Gene therapy for inborn errors of metabolism of the liver

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CHILDREN'S MEDICAL RESEARCH INSTITUTE
Jeans for Genes®

Gene Therapy Research Unit (GTRU)

Children's Medical Research Institute

The Children's Hospital at Westmead

Head of Unit: Prof Ian Alexander

(*Senior Staff Specialist, Metabolic Disorders Services*)

The laboratory bench

Basic and pre-clinical studies

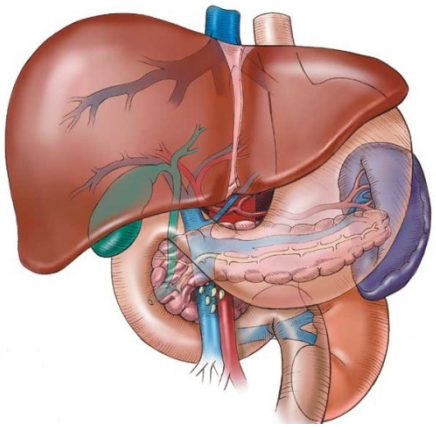
→

The patient bedside

Clinical trials

Interests of the GTRU

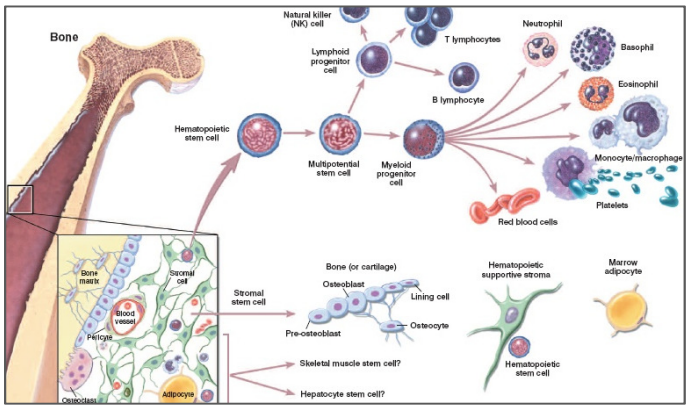
Liver



→ Metabolic disorders

(Urea cycle disorders)

Haematopoetic Stem Cells (HSCs)



→ Immune diseases

(SCID-X1)

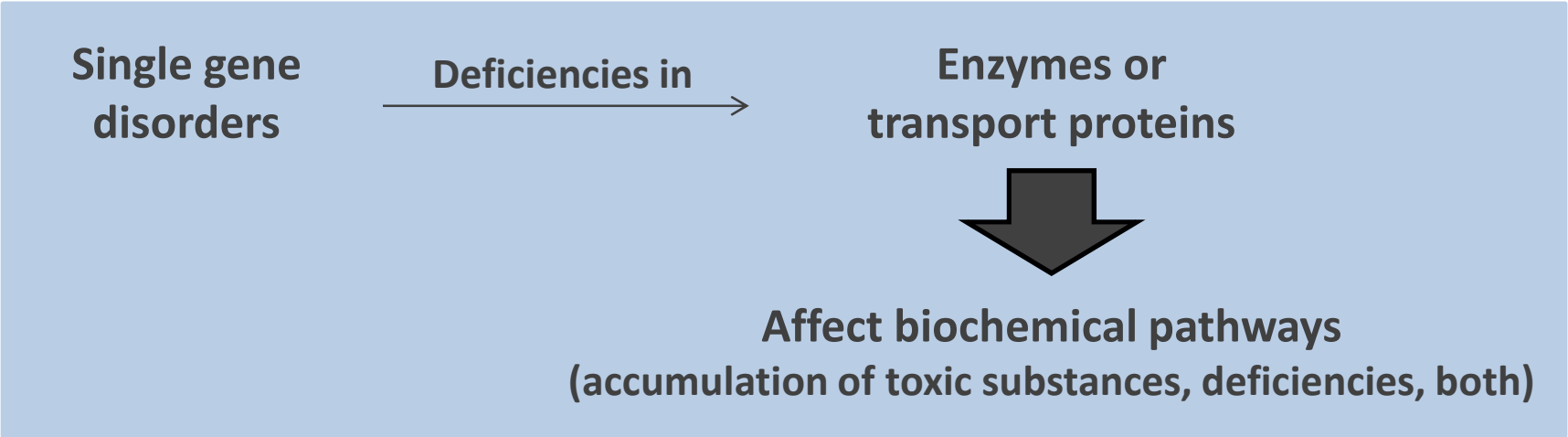
→ Cancer

(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).



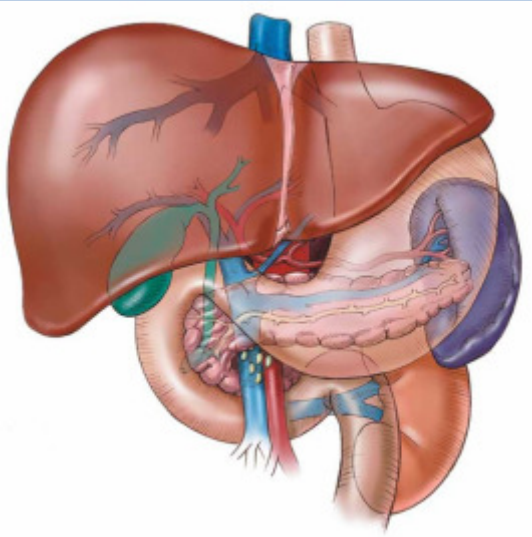
- **Many tissues and organs are affected including:**
  Liver, skeletal/cardiac muscle, central nervous system, hematopoietic compartment, among others.

Metabolic processes in the liver

- Highly complex organ, carries out many vital functions:

Intermediary metabolism
(carbohydrate, lipid, protein)
UCDs, PKU, Tyrosinaemia Type 1

Detoxification
(xenobiotics, metabolic endproducts)
Ammonia



Storage
(glycogen, vitamins, iron, copper)

