Gene therapy for inborn errors of metabolism of the liver

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The laboratory bench

Basic and pre-clinical studies

The patient bedside

Clinical trials
Interests of the GTRU

Liver → Metabolic disorders
(Urea cycle disorders)

Haematopoetic Stem Cells (HSCs)

→ Immune diseases
(SCID-X1)

→ Cancer
(myeloprotection with MGMT)
(Clinical trials)
Inborn errors of metabolism

- **Significant cause of childhood disability and death:** Individually rare, collectively common (~1 in every 500 newborns).

- **Many tissues and organs are affected including:**
  Liver, skeletal/cardiac muscle, central nervous system, hematopoietic compartment, among others.
Metabolic processes in the liver

- Highly complex organ, carries out many vital functions:

<table>
<thead>
<tr>
<th>Intermediary metabolism</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>(carbohydrate, lipid, protein)</td>
<td>(glycogen, vitamins, iron, copper)</td>
</tr>
<tr>
<td>UCDs, PKU, Tyrosinaemia Type 1</td>
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<table>
<thead>
<tr>
<th>Detoxification</th>
<th>Biosynthesis</th>
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</thead>
<tbody>
<tr>
<td>(xenobiotics, metabolic endproducts)</td>
<td>(plasma proteins, bile acids)</td>
</tr>
<tr>
<td>Ammonia</td>
<td></td>
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</tbody>
</table>

High incidence of disease-causing mutations (~1 in 800 births).

⇒ Liver is an attractive target for developing new therapies.
Urea Cycle Disorders

- A paradigm for inborn errors of liver cell (hepatocyte) metabolism.
- Ammonia detoxification by nitrogen removal (byproduct of protein metabolism).

- Elevated plasma ammonia (hyperammonaemia) → highly neurotoxic.
- Orotic aciduria, amino acid abnormalities (incl. citrulline, arginine, glutamine).

- 5 primary enzymes
- 1 co-factor producer
- 2 transport proteins
Management of severe early-onset UCDs is highly challenging

• **Severe neonatal presentation:**
  Hyperammonaemia, encephalopathy, respiratory alkalosis, coma, death if untreated.

• **Haemofiltration.**

• **Ongoing management (pharm/dietary):**
  • Alternative pathway therapy to remove nitrogen (sodium benzoate/sodium phenylacetate)
  • Arginine/citrulline supplementation.
  • Rigorous protein restriction.

• **Liver transplant for long-term survival:**
  • Waiting lists.
  • Metabolic crisis difficult to control.
  • Life-long immunosuppressive therapy.

➢ **Gene therapy - an attractive alternative!**
What is gene therapy?

“The insertion of genetic material into cells to correct a genetic defect by replacing, altering or supplementing a gene that is absent or abnormal”

Genes as medicine!
Gene delivery systems

Non-viral
- Naked DNA
- DNA-chemical complexes

Viral
- Adenovirus
- Adeno-associated virus (AAV) (Non-integrating vectors)
- Retrovirus
- Lentivirus (Integrating vectors)

(travel via the bloodstream)

Target cell
- cytoplasm
- nucleus

“taxi”
Adeno-associated viral vectors (rAAV)

- Targets liver very efficiently.
- Non-pathogenic parvovirus.
- Single-stranded DNA genome surrounded by a protein “coat” (capsid):
  
  **Virus**
  
  ![Virus Diagram]

  - Virus is “gutted” – viral genes removed.

  **Vector**
  
  ![Vector Diagram]

  - “coat variations” pseudoserosertype with different capsids depending on cell types/target species
Tools for testing a new vector

Promoter ("on switch")

GFP (reporter gene)

GFP “green fluorescent protein” (from jellyfish)

Cells “in vitro”

rAAV-LSP.GFP

Animal models “in vivo”
The journey to the clinic...

