Gene Therapy for Fanconi Anaemia

Hope or hype?

Dr Phil Ancliff
Great Ormond Street Hospital / UCL Institute of Child Health

Twycross Zoo October 2017
“Miranda Bailey” had the idea to use deactivated HIV to deliver the enzyme to Braden’s body. His parents were initially concerned about the use of HIV, but Dr. Bailey assured them that she was not going to give Braden HIV.

“After receiving chemotherapy to kill off the enzyme-deficient bone marrow, Braden contracted a viral infection, concerning his parents. Worried that the treatment would make Braden even sicker, Teresa withdrew consent for the gene therapy. Unbeknownst to the Morrises, Bailey proceeded with the gene therapy as planned.”
What really is gene therapy?

• Genes code for proteins
• Alteration in code can produce a non-functional protein
• This may result in disease

• Replace the gene with a normally functioning copy
• Insert a new copy
• Inactivate the mutated gene
The Human Genome

- The human genome is the complete set of human genetic information stored as DNA sequences within 23 chromosome pairs in the cell nucleus.
- A gene is a stretch of DNA corresponding to a unit of inheritance that codes for a protein.
- The human genome contains ~30,000 genes.
Which diseases could be treated?

- Condition must arise from defined mutations in a gene
- Know which gene is involved
- Know the biology of the disease
- Show that adding a normal copy of the gene fixes the problem
- Deliver that normal copy of the gene to the affected cells/tissues
Clinical trials

Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 64.3% (n=1223)
- Monogenic diseases 8.8% (n=1667)
- Cardiovascular diseases 8.3% (n=158)
- Infectious diseases 8% (n=153)
- Neurological diseases 1.9% (n=36)
- Ocular diseases 1.5% (n=28)
- Inflammatory diseases 0.7% (n=13)
- Other diseases 1.4% (n=27)
- Gene marking 2.6% (n=50)
- Healthy volunteers 2.5% (n=42)
Conditions you’ve heard of...
Need to get the gene into the patient

• **Viral vectors**
  - Adenovirus
  - Adeno associated virus (AAV)
  - Retrovirus
  - Lentivirus

• **Non-viral vectors**
  - Liposomes
  - Naked DNA
Why viruses?

**Viruses are active gene transfer vehicles**

Viruses have evolved to deliver genetic information to cells

They have methods of avoiding immune systems

They exploit cellular mechanisms (receptors, endosomal processing, nuclear transport)

*BUT* they are normally associated with disease
Retroviral life cycle
In vivo gene therapy
Ex vivo gene therapy
Successful gene therapy

- Selective for target cell
- Physiological expression
- Low immunogenicity
- Site specific integration
- Non-toxic
Gene therapy and paediatric conditions

- Skin conditions
  - EB
- Inborn errors
  - OCT, Hurlers
  - X-ALD
- Haemophilia
- Duchenne’s MD
- Retinal abnormalities
- Cystic fibrosis
- Severe immunodeficiencies
- Fanconi’s anaemia
- Haemoglobinopathies
- Acute leukaemias
The formation of blood cells from stem cells

- HSC-multi
- CMP
- CLP
- erythrocyte
- mast cell
- megakaryocyte
- neutrophil
- monocyte
- osteoclast
- thymus
- T cell
- NK cell
- B cell
Correcting Fanconi anaemia

stem cells

red blood cell

platelets

white blood cells

neutrophils /monocytes

white blood cells / Immune cells
Correcting Fanconi anaemia

stem cells

red blood cell

platelets

white blood cells

neutrophils /monocytes

white blood cells / Immune cells
SCID – The boy in the bubble disease
Survival after transplant... (SCID)

10 years Survival rate

- Geno: 84% (n=132)
- Pheno: 65% (n=65)
- MUD: 68% (n=82)
- mmRel: 52% (n=396)

P < 0.0001
How It Works | The procedure the SCID-X1 trial will use:

1. Stem cells are isolated from bone marrow harvested from a baby's hip.
2. The normal gene is inserted into the stem cells in the lab.
3. The corrected cells are then transfused back into the baby and populate over time, repairing the baby's faulty immune system.

