Gene therapy update

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Overview

• Present data from our ongoing Phase I/II clinical trial of gene therapy of haemophilia B with a specific focus on:
  – Long term follow up of 6 subjects in the initial dose escalation cohort (NEJM Dec 2011) and
  – 4 subjects recruited to an expanded high dose cohort
    • Is expression stable over time?
    • Can we achieve consistent level of FIX expression for a given dose?
    • What is the toxicity profile?

• Discuss plans for gene therapy of haemophilia A
Why gene therapy for haemophilia?

- Current treatment with Plasma or rec. FVIII or FIX is safe and effective but invasive, expensive and demanding
- Gene therapy offers the potential for a cure by continuous endogenous expression of FVIII/FIX protein at therapeutic levels following a single administration of vector

Haemophilies are well suited for gene therapy approaches

- Single gene defect (defect in the FIX or FVIII gene)
- Therapeutic goal modest; An increase in plasma FIX/FVIII levels above 5% would be sufficient ameliorate the bleeding phenotype
- Efficacy can be assessed by validated routine laboratory assay
- Tight regulation not required
Adeno-associated viral vectors for haemophilia gene therapy

– Best safety profile amongst viral vectors
  • Non-pathogenic single stranded DNA based parvovirus
  • Replicates only in the presence of helper virus
– Easy to render “Gutless” : reduced risk of immune response to viral proteins

– A single administration of the vector into liver results in stable long term transgenic FIX expression from episomal proviral genomes
  • Integration does occur but is rare
– Multiple serotypes with distinct tissue tropism and immunology
Three distinct features:

1. Vector pseudotyped with AAV8 capsid because of low pre-existing immunity to AAV8 in humans (~4.5% in adult Haemophilia B compared with 59% for AAV2)

2. Vectors contained a liver specific codon optimised hFIX expression cassette packaged as self complementary genomes to improve potency

3. Vector administered in the peripheral circulation
   A simple mode of vector delivery that takes advantage of the strong tropism of AAV8 for the liver, the native site for FIX synthesis
**Brief outline of trial**

**Phase I dose escalation design**

- **Objective:** Assess the safety and efficacy of a bolus peripheral vein infusion of our novel scAAV2/8-LP1-hFIXco
  - In subjects with severe haemophilia B (FIX:C<1% of normal)
  - Without the administration of upfront immunosuppression
- **Key entry criteria:**
  - No evidence of pre-existing immunity to AAV8
  - No evidence of active infection with Hepatitis B, C or HIV
- **Elaborate 3 stage informed consent process**
  - Independent ombudsman assessment
- **Main Study sites:** Royal Free Hospital & SJCRH
Peripheral vein infusion of vector is well tolerated

Vital signs
- SBP
- Pulse
- Temp
- Resp rate

Vector shedding
- Plasma
- Stools
- Saliva
- Urine
- Semen
Low vector dose

Plasma FIX levels, Subject 1: $2 \times 10^{11} \text{vg/kg}$

31Y: Missense mutation; X2/week prophylaxis; 3 bleeds/year

Off prophylaxis for >4 years but no spontaneous haemorrhage
Low and Intermediate vector dose

Summary of FIX expression

<table>
<thead>
<tr>
<th>Sub</th>
<th>Geno-type</th>
<th>Age (years)</th>
<th>Pre gene therapy prophylaxis</th>
<th>Annual bleeds</th>
<th>Vector dose (vg/kg)</th>
<th>hFIX:C level post gene transfer (% of normal)</th>
<th>Duration of expression (Years)</th>
<th>Post gene therapy prophylaxis</th>
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<tbody>
<tr>
<td>2</td>
<td>Null</td>
<td>64</td>
<td>X2/week</td>
<td>13</td>
<td>2x10^{11}</td>
<td>1</td>
<td>&gt;4.2</td>
<td>~X1/week</td>
</tr>
<tr>
<td>3</td>
<td>Mis-sense</td>
<td>43</td>
<td>X2/week</td>
<td>12</td>
<td>6x10^{11}</td>
<td>2</td>
<td>&gt;4</td>
<td>~X1/14 days</td>
</tr>
</tbody>
</table>