- Cell culture
- Small animal models
- Large animal models
- Children
- Adults
OTC deficiency

• Most common UCD; X-linked recessive (males more severe)

*Spf*<sup>ash</sup> mouse model of OTC deficiency

• Sparse fur, abnormal skin and hair (amino acid abnormalities; normal by adulthood)

• Mild metabolic phenotype:
  – Affected males 3-5% normal OTC activity.
  – Not hyperammonemnic.
  – Elevated urinary orotic acid (surrogate marker).

➢ We have successfully cured adult mice using gene therapy!
Curing OTC deficiency in the adult mouse

- Adult mice (8-10 weeks)
- 3 doses (low, mid, high)
- Injected intraperitoneally

**Analysis at 2 weeks post-injection:**
- Orotic acid (urine)
- OTC enzyme activity (liver)
Curing OTC deficiency in the adult mouse

OTC enzyme activity

![Graph showing OTC enzyme activity with different dose levels for wildtype (normal) and Spfash (treated) groups.]

Urinary orotic acid

![Graph showing urinary orotic acid levels with different dose levels for wildtype (normal) and Spfash (treated) groups.]

Liver sections

![Liver sections images showing wildtype (normal) and Spfash (treated) conditions.]
Liver-targeted AAV gene therapy for Hemophilia B

Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B


ABSTRACT

November 29, 2014
Our challenges are far greater...

**Hemophilia B**
- “low hanging fruit”.
- Made in the cell, but secreted to bloodstream.
- Only need to “supercharge“ a few cells.

**Urea cycle disorders**
- “cell autonomous” (made and functions within the same cell)
- Minimum threshold of cells need to be fixed AND maintained (challenge in the growing liver with our system)
Maintaining stable gene correction in a growing liver

AAV efficiently targets liver cells but does not integrate into target cell DNA:

- **Stable** in quiescent cells (adult liver).
- Lost from rapidly dividing cells (neonatal liver).

Cunningham et al. Mol Ther (2008)

Mouse liver sections showing eGFP-expressing cells

1 wk  | 3 wk  | 6 wk  | 6 mth | 12 mth

Neonate: ~100% efficiency

only ~5% cells remain stably gene-modified

Cunningham et al. Mol Ther (2008)
The minimum threshold for correction can be achieved in the growing liver by vector re-delivery

- Cindy Kok (PhD student)
- Mouse model of Citrullinaemia (ASS deficiency – another UCD)
- Neonatal lethal - mice die within 24 hours with elevated blood ammonia.
The minimum threshold for correction can be achieved in the growing liver by vector redelivery.

Survival

- 2 doses – sick within 2-4 wks
- 3 and 4 doses – did not get sick

Liver section

- 15% wt ASS activity
- 25% gene-modified cells
Our trajectory to the clinic

• Collaboration with metabolic team at Greater Ormond Street Hospital for Children (University College London).
• Pre-clinical studies in non-human primates.
• “Bridge-to-transplant” clinical trial in paediatric patients.
Future technologies

Mutated gene

Gene addition

Gene repair (editing)

CRISPR/Cas9 (molecular scissors that “cut and fix” DNA)
A mouse model with “humanised” mouse liver

FRG mouse (Tyrosinaemia Type 1):

- Human hepatocytes can be engrafted and selectively expanded – “humanised mouse liver”
- Immunodeficient (no rejection of human cells)
- Fah-negative (expand “normal” cells)

Building a repository of human hepatocytes with metabolic deficiencies:

OTC, CPS1, ASL
Mouse liver engrafted with OTC-deficient human liver cells

OTC-deficient human hepatocytes engrafted in an FRG mouse (human albumin staining).

Red cells = human cells

Adjacent section stained for in situ OTC activity (brown).

Intensity of brown stain = level of OTC activity
AAV vector development in the humanised mouse model

⇒ AAV-LK03 is our vector of choice for our OTC clinical trial in paediatric patients.
Exciting times ahead for liver-targeted gene therapy...

• AAV in adult liver is already showing great success in the clinic.

• An OTC clinical trial in paediatric patients with a human-specific AAV is looking highly likely.

• Further development of the “gene editing” platform will benefit gene therapy in the paediatric liver.

• These tools can be transferred to other conditions such as PKU and Tyrosinaemia.
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