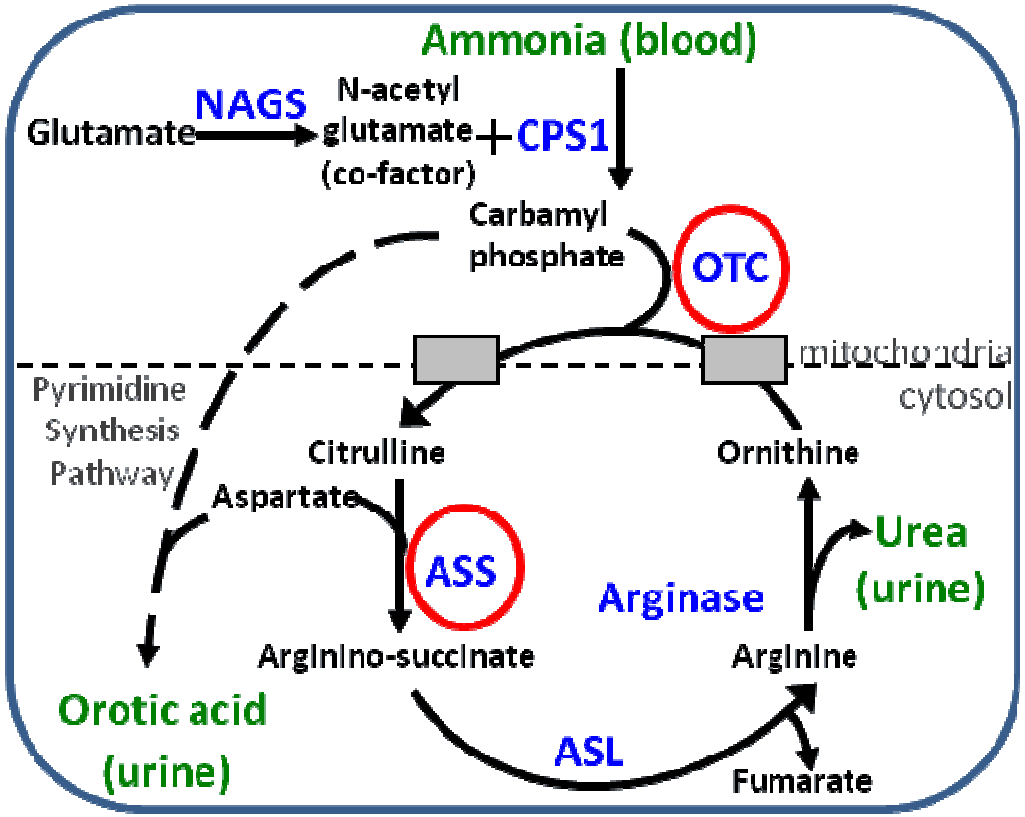
Biosynthesis
(plasma proteins, bile acids)

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).



- 5 primary enzymes
- 1 co-factor producer
- 2 transport proteins

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

Management of severe early-onset UCDs is highly challenging

- **Severe neonatal presentation:**

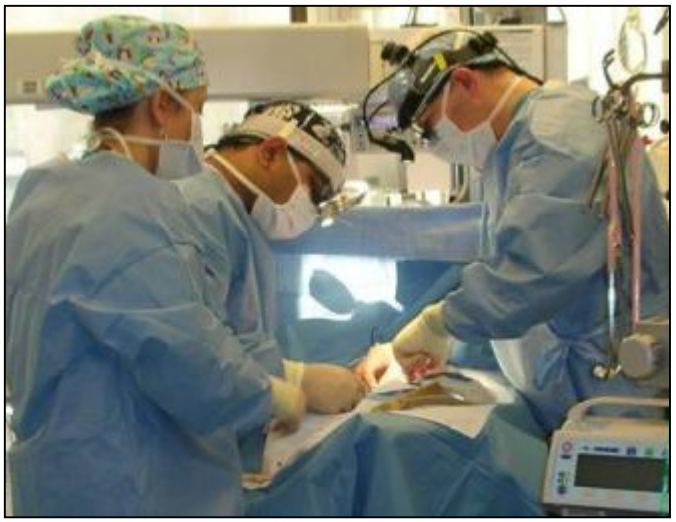
  Hyperammonaemia, encephalopathy, respiratory alkalosis, coma, death if untreated.

- **Haemofiltration.**

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

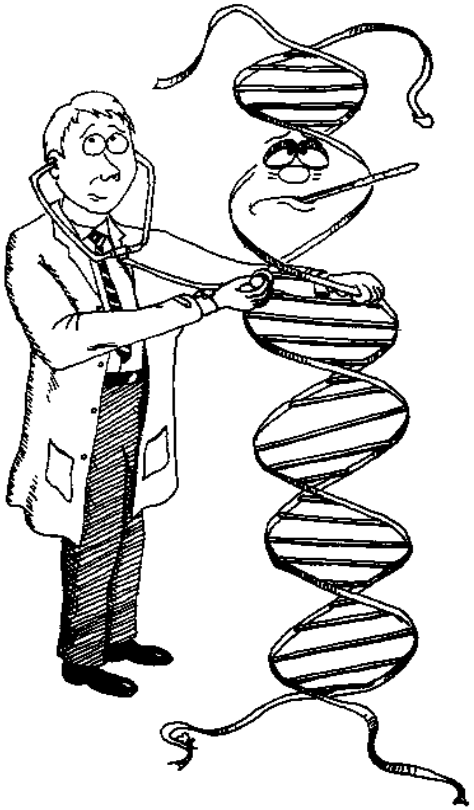
- **Liver transplant for long-term survival:**
  - Waiting lists.
  - Metabolic crisis difficult to control.
  - Life-long immunosuppressive therapy.

➢**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

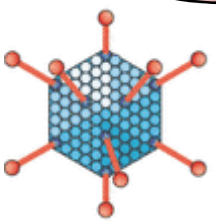
(travel via the bloodstream)

Non-viral

Naked DNA

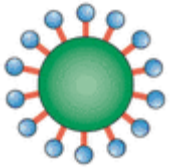
DNA-chemical complexes

Viral



Adenovirus

Adeno-associated virus (AAV)



