Cell Based Therapy for α1-antitrypsin deficiency

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Clinical Lecturer
Department of Medicine
Cell Based Therapy for α1-antitrypsin deficiency

• I have no conflicts of interest to declare
Overview

Patient

Patient-derived somatic cells

Reprogramming factors

Patient-specific iPS cells

Directed differentiation

Hepatocyte

Drug screening for disease-specific therapeutics

Transplantation (autologous)
$\alpha_1$-antitrypsin deficiency (Z)

- Chromosome 14
- Serapina 1 gene
- Polymerisation
- Hepatic accumulation
- Disease

$^{342}$Glu to Lys
Single ‘curative’ treatment

Whole organ transplantation
Liver transplantation

1. **Short term** – 10% operative risk

2. **Long term** - Immune suppression related morbidity

3. **Big Challenge** – donor shortage
Alternative to whole organ transplant?
Cell therapy - competitive advantage of wild type cells

Ding et al JCI 2011
1. Unlimited numbers
2. Any cell
3. Patient specific
4. Autologous
5. Less ethical concerns

Thompson (1998)

Somatic cells (Skin Cells)

Oct-4 / Sox2 / Klf4 / C-Myc

iPSCS

Yamanaka (2006)
Reprogramming patient skin samples

Rashid et al., J Clin Invest 2010
hIPSCs can be differentiated in vitro into hepatocytes

- **hIPSCs**
  - Day 0
  - CDM + Activin/FGF

- **Endoderm**
  - Day 1 to Day 3
  - CDM + Activin/FGF/BMP4/Ly

- **Hepatic specification**
  - Day 4 to Day 8
  - RPMI + Activin

- **Hepatocyte maturation**
  - Day 9 to Day 25
  - Maturation medium + HGF + OSM

Images show the progression from hIPSCs to hepatocytes.
Gene expression profiles replicate mammalian development

Rashid et al, J Clin Invest 2010
Protein expression replicates mammalian development

Rashid et al, J Clin Invest 2010
hiPS derived cells share key functions of adult hepatocytes

Albumin

Glycogen

LDL incorporation

Albumin secretion

Cyp3A4 activity

Rashid et al, J Clin Invest 2010
Staining for mutant AAT confirms disease specific phenotype

Z

All AAT  Polymeric AAT  All + Polymeric
Overview

Patient

- Patient-derived somatic cells

Reprogramming factors

- Patient-specific iPS cells

Directed differentiation

- Hepatocyte

- Drug screening for disease-specific therapeutics

- Transplantation (autologous)
Gene correction strategy

Zinc finger nuclease

Donor correction vector

Sangamo (California, USA)

Kosuke Yusa (Bradley Lab, Sanger)
Clean correction of single point mutation

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% Bi allelic correction

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Clinical grade reprogramming
Genetic stability (Karyotype)
Genetic stability (aCGH)

- Agilent 244k array -
### Genetic stability (single base pair)

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Gene correction prevents mutant AAT formation

Rashid* & Yusa* et al., Nature 2011
Gene correction prevents AAT entrapment in the ER

Rashid* & Yusa* et al., Nature 2011
Gene correction restores AAT enzymatic function
Testing cell functionality in vivo

Corrected IPS - hepatocytes

Liver

uPA

Liver

FRG
Post engraftment function

ELISA

IMMUNO

Human ALBUMIN

Human AAT

Rashid* & Yusa* et al., Nature 2011
## Engraftment in different murine models

### Primary

![Graph showing engraftment over weeks after transplantation]

*Azuma et al NBT 2007*

### IPS

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<th>No of mice</th>
<th>Avg duration</th>
<th>Avg Albumin</th>
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<td>20</td>
<td>26 weeks</td>
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*Rashid et al (unpublished)*
IPS derived cell engraftment = fetal engraftment and **no tumours**

- **Fetal primary hepatoblasts**
  
  hAlbumin = ng/ml

  *Haridass et al. Am J Pathol. 2009*

- **Fetal cultured liver progenitors**
  
  hAlbumin = ng/ml

  *Strick-Marchand et al. Unpublished*

- **ES derived hepatocytes**
  
  hAlbumin = ng/ml

  *Touboul et al. Hepatology 2010*

- **iPS derived hepatocytes**
  
  hAlbumin = ng/ml

  *Rashid & Yusa et al. Nature 2011*

- **Primary adult hepatocytes**
  
  hAlbumin = μg/ml to mg/ml
Clinical prospects – cells + niche

1. Acute
2. Chronic
3. Cancer

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<td>niche</td>
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Re-defining the niche (ECM) in chronic liver disease by semi-quantitative mass spec

**Human fibrotic liver tissue**

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<td><strong>ADAMTS1</strong> ADAMTS-1</td>
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<td><strong>SDF1</strong> Stromal cell-derived factor 1</td>
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**Rashid & Humphries et al J Prot Research 2012**
Clinical prospects – cells + niche

1. Acute
   - ALF

2. Chronic
   - Inherited
   - Mild
   - Severe

3. Cancer
   - Childs A

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Summary

- Patient specific hIPSC-heps from skin
- Disease model
- Clean genetic correction
- Erasure of phenotype in vitro
- Functionality in vivo
- Safety validation
- Niche to transplant cells
- Patient selection
Patients

Supervisors
David Lomas, Ludovic Vallier, Allan Bradley, Martin Humphries

Collaborators
Helene Strick-Marchand, James di Santo (Pasteur, Paris)
Graeme Alexander & Dept of Hepatology
Andrew Bradley & Dept of Surgery
Sir Aaron Klug & Sangamo (USA)
DNAVec (Japan)
Floriane Fusil, Francois Loic Cosset (Lyon)

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