 h – 5x rFIX
- Better plasma recovery than rFIX

Ostergaard, Blood, 2011

Negrier, Blood, 2011
Pharmacokinetic Modeling of glycoPEGylated FIX: Prophylaxis

Fig. 3. The steady-state predicted profiles for N9-GP dose regimens of 10 (light pink) and 40 (dark pink) U kg\(^{-1}\) once-weekly vs. standard FIX dose regimens of 40 IU kg\(^{-1}\) rFIX (blue) and pdFIX (green) every 3 days. Blue horizontal dashed line, 3 IU dL\(^{-1}\) or U dL\(^{-1}\) FIX activity; dashed lines, mean predicted values; shaded regions, 95% prediction intervals.
Pharmacokinetic Modeling of glycoPEGylated FIX: On-demand

Collins, JTH (2012) 10:2305-12
Preclinical Safety with Other PEGylated Therapeutics

- Organ-specific vacuolation observed in some animal studies
  - Findings are not consistent
    - PEG-20kDa TNF-binding protein administered by chronic dosing to rats yielded kidney vacuolation
    - Same protein conjugated with PEG-50kDa showed minimal or no effects
    - In both cases, no changes in kidney function
  - PEGylated hemoglobin administered to monkeys induced vacuolation in liver, and renal tubules and macrophages (in bone marrow, spleen and lymph nodes) when at high dose (ie. replacing 30% of blood volume)
    - Changes were dose-dependent, transient and without toxic effects

Ivens, Haemophilia (2013) 19:11-20
In vivo assessment of rFVIII-PEG60kDa

• Single high dose acute toxicity study in rats
  • Up to 210 mg/kg PEG-60
  • No adverse effects including by light microscopy of all organs and tissues

• Repeat administration study in rats
  • Up to 11 mg/kg PEG-60 administered every other day for 4 weeks
    • Equivalent to estimated total cumulative lifetime dose if used in humans
    • No adverse effects including by light microscopy of all organs and tissues

• BAY94-9027 preclinical program
  • No vacuolation of any organ or tissue
PEG Doses and PEG-related Cellular Changes

Ivens, Haemophilia (2013) 19:11-20
Safety with PEGylated Coagulation Factors

- GlycoPEGylated rFVIIa (N7-GP)
  - No serious adverse events
    - Including no thromboembolic events and no neutralizing antibodies

- GlycoPEGylated rFIX (N9-GP)
  - No safety findings identified in the toxicology program
  - Treatment-associated adverse events in 6 of 16 patients
    - 10 events in 5 patients rated as moderate or mild
      - 3 rated as probably or possibly related to N9-GP
        - Fatigue, myalgia
      - 1 serious adverse event
        - Hypersensitivity reaction in a 25 year old, not associated with an antibody response, fully resolved within 8 hrs of onset with supportive care and antihistamines; patient resumed therapy with pdFIX

- PEG has been present in some plasma-derived clotting factors used for decades

# Evaluating the Risks

Tab. 3

<table>
<thead>
<tr>
<th>study</th>
<th>species</th>
</tr>
</thead>
<tbody>
<tr>
<td>tail tip bleeding</td>
<td>FVIII ko mouse</td>
</tr>
<tr>
<td>carotid occlusion</td>
<td>FVIII ko mouse</td>
</tr>
<tr>
<td>thrombogenic potential</td>
<td>rabbit</td>
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<tr>
<td>cardiovascular effects (telemetry)</td>
<td>macaque</td>
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<tr>
<td>pharmacokinetics</td>
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<tr>
<td>single dose</td>
<td>FVIII ko mouse</td>
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<tr>
<td>two doses</td>
<td>macaque</td>
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<tr>
<td>ADME</td>
<td>rat</td>
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<tr>
<td>repeated dose toxicity</td>
<td>macaque</td>
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<tr>
<td>including TK</td>
<td>macaque</td>
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<td>comparative immunogenicity</td>
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<tr>
<td>in vitro</td>
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<tr>
<td>in vivo</td>
<td>human plasma</td>
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<tr>
<td></td>
<td>E17 FVIII k.o.</td>
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<tr>
<td></td>
<td>- human MHC-class II (HLADR15) transgenic mice</td>
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<tr>
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<td>- mice on a Balb/c background</td>
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<tr>
<td></td>
<td>- mice on a C57BL/6 background</td>
</tr>
<tr>
<td></td>
<td>- human FVIII transgenic mice</td>
</tr>
<tr>
<td></td>
<td>macaque</td>
</tr>
</tbody>
</table>
Summary

- PEGylation is a rational and proven strategy for half-life extension of biologics
- Modern chemistry applications have enabled more precision in achieving targeted conjugation to avoid unwanted effects on protein function
- PEGylated proteins have been administered clinically for more than 2 decades with an excellent safety experience
- Preclinical and clinical programs with PEGylated clotting factors have not demonstrated any toxicity
Advantages of Fc fusion technology