Source: Children's Hospital Boston
Photo: Getty Images
Gene therapy labs
Successful recovery of the immune system
Serious Adverse Events in Clinical trials

A Serious Adverse Event after Successful Gene Therapy for X-Linked Severe Combined Immunodeficiency


**Research Article**

**LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1**

S. Hacein-Bay-Abina, 3,20 C. Von Kalle, 6,7,8 M. Schmidt, 6,7 M. P. McCormack, 9 N. Wulffraat, 10 P. Leboulch, 11 A. Lim, 12 C. S. Osborne, 13 R. Pawlik, 14 E. Morillon, 2 R. Sorrensen, 19 A. Forster, 9 P. Fraser, 13 J. I. Cohen, 15 G. de Saint Basile, 1 I. Alexander, 16 U. Wintergerst, 17 T. Frebourg, 18 A. Auras, 19 D. Stoppa-Lyonnet, 20 S. Romana, 3 I. Radford-Weiss, 9 F. Gross, 5 F. Valensi, 4 E. Delabesse, 4 E. Macintyre, 4 F. Sigaux, 20 J. Soulier, 21 L. E. Leiva, 18 M. Wissler, 6,7 C. Prinz, 6,7 T. H. Rabbitts, 9 F. Le Deist, 1 A. Fischer, 1,1,1 M. Cavazzana-Calvo, 1,2

Science 17 October 2003
Engineering safer vectors...
Why pick Fanconi anaemia?
Corrected cells have an advantage

stem cells

red blood cell

platelets

white blood cells

neutrophils / monocytes

white blood cells / Immune cells
Mosaic reversion ...natural gene therapy

- Hemoglobin
- Leukocyte
- Thrombocyte
Not all children have a donor

<table>
<thead>
<tr>
<th>Reference</th>
<th>Conditioning regimen</th>
<th>GVHD prophylaxis</th>
<th>No. of cases</th>
<th>Recipient age (range)</th>
<th>Sustained engraftment</th>
<th>Acute GVHD</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guardiola et al (2000)</td>
<td>Varied</td>
<td>Varied</td>
<td>69</td>
<td>10±8 years (4±0–37±4 years)</td>
<td>83%*</td>
<td>34%</td>
<td>35% alive at 3 years</td>
</tr>
<tr>
<td>Wagner et al (2007)</td>
<td>Varied</td>
<td>Varied</td>
<td>98</td>
<td>12 years (0±8–33 years)</td>
<td>89% (FLU)</td>
<td>21% (TCD)</td>
<td>52% (FLU) at 3 years 13% (no FLU) at 3 years</td>
</tr>
<tr>
<td>Gluckman et al (2007)</td>
<td>Varied</td>
<td>Varied</td>
<td>93</td>
<td>8±6 years (1±1–45 years)</td>
<td>60% ± 5%</td>
<td>32% ± 5%</td>
<td>74% ± 13% (HLA 6/6, n = 12) 48% ± 9% (HLA 5/6, n = 35) 25% ± 7% (HLA 3–4/6, n = 45) 14/21 alive</td>
</tr>
<tr>
<td>Chaudhury et al (2008)</td>
<td>CY 40 mg/kg TBI 450 cGy rATG 10 mg/kg</td>
<td>TCD</td>
<td>21</td>
<td>21/21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MacMillan et al (2009)</td>
<td>CY 40 mg/kg TBI 300/150 cGy with thymic shielding FLU 140 mg/m² ATG 150 mg/kg</td>
<td>TCD + CSA</td>
<td>24</td>
<td>8±8 years (4±0–21±2 years)</td>
<td>22/22 (TBI 300 cGy)</td>
<td>0/2</td>
<td>19/22 alive 2/2 alive</td>
</tr>
</tbody>
</table>

CY, cyclophosphamide; TBI, total body irradiation; rATG, rabbit anti-thymocyte globulin; FLU, fludarabine; TCD, T cell depletion; TCD, CSA, cyclosporine A.