Transgene expression of <3% is not sufficient to permit discontinuation of prophylaxis in subjects with severe pre-existing arthropathy
Intermediate vector dose

Plasma FIX levels, Subject 4: $6 \times 10^{11} \text{vg/kg}$

29Y: Missense mutation; X1/Week prophylaxis; 12 bleeds/year

Off prophylaxis for > 3 years. Continues to engage in contact sports (soccer and cricket) but now without targeted prophylaxis.
High vector dose

Plasma FIX levels, Subject 5: $2 \times 10^{12}$ vg/kg

32Y: Missense mutation; X2/Week prophylaxis; 15 bleeds/year

Currently on X1/10 days prophylaxis
High vector dose

Plasma FIX levels, Subject 6: $2 \times 10^{12}$ vg/kg

27Y: Promoter mutation; X3/Week prophylaxis; 3 bleeds/year

- Off prophylaxis for > 3.5 years
- Not required on-demand FIX treatment

"I have not needed any of my normal treatment, either preventative or on-demand as a result of an injury. Previously, I used to infuse at home three times a week … I play football, run and take part in triathlons - and previously I might have had to infuse both before I took part and possibly after as well. Not having to do that has been absolutely brilliant."

ALT (IU/l)

hFIX:C (IU/dL)

0 0.1 0.2 0 2 4 6 8 10 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

0 0.5 1.5 2.5 3.5

3 post vector infusion
Summary: Initial phase of the study

- 6 subjects received our novel scAAV2/8-LP1-hFIXco: 2 at each of 3 dose levels

- Evidence of sustained FIX expression at 1-5% in all 6 subjects for > 3 years
  - 3/6 able to stop prophylaxis, with significant improvement in quality of life
  - 3/6 remain on prophylaxis but at extended intervals of X1/10-14 days

- AAV8 capsid-targeted cell mediated immunity is a concern at the high dose level
  - controlled by a short course of prednisolone, without loss of transgene expression
  - No late occurrence of liver inflammation
Expanding the high dose cohort

4 new subjects recruited in 2012

To answer the following questions:

1. Will we observe consistent level of gene transfer?
2. Will perturbation of liver enzymes occur in all subjects treated at the high dose level?
3. Will early treatment with steroids reduce the level of transaminitis and preserve stable expression of FIX?
4. Should all high dose subjects receive prophylactic steroids between 6-12 weeks after gene transfer?
Plasma FIX level: Subject 7; 2x10^{12} vg/kg

22Y: Null mutation; X2/Week prophylaxis; 2 bleeds/year

- Off prophylaxis for >2.5 years post gene transfer
*Plasma FIX level: Subject 8; 2x10^{12} vg/kg*

38Y: Missense mutation; on demand: 1Xweek; 22 bleeds/year

- No spontaneous bleeding episodes following gene transfer
- No transaminitis; **No need for steroids**
- Not required FIX concentrates following gene transfer
**Plasma FIX level: Subject 9; 2x10^{12}vg/kg**

44Y: Missense mutation; on demand X1 week; 36 bleeds/year

- No spontaneous bleeding episodes over post gene transfer
- No transaminitis; **No need for steroids**
Plasma FIX level: Subject 10; 2x10^{12} vg/kg

33Y: Null mutation; on demand X1/Week; 29 bleeds/year

- Required FIX concentrates for 4 episodes of traumatic bleeds
**Nathwani et al.'s Generation 1 Study**

- **Severe Patients only**
  - All <1% FIX levels
- **Peripheral vein infusion**
- **AAV8 self-complementary cassette**
- **Sustained multiyear efficacy**
  - First patient 4.5+ yrs
- **Immune response in some patients controlled with simple tapered steroid dose**
- **Dramatic improvement in annual bleed rate**
- **Significant reduction in recombinant FIX use**

**Graphs:**
- **First 10 Week Peak Levels**
- **Steady state FIX levels**
- **Annual bleeding rate prior to gene transfer**
- **Annual bleeding rate post gene transfer**

Change in bleeding phenotype

Annualised bleeding rate

FIX concentrate usage

Savings from reduction in FIX usage so far is £ 2.0M and accruing
• In total 10 subjects received scAAV8-LP1-hFIXco