- Improved therapeutic half-life
- Relatively high expression in mammalian culture systems and ease of purification\(^1\)
- Fc-fusion proteins may confer tolerogenic properties on otherwise immunogenic therapeutic proteins\(^2\)
- Further bioengineering of the Fc portion can enhance FcRn interaction to increase circulating half-life
- Multiple approved indications for arthritis, psoriasis, transplant rejection, macular degeneration, and ITP
- Late phase clinical trials in hemophilia, SLE, glioblastoma, MDS, ovarian cancer and anemia

1. Ghose, 2006
First Human Clinical Trial with rFIXFc

- Phase I/IIa trial completed in 2010
- 14 adults (age range 18-76) with severe and moderate Hemophilia B
- No adverse events including no inhibitors
- 3- to 6-fold longer half-life (64h + 15h) compared to rFIX
- Greater recovery compared to rFIX

Shapiro, Blood, 2012;119(3):666-72
rFIX-Fc Phase III Results

- Weekly treatment, starting dose 50 IU/kg:
  - 2.95 bleeding episodes per year
- Dosing 100 IU/kg at variable (median 14d) intervals:
  - 1.38 episodes per year
- Dosing only after bleeding episodes began:
  - 17.69 episodes per year
  - 90.4% controlled with a single injection
- No serious adverse events including no inhibitors

Biogen Idec, press release, 9-26-12
rFVIIIFc

Figure 1. Schematic representation of rFVIIIFc monomer.

A and B: Graphs showing changes in antigen concentration and FVIII activity over time.
Phase I trial of rFVIIIFc

- 16 adult men with severe hemophilia A
- No serious adverse events including no inhibitors
- 1.5 to 1.7-fold longer half-life compared to rFVIII

Powell et al., BLOOD (2012)
rFVIII-Fc Phase III Trial Results

- 165 patients with severe hem A ≥12 y.o.
- 3 arms
  - Individualized prophylaxis (25-65 IU/kg, q3-5d)
    - Median dosing interval:
      - 3.5 days, 30% of patients on q5d last 3 months of the trial
      - 1.6 annualized bleed rate
  - Weekly prophylaxis (65 IU/kg)
    - 3.6 annualized bleed rate
  - On-demand
    - 98% of bleeds controlled with 1-2 infusions
    - 33.6 annualized bleed rate
- No inhibitors and no drug-related serious adverse events
• Albumin is a large, globular transport protein present in high concentrations in plasma with an extraordinary half-life (19 days)
  • Size prevents renal excretion
  • Takes advantage of FcRn-dependent recycling
• Unlikely to be recognized as a foreign molecule when administered as part of a fusion protein
• Number of albumin-fusion proteins in development for several potential applications (type-2 diabetes, neutropenia, and clotting factors)
Santagostino, BLOOD (2012)
FVIII and VWF

- Primary determinant of FVIII residence in plasma is association with VWF
  - Protects from proteolysis and cellular uptake
  - Half-life extended from ~2 hr to ~12 hr
- Pre-infusion VWF levels correlate with FVIII half-life
- FVIII clearance may be related to VWF clearance
- Dependence of bioengineered FVIII variants on VWF interaction continues to limit half-life extension strategies
- New directions:
  - FVIII variants with enhanced VWF affinity
  - Bioengineered VWF for extended half-life

Fischer, PLoS One 2009; 4:e6745
Enhanced VWF Affinity Through FVIII Bioengineering

- Heavy and light chains of FVIII held together by a labile metal ion bridge
  - Contributes to FVIII instability

- Recombinant single-chain FVIII with a covalent bond between heavy and light chains (rVIII-SingleChain)
  - Demonstrated increased affinity for VWF

- Hypothesis that rVIII-SingleChain clearance would more closely approximate the clearance of VWF (~1.5X longer than FVIII)

Schulte, Thromb Res, 2013
Long Acting Clotting Factors

- Prolonged half-life will yield:
  - Reduced infusions per week/month/year
  - Reduced overall factor consumption in units/year

- Prolonged half-life may yield:
  - Improved convenience and compliance
  - Improved QOL
  - Improved joint outcomes
  - Reduced reliance on CVLs
What’s Next?

- **Novel biologics in pre-clinical testing**
  - Bispecific antibody substitution for FVIII
  - Factor IX muteins as bypass therapy
  - Zymogen-like Factor Xa
  - Anti-TFPI antibody

- **Non-protein therapies**
  - Anti-TFPI
    - Natural – fucoidan
    - Synthetic – aptamers, small molecule inhibitors
  - Anti-protein C
  - Anti-antithrombin

\[
\text{Peptide inhibitors} \\
\text{siRNA}
\]