*Probability of secondary graft failure was 19%.

Clinical outcomes from alternative-donor bone marrow transplantation in patients with FA
Complications of BMT

- No FA BMT is straightforward
- Potential increase risk for SCC after conditioning
Problems for gene therapy in FA

• Obtaining sufficient cells:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cycle No.</th>
<th>Circulating PB CD34&lt;sup&gt;+&lt;/sup&gt; cells after G-CSF (per μl)</th>
<th>Infused CD34&lt;sup&gt;+&lt;/sup&gt; cells</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Total (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>Dose (10&lt;sup&gt;6&lt;/sup&gt;/kg)</td>
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<tr>
<td>1</td>
<td>1</td>
<td>0–1.1</td>
<td>0.6</td>
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<tr>
<td></td>
<td>2</td>
<td>0.7–3.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0</td>
<td>1.0</td>
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<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>0.12</td>
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<td>2</td>
<td>1</td>
<td>43</td>
<td>28.1</td>
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<td>0.9–4.1</td>
<td>0.18</td>
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<td>3</td>
<td>33.6–116</td>
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Add Plerixafor and collect early
Problems for gene therapy in FA

- Achieving successful transduction:

- Two published GT studies in FA
  - Only 6 patients infused with transduced product
  - Detectable vector in 4
  - One patient had small increase in haematological values

Optimise protocol

Lentiviral vector
Minimal cell culture
Role of conditioning
Engraftment and in vivo proliferation advantage of gene-corrected mobilized CD34+ cells from Fanconi anemia patients

Paula Río,1-3,* Susana Navarro,1-3,* Guillermo Guenechea,1-3 Rebeca Sánchez-Domínguez,1-3 María Luisa Lamana,1-3 Rosa Yañez,1-3 Jose A. Casedo,1-3 Parinda A. Menta,4 Maria Roser Pujol,3,5,6 Jordi Surralles,3,5,6 Sabine Charrier,7 Anne Gailly,7 José C. Segovia,1-3 Cristina Díaz de Heredia,8 Julián Sevilla,3,9,10 and Juan A. Bueren1-3

1Division of Hematopoietic Innovative Therapies, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas, Madrid, Spain; 2Advanced Therapies Unit, Instituto de Investigación Sanitaria Fundación Jiménez Díaz, Madrid, Spain; 3Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid, Spain; 4Division of Bone Marrow Transplantation and Immune Deficiency, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; 5Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, Barcelona, Spain; 6Genetics Department and Institute of Biomedical Research, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; 7Genethon and UMR S951 Genethon, INSERM, Université Evry, Ecole Pratique des Hautes Études, Evry, France; 8Servicio de Oncología y Hematología Pediátrica, Hospital Universitario Vall d’Hebron, Barcelona, Spain; and 9Servicio Hemato-Oncología Pediátrica and 10Fundación Investigación Biomédica, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
Phase I/II open label, single centre clinical trial to assess the safety and efficacy of autologous CD34+ cells transduced with lentiviral vector for patients with Fanconi anaemia subtype A

Funder (s): EC FP7 Funded
EudraCT no: 2011-006197-88
Active IMP(s): PGK-FANCA lentiviral vector transduced patient CD34+ cells
Inclusion criteria

- Patients diagnosed with Fanconi anaemia subtype A
- Age 1-15 years
- At least one of the following parameters must exceed the values indicated: haemoglobin: 8.0 g/dL; neutrophils: 750/mm$^3$; platelets: 30000/mm$^3$
- Patients lacking a fully matched donor for haematopoietic stem cell transplant
- Evidence of hypocellular bone marrow on bone marrow biopsy
- Lansky index > 60%
- Left ventricular ejection fraction > 50%
- Provide informed consent in accordance with current legislation
Trial summary
When?

• Q1 2018....

Thank you!