Cellular therapy to Tweak Liver Regeneration

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I have no financial relationships to disclose within the past 12 months relevant to my presentation.
The need for therapy for liver disease

Deaths from cirrhosis have doubled during the past ten years

In the UK, Cirrhosis now kills more women than cervical cancer

Deaths from chronic liver disease cause 1 in 50 of all Scottish deaths

Mortality from Liver Disease

Transplantation
Steady supply : Rising demand

Leon and McCambridge Lancet 2006 52-56

Leon Lancet 2006

NHSBT 2012
Potential targets for stem cell therapy

- Fulminant liver failure
- Metabolic liver disease
- End stage liver cirrhosis
- Liver cirrhosis plus cancer
Hepatocyte transplant for metabolic liver disease

Early hepatocyte transplant reports in non cirrhotics

<table>
<thead>
<tr>
<th>Ref</th>
<th>Disease</th>
<th>Age at LCT (months)</th>
<th>Viable cells (x10⁶)</th>
<th>% of fresh cells</th>
<th>Immunos</th>
<th>Follow-up</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Ref. Prat</td>
<td>120</td>
<td>7.5</td>
<td>100</td>
<td>Tacro (10–15) + Pred</td>
<td>↓ 60% bilirubin, liver (a) for enzyme activity</td>
<td>Persistence up to 11 months</td>
</tr>
<tr>
<td>7</td>
<td>Ref. Tavakkol</td>
<td>96</td>
<td>6</td>
<td>32</td>
<td>Tacro (6–8) + Pred</td>
<td>↓ 40% bilirubin for 6 months</td>
<td>CLT after 20 months</td>
</tr>
<tr>
<td>8</td>
<td>Ref. Sveda</td>
<td>108</td>
<td>7.5</td>
<td>100</td>
<td>Tacro + Pred</td>
<td>↓ 50% bilirubin for a few weeks</td>
<td>CLT after 8 months</td>
</tr>
<tr>
<td>9</td>
<td>Ref. Kajdas</td>
<td>18</td>
<td></td>
<td>100</td>
<td>Tacro + Pred</td>
<td>↓ 50% bilirubin for 7 months</td>
<td>Under F-up</td>
</tr>
<tr>
<td>10</td>
<td>Ref. Hubschke</td>
<td>20</td>
<td>4</td>
<td>30%</td>
<td>Tacro + Pred</td>
<td>↓ 30% bilirubin</td>
<td>Lost on F-up</td>
</tr>
<tr>
<td>11</td>
<td>Ref. Debreceni</td>
<td>9</td>
<td>2.2</td>
<td>18</td>
<td>Tacro + Pred</td>
<td>↓ 50% bilirubin for 5 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hyper-cholestodermia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Ref. Okada</td>
<td>336</td>
<td>1.1</td>
<td>100</td>
<td>None</td>
<td>↓ 20% cholesterol, LDL &amp; apo B</td>
<td>Persistence up to 28 months</td>
</tr>
<tr>
<td>13</td>
<td>Ref. Okada</td>
<td>144</td>
<td>1.3</td>
<td>100</td>
<td>None</td>
<td>No effect</td>
<td>Persistence up to 19 months</td>
</tr>
<tr>
<td>14</td>
<td>Ref. Okada</td>
<td>84</td>
<td>1.0</td>
<td>100</td>
<td>None</td>
<td>↓ 6% cholesterol, LDL &amp; apo B</td>
<td>Persistence up to 7 months</td>
</tr>
<tr>
<td>15</td>
<td>Ref. Okada</td>
<td>452</td>
<td>3.2</td>
<td>100</td>
<td>None</td>
<td>Minor effect</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Ref. Okada</td>
<td>132</td>
<td>1.5</td>
<td>100</td>
<td>None</td>
<td>↓ 20% cholesterol, LDL &amp; apo B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Factor VII deficiency</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Ref. Okada</td>
<td>3</td>
<td>1.1</td>
<td>0</td>
<td>Tacro + Pred</td>
<td>↓ 80% need for factor VII up to 6 months</td>
<td>CLT after 7 months</td>
</tr>
<tr>
<td>18</td>
<td>Ref. Okada</td>
<td>35</td>
<td>2.2</td>
<td>50</td>
<td>Tacro + Pred</td>
<td>↓ 80% need for factor VII up to 6 months</td>
<td>CLT after 8 months</td>
</tr>
<tr>
<td>19</td>
<td>Ref. Okada</td>
<td>35</td>
<td>2.2</td>
<td>50</td>
<td>Tacro + Pred</td>
<td>↓ 80% need for factor VII up to 3 months</td>
<td>Under F-up</td>
</tr>
<tr>
<td>20</td>
<td>Ref. Okada (disease not specified)</td>
<td>564</td>
<td>2</td>
<td>100</td>
<td>Tacro + MMF</td>
<td>↑ fasting time from 3 to 7 h, ↓ 30–40% triglyceride</td>
<td>Persistence up to 18 months</td>
</tr>
<tr>
<td>21</td>
<td>Ref. Okada (disease not specified)</td>
<td>48</td>
<td>2</td>
<td>50</td>
<td>Tacro + Pred</td>
<td>↓ 40% of abnormal metabolites</td>
<td>Persistence up to 18 months</td>
</tr>
<tr>
<td>22</td>
<td>Ref. Okada (disease not specified)</td>
<td>0.3</td>
<td>100</td>
<td>Tacro + Pred</td>
<td>No clear benefit</td>
<td></td>
<td>Persistence up to 18 months</td>
</tr>
<tr>
<td>23</td>
<td>Ref. Okada (disease not specified)</td>
<td>0.3</td>
<td>100</td>
<td>Tacro + Pred</td>
<td>No clear benefit</td>
<td></td>
<td>CLT after 5 months</td>
</tr>
</tbody>
</table>
iPS Mediated Hepatocyte Generation