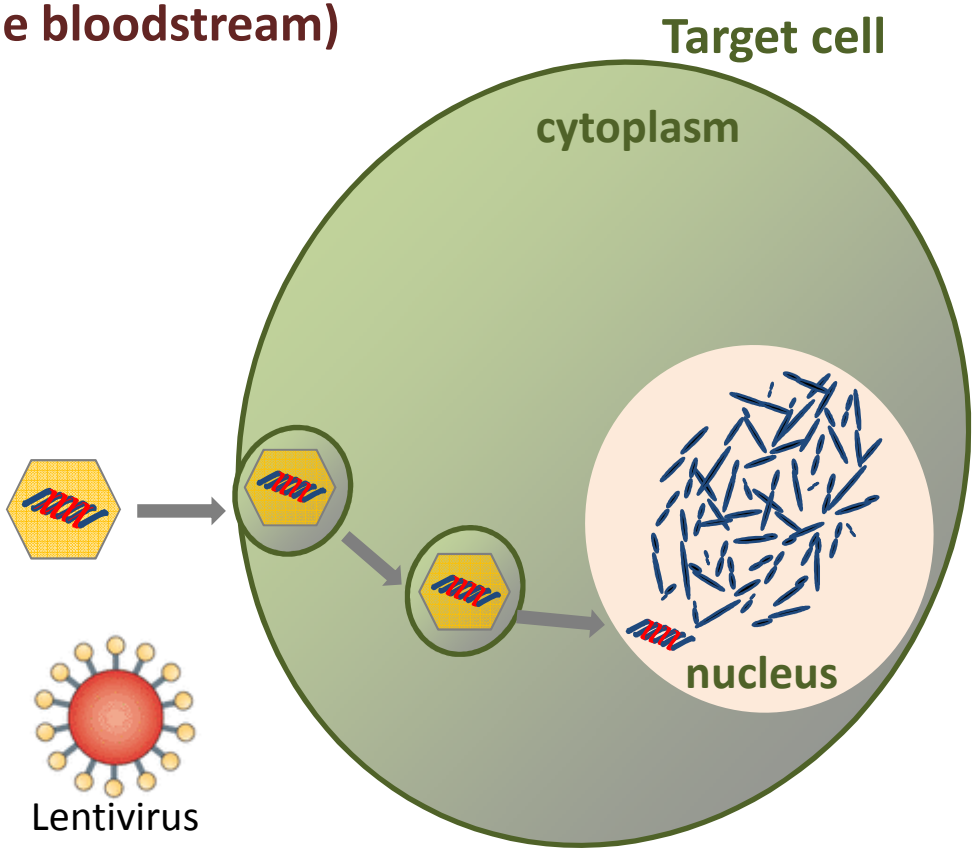
Retrovirus

Lentivirus

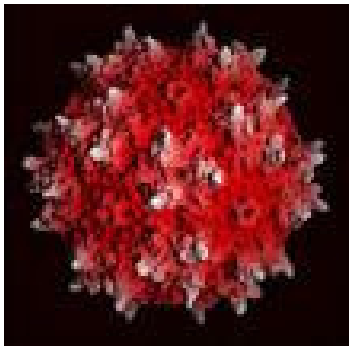
(Non-integrating vectors)

(Integrating vectors)

"taxi"

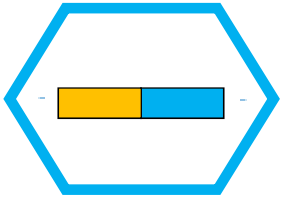


Adeno-associated viral vectors (rAAV)



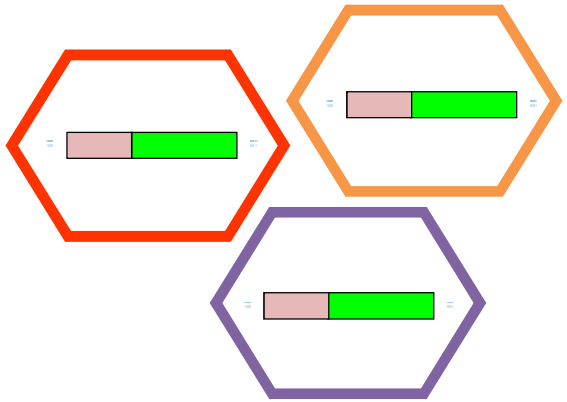
- Targets liver very efficiently.
- Non-pathogenic parvovirus.
- Single-stranded DNA genome surrounded by a protein “coat” (capsid):

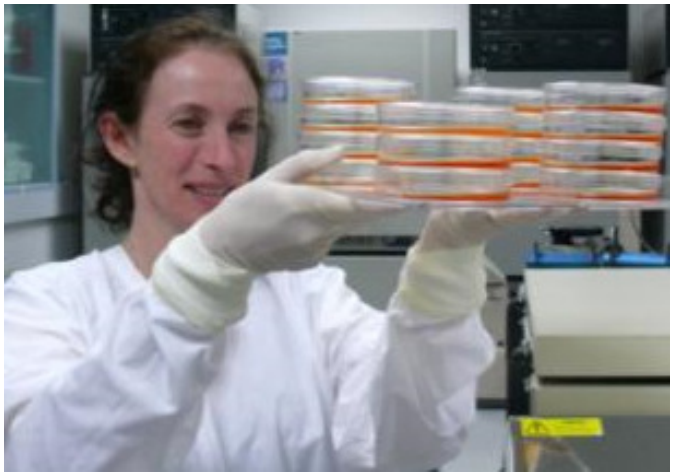
Virus



- Virus is “gutted” – viral genes removed.

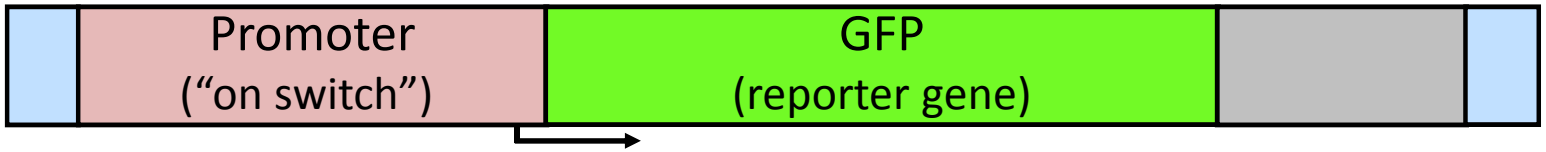
Vector

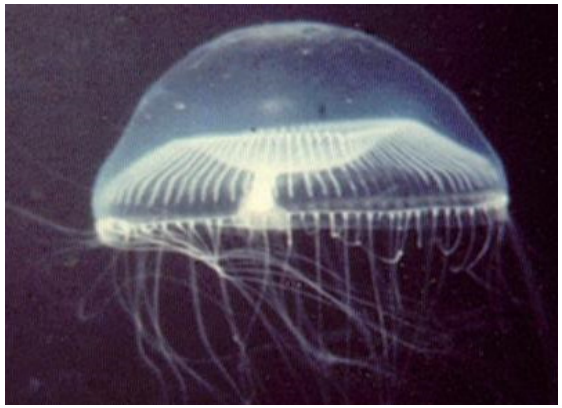




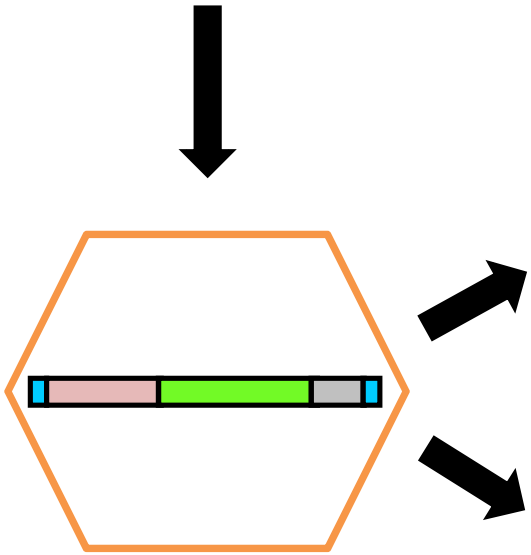
“coat variations”
pseudoserotype with different capsids
depending on cell types/target species