• Evidence of vector mediated human FIX expression in all 10 subjects treated to date with follow-up between 1-4.5 years
  – Expression consistent at high dose level of ~5%
  – 4/7 able to stop prophylaxis, with significant improvement in quality of life and no spontaneous bleeds

• Perturbation of liver enzymes has been observed in 4/6 subjects treated at the high dose level
  – Use steroids prophylactically between 7-9 weeks after gene transfer

• **Preliminary results are encouraging and support further evaluation of this approach**
Current status of haemophilia B gene therapy

UCL/St Jude
Evaluating a new high purity AAV preparation with fewer empty capsids

Other FIX trials in the pipe line

Baxter: PI: Monahan
1. scAAV vector pseudotyped with AAV8 capsid
2. FIX cDNA containing a hyperactive (Padua) FIX mutation
3. Study is open and 3 subject recruited at 1e11vg/kg

Sparke: PI= Kathy High
1. ssAAV vector pseudotyped with AAV8 capsid
2. Strong liver specific promoter: HCR-hAAT
3. 3 subject recruited
<table>
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<tr>
<th>US</th>
<th>Europe</th>
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<tr>
<td>4D molecular Therapeutics</td>
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<tr>
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<td>NeuralGene</td>
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RoW
High et al.'s Generation 1 Study

- Severe Patients only
  - All <1% FIX levels
- Hepatic portal vein infusion
- AAV2 single stranded cassette
- Transient efficacy
- Immune response in some patients not controlled

Source: Manno et al., Nature Medicine 2006
Baxter Generation 1 Study

- Severe Patients only
  - All <1% FIX levels

- Peripheral vein infusion

- AAV8 self-complementary cassette

- Immune response in some patients
  - Tapered steroid dosing

- Continued FIX infusions for all but 1 patient

Source: Presentation at the 2015 International Society on Thrombosis and Haemostasis (ISTH) Congress, Toronto
Status of haemophilia A gene therapy
Major hurdles to safe and effective Haemophilia A gene therapy with AAV

- FVIII gene specific: 4 fold lower expression than FV

- AAV specific: Limited packaging capacity ~5.2kb

What about B domain deleted FVIII variants?

Deletion of B-domain improves FVIII expression by 3X (Pittman et Blood 1993)

Addition of 226 aa of B-domain improves (N6) FVIII expression by 10X (Miao et Blood 2004)
**In-vivo potency of AAV-FVIII-variants**

New codon optimised FVIII leads to 10 fold higher expression in mice following rAAV mediated gene transfer.
**Vector dose – hFVIII-V3 expression relationship**

AAV8-HLP-codop-hFVIII-V3 IV C57Bl/6 mice

**Extrapolation of mouse to humans**

<table>
<thead>
<tr>
<th>Dose vg/kg</th>
<th>2x10^{11}</th>
<th>6 x 10^{11}</th>
<th>2 x 10^{12}</th>
</tr>
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<tbody>
<tr>
<td>FVIII Levels in mice</td>
<td>14%</td>
<td>37%</td>
<td>135%</td>
</tr>
<tr>
<td>Predicted FVIII levels in humans</td>
<td>0.7%</td>
<td>2%</td>
<td>7%</td>
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Therapeutic dose range in humans likely to be between 2e11 to 2e12 vg/kg. Same dose range as the current FIX study.
Summary

• Bioengineering of the FVIII has resulted in a potent HLP-codop-hFVIII-V3 expression cassette that is efficiently packaged into AAV vectors
• Dose finding studies suggested that doses that were effective in haemophilia B will produce a therapeutic level of FVIII in haemophilia A
• Gene therapy trial for haemophilia A using AAV has started in Q3 of 2015
Conclusion

AAV mediated gene therapy has the potential to be a new paradigm in treatment for haemophilia

A single administration of AAV vector can result:

– Safe and consistent long-term expression of transgene (> 5 years)
– Reduction in spontaneous bleeding episode
– Reduction in clotting factor usage
– Improvement in quality of life

Commercialization of this approach will require improved vector production, demonstrated efficacy in children and elimination of immune response to AAV mediated gene transfer
• The patients

Without whose altruism and bravery in entering these trials without any expectation of benefit, none of these advances could have happened or will happen in future.