Induced Pluripotent Stem Cells

- Reprogramme adult cells to somatic stem cells

Donor cell → iPS induction → +/- Genetic Manipulation → Pluripotent stem cell → Differentiation + Expansion → Hepatocytes

- Potential for hepatocyte therapy
  - Transplantation
  - Extracorporeal devices

Hepatocyte transplantation during cirrhosis

Problem of cell based therapy in cirrhosis

Cell transplantation reports in decompensated liver disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age at LCT (yr)</th>
<th>Number of cells (10^7)</th>
<th>Route of infusion</th>
<th>Encephalopathy</th>
<th>Results and outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha1AT deficiency</td>
<td>52</td>
<td>2.2</td>
<td>Spleen</td>
<td>4</td>
<td>UNH4, OLT after 2 days</td>
</tr>
<tr>
<td>Alpha1AT deficiency</td>
<td>0.3</td>
<td>0.8</td>
<td>Spleen</td>
<td>1</td>
<td>OLT after 4 days, Enceph</td>
</tr>
<tr>
<td>HCV</td>
<td>40</td>
<td>0.8</td>
<td>Spleen</td>
<td>4</td>
<td>Death on day 2, UNH4</td>
</tr>
<tr>
<td>TPN/sepsis</td>
<td>0.5</td>
<td>5.2</td>
<td>Spleen</td>
<td>4</td>
<td>Death on day 7, Discharged</td>
</tr>
<tr>
<td>Cirrhosis – alcohol</td>
<td>62</td>
<td></td>
<td>Spleen</td>
<td>2–3</td>
<td>Death after 33 days</td>
</tr>
<tr>
<td>Cirrhosis – alcohol</td>
<td>46</td>
<td></td>
<td></td>
<td>2–3</td>
<td>Death after 50 days</td>
</tr>
</tbody>
</table>
Bone marrow derived therapy for liver disease

Bone marrow-derived mesenchymal stem cells normalized experimental liver fibrosis in rats

Dong-Chang Zhao, Jun-Xia Lei, Rui Chen, Wei-Hua Yu, Xi-Ming Zhang, Xian-Yuan Li

A subpopulation of bone marrow cells depleted by a novel antibody, anti-Liv8, is useful for cell therapy to repair

Naoko Kitazaki

Purified hematopoietic stem cells can differentiate into hepatocytes in vivo

Eric L. Weissman & Markus Grompe

© 2000 Nature America Inc. • http://medicine.nature.com

Therapeutic Application

Hanako Yamamoto, Gary Quinn, Akira Asari, Hiroko Yamanokuchi, Takumi Teratani, Masaaki Terada, and Takahiro Ochiya
Cell therapy to Influence Regeneration

Stem cell mediated regeneration
Proliferation and cell fate choice
Liver stem cells

- Two tier regeneration
  - Differentiated parenchyma
  - Stem cells
Liver stem cells

- Two tier regeneration
  - Progenitor Cells

Massive or Chronic injury

- Hepatocytes
- Stem Cell
- Bile duct
Macrophages and the HPC niche

Stereotypical niche of macrophages and myofibroblasts extends with HPCs

Recruitment of circulating macrophages to the sites of regeneration

A

Female wild type

Baytril 4 weeks

Baytril 4 weeks

3 weeks recovery

CDE 2 weeks

10.5 Gy irradiation

BM injection from male donor

Tissue analysis

B

panCK Y Chr DAPI

F4/80 Y Chr DAPI

HPCs Y Chromosome
Leukocytes as a potential regenerative therapy

Can Macrophages influence HPC behaviour?