Tools for testing a new vector

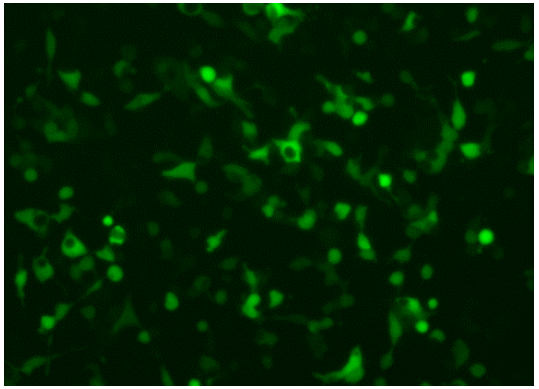




GFP "green fluorescent protein" (from jellyfish)



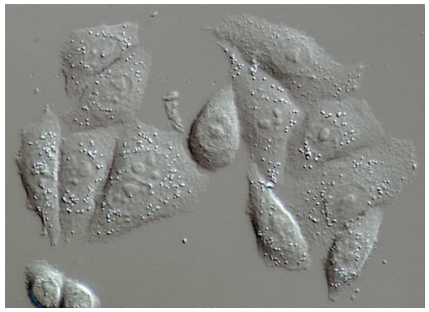
rAAV-LSP.GFP



Cells "*in vitro*"

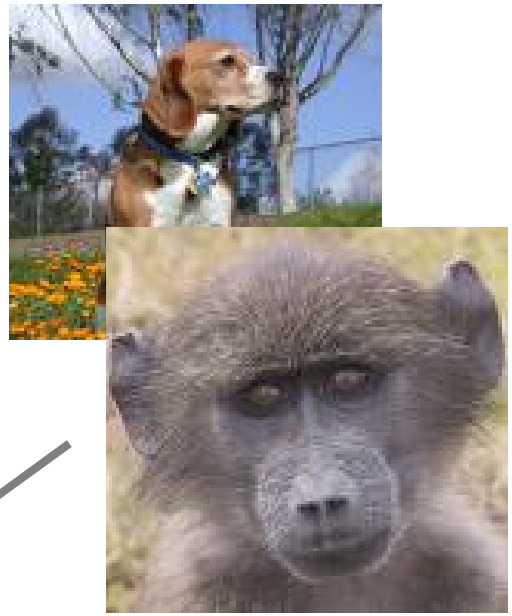
Animal models *"in vivo"*

The journey to the clinic…

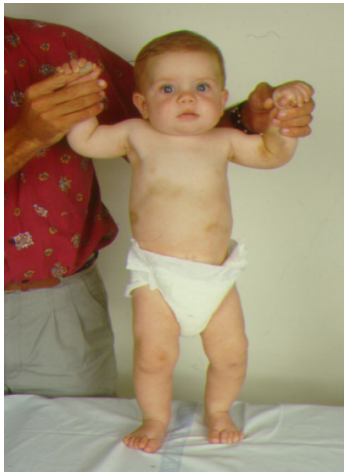


Cell culture

Small animal models



Large animal models



Children

Adults

OTC deficiency

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

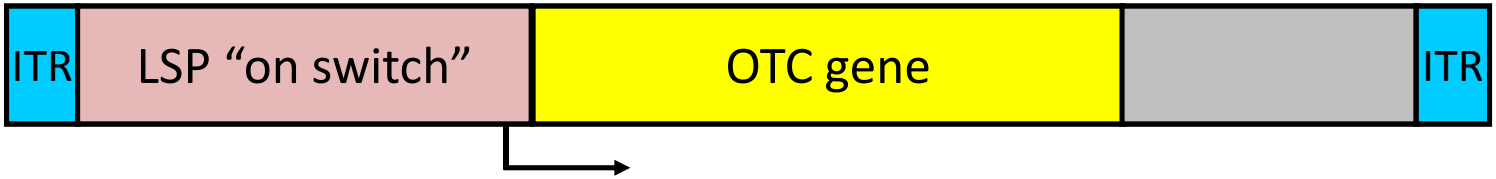
Spf^{ash} mouse model of OTC deficiency

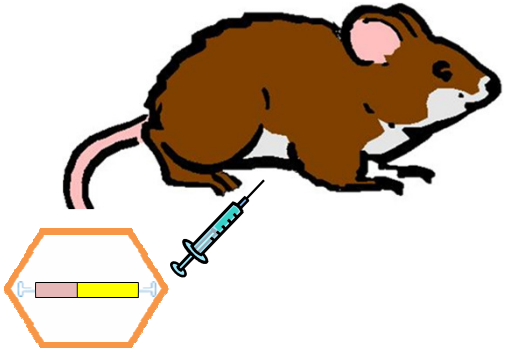
- Sparse fur, abnormal skin and hair (amino acid abnormalities; normal by adulthood)
- Mild metabolic phenotype:
  - Affected males 3-5% normal OTC activity.
  - Not hyperammonemic.
  - Elevated urinary orotic acid (surrogate marker).

➢ ***We have successfully cured adult mice using gene therapy!***

Curing OTC deficiency in the adult mouse

AAV viral vector





Adult mice (8-10 weeks)

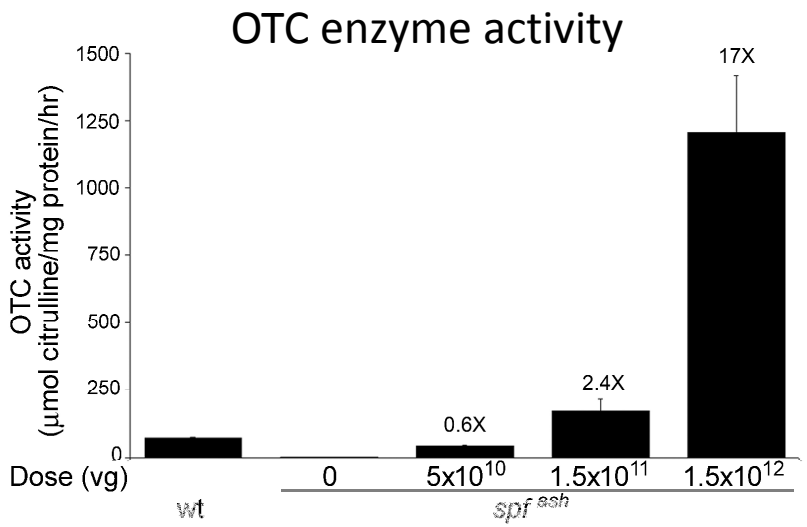
3 doses (low, mid, high)

