Gene Therapy for Haemophilia

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University College London
OCH Annual meeting 2019
Declaration of financial interests by Edward Tuddenham

• I am a named inventor of patents in the field of gene therapy for haemophilia held by UCL
• I am a consultant to Biomarin Inc.
• I am a consultant to Freeline Therapeutic Ltd.
AAV Transduction Pathway
**AAV Vectors: Gene Transfer**

<table>
<thead>
<tr>
<th>Particle radius</th>
<th>25nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td></td>
</tr>
<tr>
<td>Protein (74%)</td>
<td>$M_r \sim 3750$ kDa</td>
</tr>
<tr>
<td>DNA (25%)</td>
<td>$M_r \sim 1350$ kDa</td>
</tr>
<tr>
<td>Total virus</td>
<td>$M_r \sim 5100$ kDa</td>
</tr>
</tbody>
</table>

**Wild-type genome**

**Vector genome**

**AAV-2**
Spark SPK 8011  
AAVspk FVIII

**AAV-8**
Sangamo SB-525  
AAV6 FVIII

**AAV-1**
BioMarin BMN 270  
AAV5-FVIII SQ

**AAV-6**
Dimension DTX201  
AAV rh10 FVIII

**AAV-7**
UCL/St Jude  
AAV8-FVIIIv3

**AAV-5**
Shire SHP654  
AAV8 FVIII

**Liver**

**Skeletal Muscle**

**Heart**

Adapted from Arruda VR & Xiao W, JTH 2007
Rationale: Gene Therapy for Hemophilia

- Hemophilia is monogenic
- Wide range of FVIII, IX effective
- Tissue-specific expression not required
- Well-characterized animal models exist
- One-time treatment required for gene transfer
- Avoids cost, morbidity, complications of current therapy
- Potential global treatment for all affected
Aim: Hemophilia Gene Transfer

- **Sustain:** long-term expression
- **Achieve:**
  - *Past:* FVIII, IX ≥ 15%
  - *Current:* FVIII, IX > 50%
- **Convert:** severe to normal
- **Avoid:** all bleeds

*den Uijl et al, Haemophilia 2011*
Two trials that established gene therapy for haemophilia B
Manno et al 2006; Nathwani et al 2010

Successful transduction of liver in hemophilia by AAV-Factor IX and limitations

Ajan S, et al. Adenovirus-associated virus vector-mediated gene transfer in
[25]:2357-2365.
Longer term follow up published in 2014 is considered a ‘landmark study’

1. Data for up to 4 years
2. Consistent and durable response
3. No lasting adverse effects
4. Liver enzyme elevation controlled by steroid treatment
5. Bleeding rate reduced substantially
6. Factor usage reduced to near zero in 6 of 10 patients.
New study in haemophilia B using high activity factor IX Padua variant

- Pioneered successful trial with R338L FIX
- Range of mean steady-state FIX activity: 14.3-76.8%
- 2 transient transaminase elevations
- 3 received steroids
- Evidence of anti-capsid response
- No inhibitors
- Profound reduction in bleeding rate and factor utilization through 2 years
Freeline study with Padua Factor IX in rAAVS3 capsid dose $6 \times 10^{11}$ vg/kg
A possible cause of “transaminitis”
Immune barriers to successful transduction by AAV vectors in humans.
Rapid increase of investment and new trials using AAV 2014-2019

Table 1 A selection of ongoing AAV interventional clinical trials

<table>
<thead>
<tr>
<th>Primary gene delivery target</th>
<th>Condition</th>
<th>AAV capsid</th>
<th>Transgene product</th>
<th>Strategy</th>
<th>Sponsor</th>
<th>Phase</th>
<th>Clinical Trials.gov identifier</th>
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</thead>
<tbody>
<tr>
<td>Brain</td>
<td>AAV6/8</td>
<td>FIX</td>
<td>Replacement</td>
<td>FhA</td>
<td>Shire</td>
<td>Phase II</td>
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<td>AAV6/8</td>
<td>FIX</td>
<td>Replacement</td>
<td>FhA</td>
<td>Pfizer</td>
<td>Phase II</td>
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<td>FIX</td>
<td>Replacement</td>
<td>FhA</td>
<td>Pfizer</td>
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<td>FIX</td>
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<td>FhA</td>
<td>Sangamo</td>
<td>Phase I</td>
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<td>AAV6/8</td>
<td>FIX</td>
<td>Replacement</td>
<td>FhA</td>
<td>St. Jude Children's Research Hospital</td>
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<td>FhA</td>
<td>Singhealth</td>
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<td>FIX</td>
<td>Replacement</td>
<td>FhA</td>
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<td>Phase I</td>
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<td>AAV6/8</td>
<td>ZFN1, ZFN2 and IEXA donor</td>
<td>Editing</td>
<td>Sangamo</td>
<td>Phase II</td>
<td>NCT0272115</td>
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<td>Sangamo</td>
<td>Phase II</td>
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<td>NCT03612869</td>
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<td>ASB</td>
<td>Replacement</td>
<td>FhA</td>
<td>Fosanzena Telethon</td>
<td>Phase II</td>
<td>NCT03135521</td>
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</tbody>
</table>
BMN 270: AAV vector construct