- Macrophage infiltration is a key event in any injured and regenerating tissue
- BMC therapy has shown promising results in human chronic liver disease
- Effects include increased cellular proliferation and improved liver function

Could BMC therapy be exerting its effects by paracrine signalling of macrophages to HPCs?
HPC activation induced in the healthy liver following bone marrow infusion

- BMC
- Healthy mice
- Activation of HPCs

**Graph:**
- **HPC activation following BMC transfer**
- **Mean number of Ck^+ HPCs per field**
- **Days following BMC transfer**
  - Control
  - 3
  - 7
  - 42
  - Irradiated 42

* indicates a significant difference.
Macrophages as conductor of liver regeneration

Role of Notch signalling during biliary regeneration

Macrophages induce HPC expression of NUMB via Wnt

Human NUMB expression

Macrophages express Wnt upon ingestion of dead hepatocytes

Murine NUMB expression

Wnt results in HPC expression and hepatocyte differentiation

Macrophage direction of HPC fate decisions

Model of macrophage direction of HPC differentiation

Biliary Injury Model

Hepatocyte Injury Model

Depletion of macrophages

Cell therapy to Influence Regeneration

Bone Marrow Therapy and Fibrosis
Fibrosis is inhibitory to regeneration

Use of mouse expressing resistant collagen

Col1a1<sup>rr</sup> collagenase (MMP2, MMP8, MMP13) resistant mouse

CDE DIET MODEL OF LIVER INJURY
Performed with baseline fibrosis

Kallis et al. Gut 2010
Fibrosis is potentially reversible

Removal of underlying aetiology may allow reversal of fibrosis

- viral hepatitis
- alcohol
- metabolic disease
- autoimmune disease
- fatty liver

BM derived macrophages (MØ) populate hepatic scars during resolution

Deletion of macrophages during recovery reduces fibrosis reversal
Macrophage infusion reduces fibrosis

12 week CCl$_4$ model of fibrosis with macrophage therapy at 8 weeks

Transient donor macrophage engraftment

Y chromosome FISH 1 day

GFP tracking 1 day
Effects of macrophage during injury

Reduction in myofibroblasts

Transient increase in scar degrading MMP production
Summary of macrophage effects during liver disease

- Activation of stem cell
- Reduction in fibrosis
- Debris ingestion and progenitor fate control

Bile duct

Reduction in fibrosis and progenitor fate control
Activation of stem cell
Ongoing multicentre clinical trial if bone marrow derived therapy

A Multicentre, Phase II, Open Label, Randomised Controlled Trial of Repeated Autologous Infusions of G-CSF mobilised CD133+ Bone Marrow Stem Cells in patients with Cirrhosis

Currently recruiting
Realistic study protocol

Recruitment: Age 18-70, MELD 12-15, Compensated Cirrhosis, Aetiology

Baseline: MELD, UKELD, ELF, Fibroscan, QoL

Randomisation: Stratified by Site and Aetiology

Treatment Group 1
n = 27

Standard Conservative Management

Treatment Group 2
n = 27

5d GCSF 15µg/kg/day SC (D1-5)

Leukapheresis, isolation of CD133+ cells, Aliquotted and Frozen (D4/5)

Reinfusion of 0.2x10^6/kg
CD133+ sorted cells via periph vein between (D6/10)

Reinfusion of 0.2x10^6/kg
CD133+ sorted cells via periph vein (D30)

Reinfusion of 0.2x10^6/kg
CD133+ sorted cells via periph vein (D60)

Day 90: MELD, UKELD, ELF, Fibroscan, QoL

Secondary Follow Up (3 monthly until 12 months)
MANY THANKS

Stuart Forbes
John Iredale
Owen Sansom
Stephi Lorenzini
Luke Boulter
Wei-Yu Lu
Belinda Knight
Lam Chan

REALISTIC study investigators