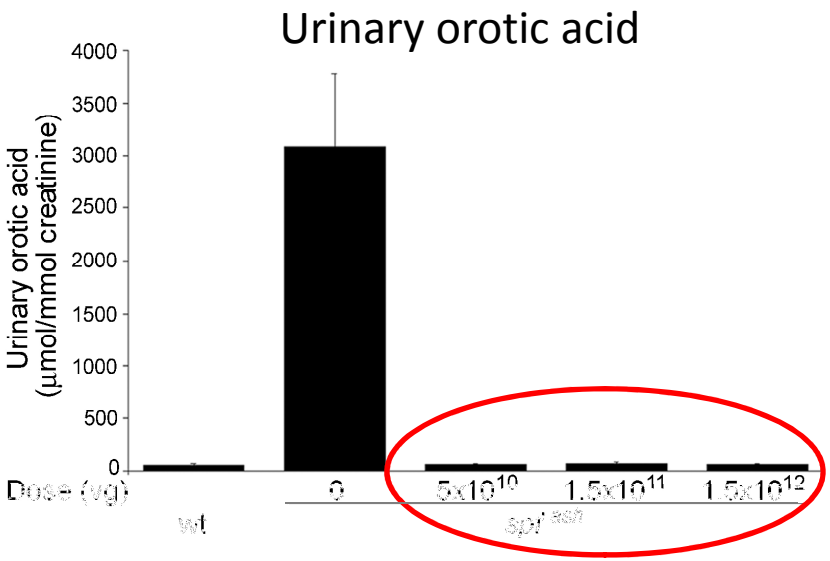
Injected intraperitoneally

Analysis at 2 weeks post-injection:

- Orotic acid (urine)
- OTC enzyme activity (liver)

Curing OTC deficiency in the adult mouse



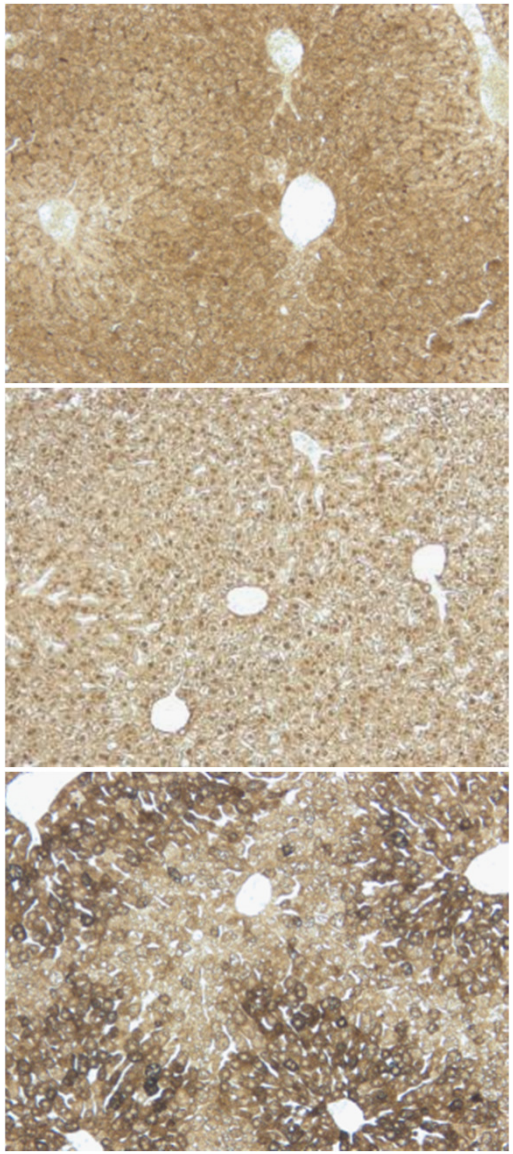


Liver sections

Wildtype (normal)

Spf^ash^

***Spf^ash^* (treated)**



Cunningham *et al.* Mol Ther 2009

Liver-targeted AAV gene therapy for Hemophilia B

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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

A.C. Nathwani, U.M. Reiss, E.G.D. Tuddenham, C. Rosales, P. Chowdary, J. McIntosh, M. Della Peruta, E. Lheriteau, N. Patel, D. Raj, A. Riddell, J. Pie, S. Rangarajan, D. Bevan, M. Recht, Y.-M. Shen, K.G. Halka, E. Basner-Tschakarjan, F. Mingozzi, K.A. High, J. Allay, M.A. Kay, C.Y.C. Ng, J. Zhou, M. Cancio, C.L. Morton, J.T. Gray, D. Srivastava, A.W. Nienhuis, and A.M. Davidoff

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

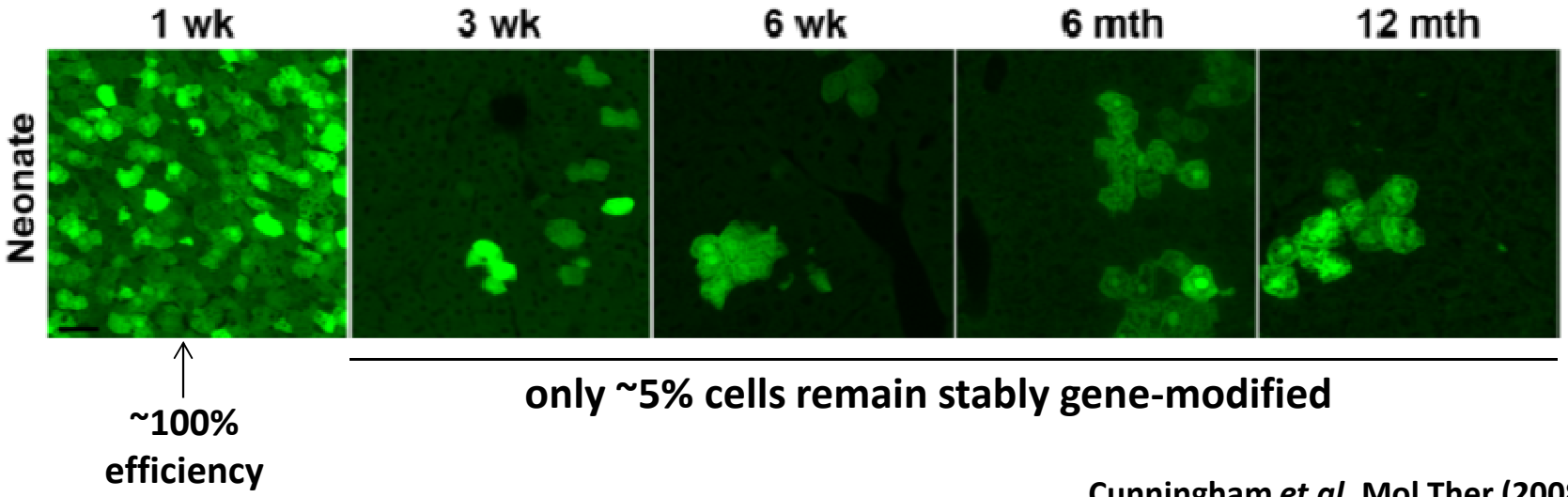
- “cell autonomous” (made and functions within the same cell)
- Minimum threshold of cells need to be fixed **AND** maintained (challenge in the growing liver with our system)

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).