- Due to packaging constraints, size of vector components minimized
- Efficient transduction of the liver by AAV5
- Liver specific promoter
- Active Factor VIII with B domain deletion of Refacto™
Clinical Findings:

- **Factor VIII Activity:** Mean peak 115-130%
- **Durability:** Maintained to > 44 weeks
- **ABR:** 91% reduction (No bleeds in 5/6)
- **FVIII Use:** 98% reduction (No FVIII in 5/6)
- **Immune response:** High-dose cohort - steroid responsive

**Conclusion:** Safe, effective, durable > 44 weeks
Steroid-responsive immune response
**MEAN FVIII ACTIVITY LEVELS SETTLING IN NORMAL RANGE (6e13 VG/KG)**

- No FVIII activity above upper limit of normal at year 2
MEAN FVIII ACTIVITY LEVELS AT HIGH END OF MILD RANGE (4e13 VG/KG)

- No FVIII activity above normal
3 year data from BioMarin study

3 Year Phase 2 6e13 vg/kg Data Demonstrates Durable Factor VIII Expression

Rate of FVIII decline continued to be expression level dependent, slowed in year 3, and appears to be approaching plateau.

<table>
<thead>
<tr>
<th>FVIII activity level (IU/dl) time point</th>
<th>Mean (Chromogenic)</th>
<th>Median (Chromogenic)</th>
<th>Mean (One Stage)</th>
<th>Median (One Stage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeks 23-26</td>
<td>68</td>
<td>57</td>
<td>127</td>
<td>100</td>
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<tr>
<td>Week 52</td>
<td>64</td>
<td>60</td>
<td>104</td>
<td>89</td>
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<tr>
<td>Week 104</td>
<td>36</td>
<td>26</td>
<td>59</td>
<td>46</td>
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<tr>
<td>Week 156</td>
<td>33</td>
<td>20</td>
<td>52</td>
<td>30</td>
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</table>

Three year results with Chromogenic Assay
## Durability: Hemophilia Gene Clinical Trials

<table>
<thead>
<tr>
<th>Factor IX</th>
<th>NCT</th>
<th>Vector/Gene</th>
<th>AAV-IR</th>
<th>Expression</th>
<th>Durability</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCL/St. Jude</td>
<td>NCT00979238</td>
<td>AAV2/8-LP1-hFIX&lt;sub&gt;co&lt;/sub&gt;</td>
<td>+ 5/10</td>
<td>2-8%</td>
<td>up to 8 years</td>
</tr>
<tr>
<td>Shire</td>
<td>NCT01687608</td>
<td>AAV8-sclIX</td>
<td>+ 2/7</td>
<td>0-25%</td>
<td>-</td>
</tr>
<tr>
<td>Spark</td>
<td>NCT02484092</td>
<td>AAV8(spk100)-hFIX</td>
<td>+</td>
<td>29-35%</td>
<td>up to 2 years</td>
</tr>
<tr>
<td>UniQure</td>
<td>NCT02396342</td>
<td>AAV5-hFIX</td>
<td>+ 1/5</td>
<td>5.2-6.9%</td>
<td>up to 2 years</td>
</tr>
<tr>
<td>Dimension</td>
<td>NCT02618915</td>
<td>AAVrh10-FIX</td>
<td></td>
<td>10-20%</td>
<td>up to 52 weeks</td>
</tr>
<tr>
<td>Sangamo</td>
<td>NCT02695160</td>
<td>AAV2/6-ZFN</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### Factor VIII

<table>
<thead>
<tr>
<th>NCT</th>
<th>Vector/Gene</th>
<th>AAV-IR</th>
<th>Expression</th>
<th>Durability</th>
</tr>
</thead>
<tbody>
<tr>
<td>BioMarin</td>
<td>NCT02576795</td>
<td>AAV5-hFVIII</td>
<td>+</td>
<td>4-300%</td>
</tr>
<tr>
<td>Spark</td>
<td>NCT03003533</td>
<td>AAV8(spk200)-hFVIII</td>
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<tr>
<td>UCL/St. Jude</td>
<td>NCT03001830</td>
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<tr>
<td>Sangamo</td>
<td>NCT03061201</td>
<td>AAV2/6-hFVIII</td>
<td></td>
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</tr>
</tbody>
</table>
Known Unknowns in Gene Therapy

- Consequences of integrating DNA into chromosomal genome?
- How to manage hepatocyte toxicity?
- Where else apart from liver does vector genome go?
- How to overcome existing immunity to AAV?
- How long will transgenic synthesis of factor last?
- Can we repeat treatment with another AAV serotype or viral type?
- Should we treat children despite their growing livers?
- What will it cost?
Where are we now?

We are learning how to do gene therapy for haemophilia one patient at a time

Questions.