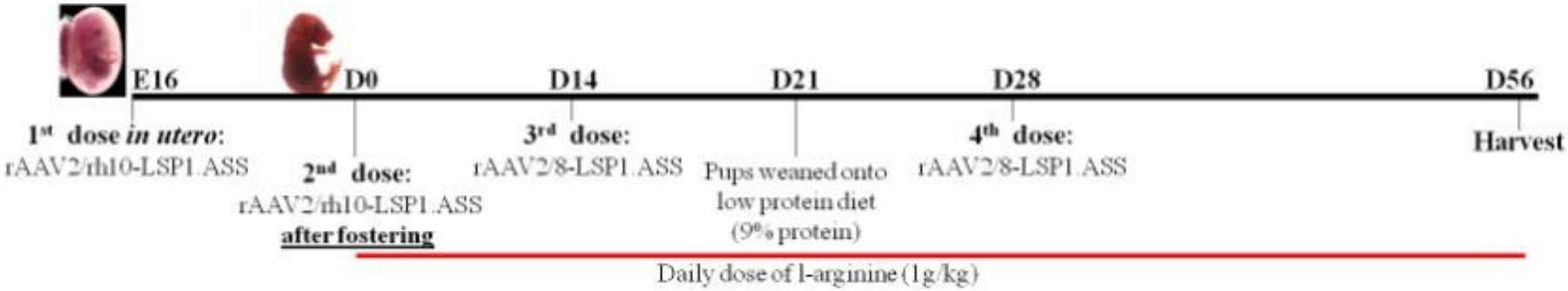
Mouse liver sections showing eGFP-expressing cells



Cunningham *et al.* Mol Ther (2008)

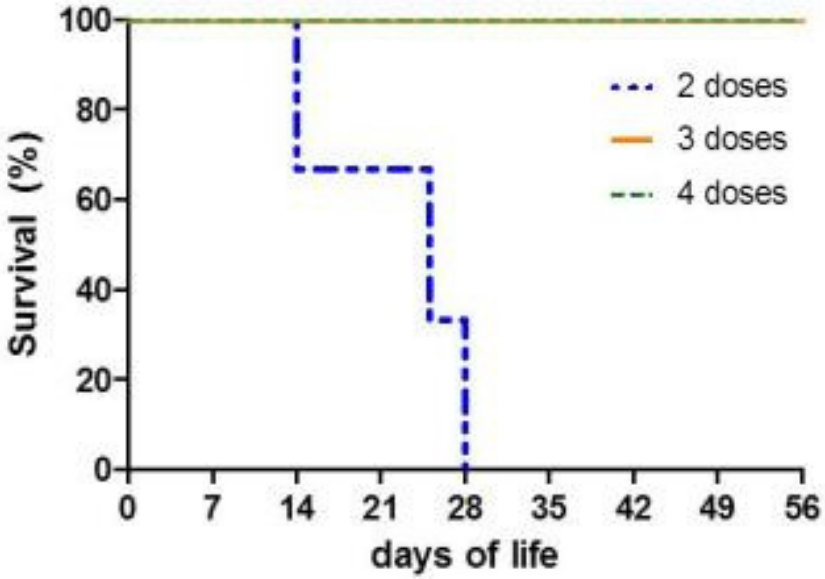
The minimum threshold for correction can be achieved in the growing liver by vector re-delivery

- Cindy Kok (PhD student)
- Mouse model of Citrullinaemia (ASS deficiency – another UCD)
- Neonatal lethal - mice die within 24 hours with elevated blood ammonia.

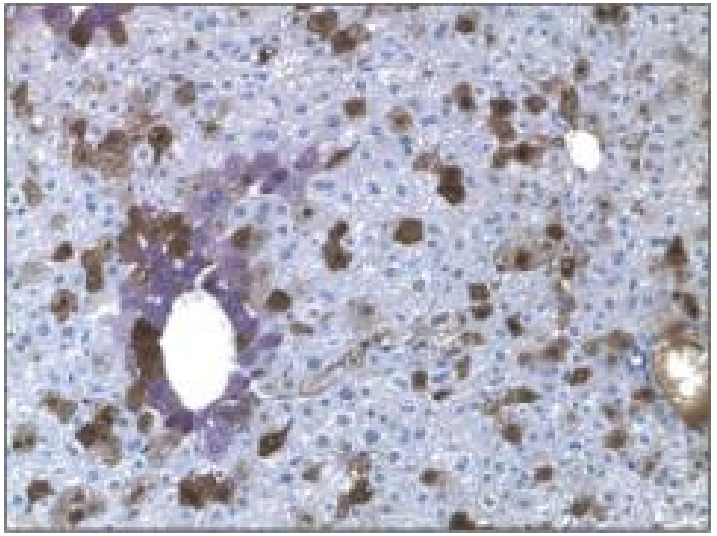


The minimum threshold for correction can be achieved in the growing liver by vector redelivery

Survival



Liver section



15% wt ASS activity

25% gene-modified cells

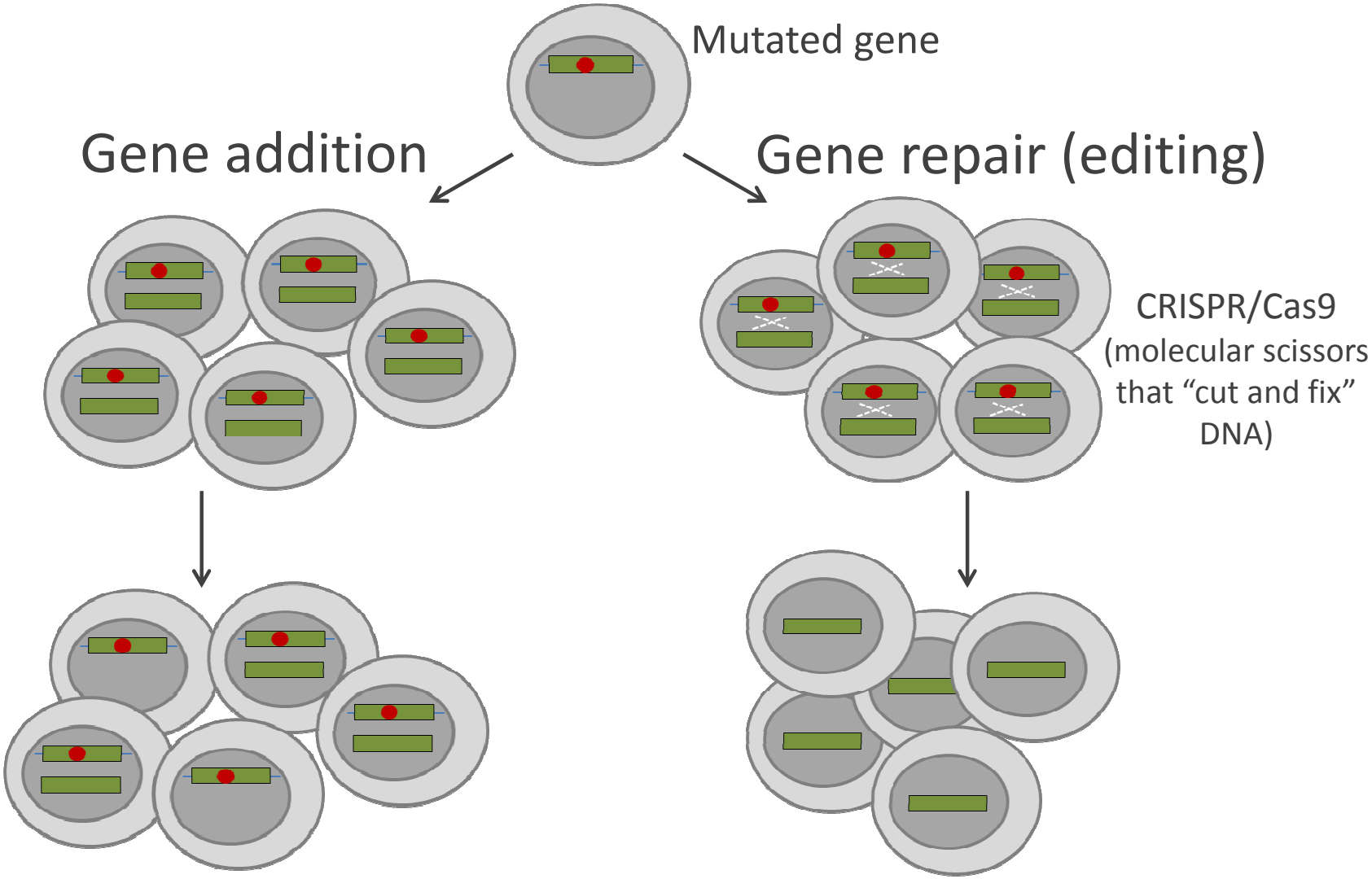
2 doses – sick within 2-4 wks

3 and 4 doses – did not get sick

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



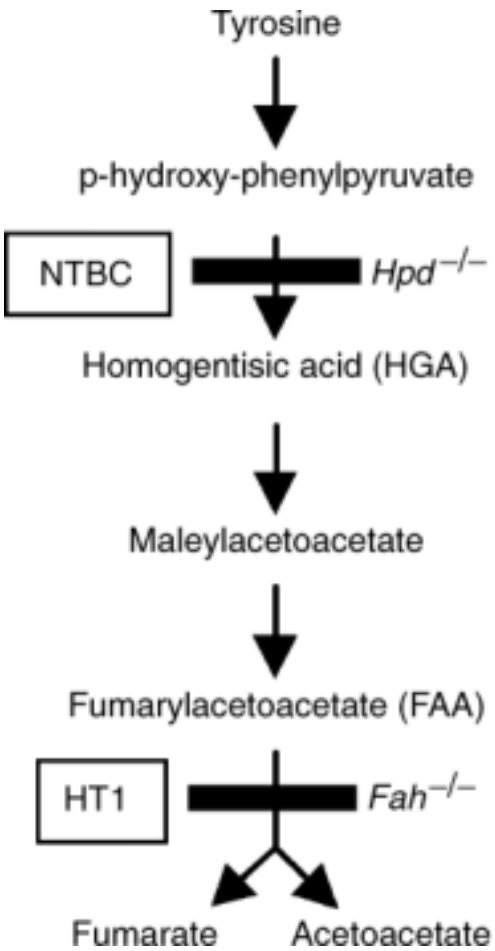
A mouse model with “humanised” mouse liver

FRG mouse (Tyrosinaemia Type 1):

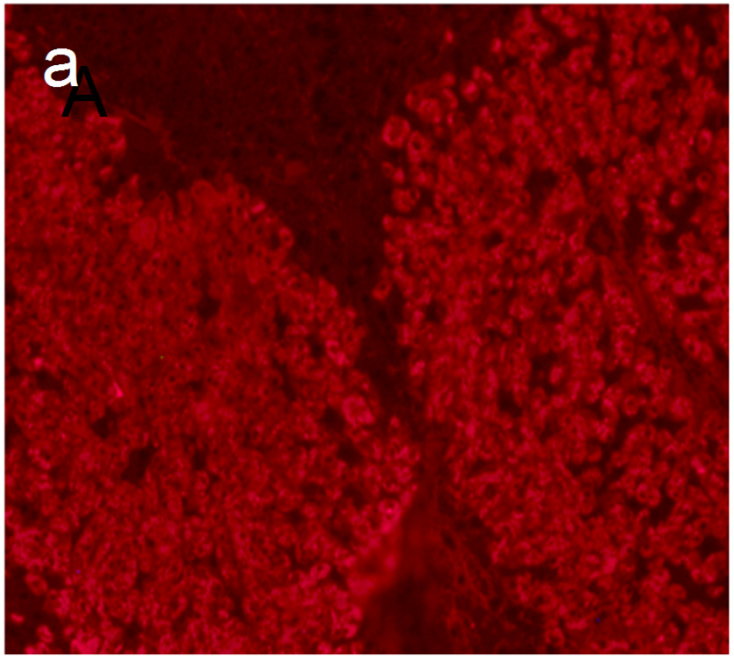
- Human hepatocytes can be engrafted and selectively expanded – “humanised mouse liver”
- Immunodeficient (no rejection of human cells)
- Fah-negative (expand “normal” cells)

Building a repository of human hepatocytes with metabolic deficiencies:

OTC, CPS1, ASL

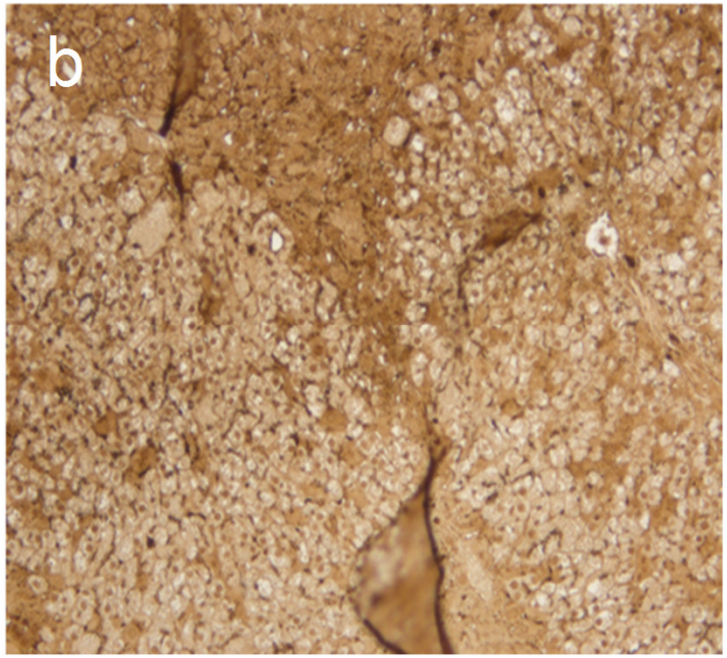


Mouse liver engrafted with OTC-deficient human liver cells



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

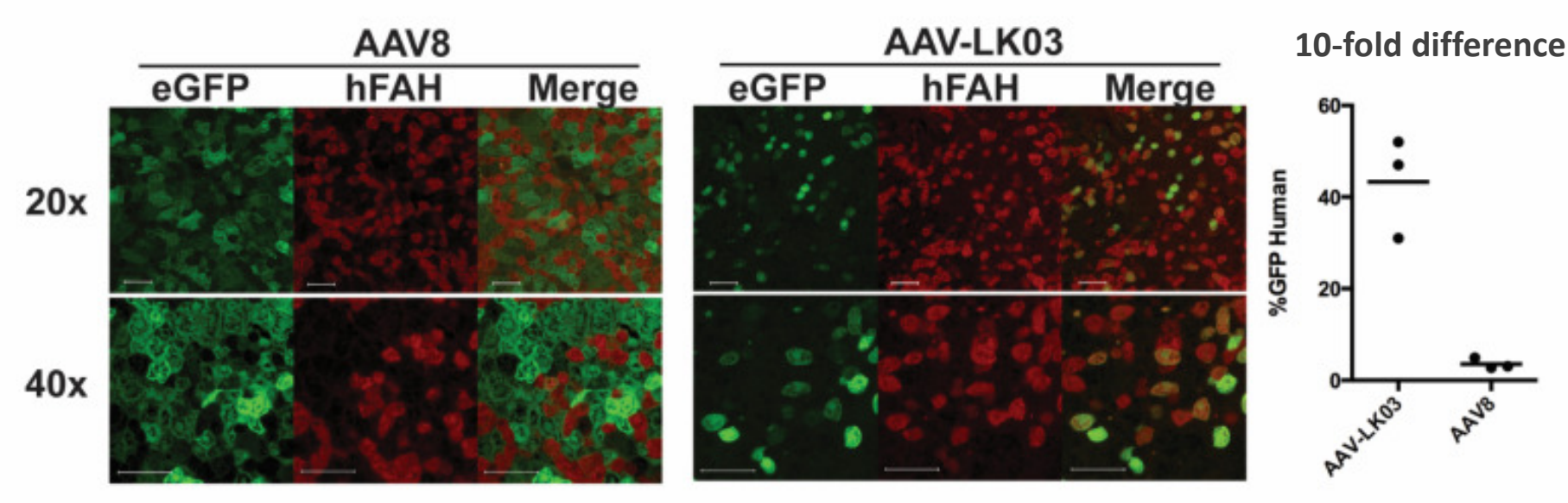
Red cells = human cells



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

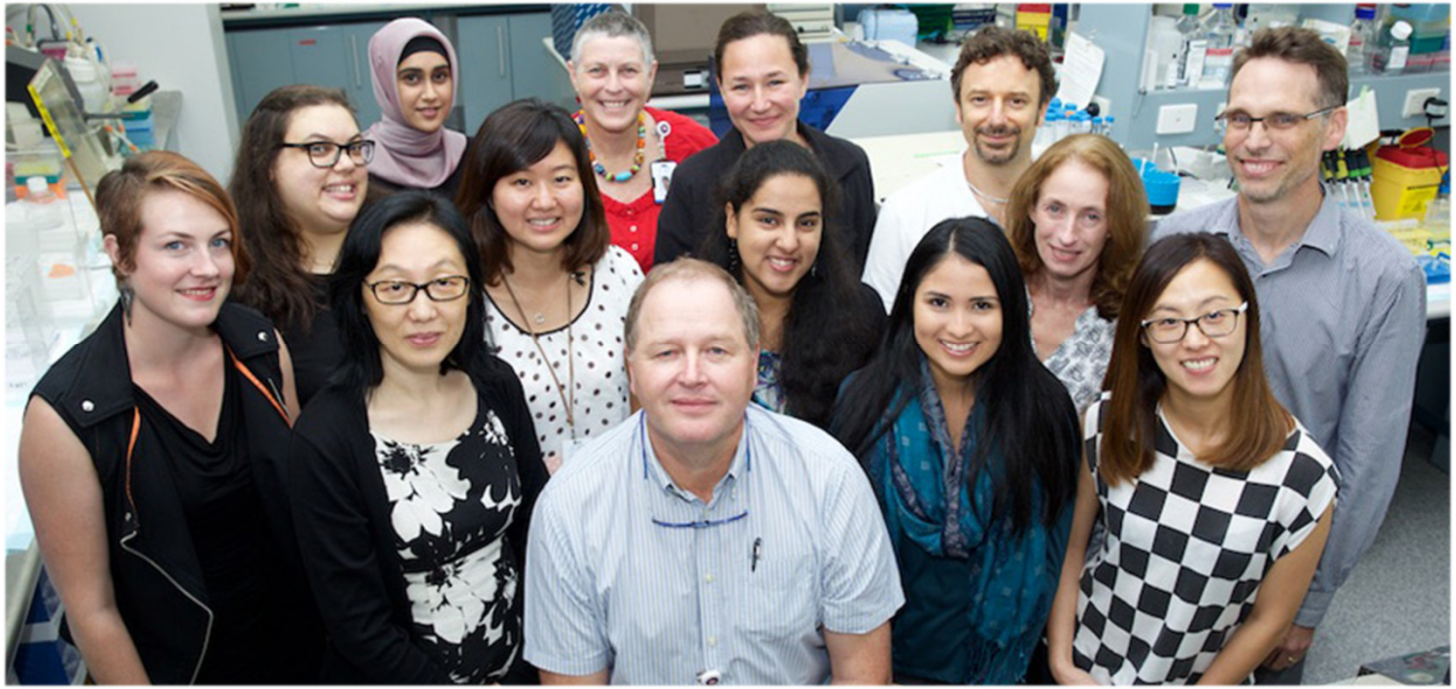


⇒ *AAV-LK03 is our vector of choice for our OTC clinical trial in paediatric patients.*

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.

Acknowledgements



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Markus Grompe	OSHU
David Russell	University of Washington
Rob Kotin	NIH
Paul Gissen	UCL / GOSH
Adrian Thrasher	UCL / GOSH