StemBANCC: iPSC Models for Drug Discovery & Safety Assessment

Overview of the Project - October, 2015

STEM cells for Biological Assays of Novel drugs and predictive toxicology

Jim Ross, University of Edinburgh
Quantum Change: Patient iPSCs* – disease modelling

Sir John Gurdon and Shinya Yamanaka
Nobel Prize in Physiology or Medicine 2012

*induced pluripotent stem cells

Grskovic et al. Nature Reviews, Dec 2011

This project has received support from the IMI Joint Undertaking (GA n°115439); financial contributions from FP7/2007-2013 and EFPIA in kind contributions
Stem cells in Discovery and Safety

Pluripotent Cells (hESCs* and iPSCs*): have the potential to differentiate into any tissue of an adult.

*hESC: human embryonic stem cells, iPSCs: induced pluripotent stem cells
## POC: iPS cells and disease

<table>
<thead>
<tr>
<th>HEART</th>
<th>LIVER</th>
<th>NEURON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long QT Type I</td>
<td>Alpha I Antirtrypsin deficiency</td>
<td>Spinal muscle atrophy</td>
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<tr>
<td>Leopard Syndrome</td>
<td>Familial Hypercholesteronemia</td>
<td>Familial Dysautonomia</td>
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<td>Pompe Disease</td>
<td>Glycogen storage disease type Ia</td>
<td>Parkinson Disease</td>
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<td>Hypertrophy</td>
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<td>Huntington’s disease</td>
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<tr>
<td>Timothy Syndrome</td>
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<td>Rett Syndrome</td>
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</table>

| | Raval et al. (2010) | Ebert et al. (2009) |
| | Foldes et al. (2010) | Lee et al. (2009) |
| | Yazawa, et al. (2011) | Schneider et al. (2007) |
| | | Chan et al. (2010) |
| | | Marchetto, et al. (2010) |
| | | Agarwal et al. (2010) |

*Adapted from from Morrow & Holder, Drug Discovery World. 2010/2011*
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Grskovic et al. Nature Reviews, Dec 2011

StemBANCC Project - Overview

500 Patients

fibroblasts

Sendai virus system

1,500 iPSC lines

Disease Areas
- Diabetes
- PNS
- CNS
- Toxicology

Identify phenotypes,
Develop differentiation protocols & assays . . .
StemBANCC at a Glance

- IMI: Innovative Medicine Initiative / EFPIA
- Consortium of 10 Pharma and 25 Academic/SME partners
- Start date: Autumn 2012
- Duration: 5 years
- Total cost: €55.6 million
- Project coordinator: Martin Graf, F. Hoffmann-La Roche Ltd
- Managing entity: Zam Cader, University of Oxford
- [www.stembancc.org](http://www.stembancc.org)
This project has received support from the IMI Joint Undertaking (GA n°115439); financial contributions from FP7/2007-2013 and EFPIA in kind contributions.
Workpackages

WP1: Management & Administration

WP2: Subject recruitment
- A) DIABETES
- B) PNS
- C) CNS
- D) Tox

WP3: Reprogramming & QC of iPSC
WP4: Biobanking and distribution

WP5: Molecular profiling (patient & IPS) (OMICS)

WP6: Data Management & Interpretation

WP7: Cell diff. PNS
WP8: Cell diff. CNS
WP9: Cell diff. DIABETES
WP10: Cell diff. Toxicology

WP11: Assay development, validation & scaling

WP12: Communication & dissemination

Diabetes  PNS  CNS  Toxicology
Subject Recruitment

**Neuropathy**: Pain channelopathies (SC9A, TRPA1, TRESK), Motor Neuropathy (GARS, HSP27), Diabetic neuropathy

**Alzheimer’s**: Monogenic (PS1, PS2, APP, MAPT, C90rf); Sporadic (ApoE4 homozygotes, ApoE4 heterozygotes, ApoE other, not yet genotyped, Others (e.g. TREM)

**Parkinson’s**: Monogenic (SCNA, LRRK2, GBA, Gaucher, Parkin, PINK1); Sporadic (PD dementia, others)

**Autism**: Non-synaptic CNVs, high functioning

**Schizophrenia**: CNVs/GWAS, sporadic

**Bipolar**: Treatment responsive, treatment resistant

**Migraine**: Monogenic (FHM1 and FHM2), Familial MA/GWAS, Sporadic

**Diabetes**: Monogenic, Early onset familial T2D, Sporadic Typical T2D

**Drug metabolism**: Long QT, Brugada, DILI, others

**Healthy Volunteers** 60
Status – October 2015

• All important SOPs in place
  • For biopsy, iPS generation, cultivation of iPS etc.

• Patient recruitment forms / ethical documents in place

• Patient recruitment almost complete

• Edinburgh set up as recruitment centre for drug-induced liver injury, cardiopathies and Alport’s syndrome
### Timeline Overview

<table>
<thead>
<tr>
<th>Activity</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
<th>Year 5</th>
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<tbody>
<tr>
<td>Project management</td>
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<td>WP1</td>
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<tr>
<td>Establish ethics framework</td>
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<td>WP2</td>
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<tr>
<td>Provision of biomaterials</td>
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<td>500 subjects</td>
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<td>Provision of Clinical Phenotypes</td>
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<td>WP2</td>
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<tr>
<td>Establish reprogramming technology &amp; QC</td>
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<td>WP3</td>
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<tr>
<td>Reprogramming to generate 1500 iPSC lines</td>
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<td>300 monogenic</td>
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<td>Differentiation protocols and standards</td>
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<td>WP4</td>
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<td>iPSC Upscaling</td>
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<td>WP4</td>
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<td>iPSC Tools</td>
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<td>WP4</td>
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<td>Genotyping/Exome sequencing of 500 subjects</td>
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<td>WP5</td>
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<td>Omics profiling of 200 iPSC lines</td>
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<td>WP5</td>
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<td>Method development for WP5 and WP11</td>
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<td>WP5</td>
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<td>Data warehousing</td>
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<td>WP6</td>
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<td>Data interpretation</td>
<td>Pilot Data</td>
<td>Monogenic disease</td>
<td>Polygenic Disease</td>
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<td>WP6</td>
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<tr>
<td>Differentiation for WP5</td>
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<td>Monogenic lines</td>
<td>Polygenic lines</td>
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<td>WP7-10</td>
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<td>Cellular phenotyping</td>
<td>Method dvlpmnt</td>
<td>Monogenic lines</td>
<td>Polygenic lines</td>
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<td>WP7-10</td>
</tr>
<tr>
<td>Higher throughput phenotyping (e.g. HCS, MEA)</td>
<td>Method dvlpmnt</td>
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<td>WP11</td>
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<tr>
<td>Translation of phenotype to drug screening assay</td>
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<td>WP12</td>
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<tr>
<td>Communication</td>
<td></td>
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<td>WP12</td>
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</table>

Figure 3.1 – General timeline of StemBANCC

This project has received support from the IMI Joint Undertaking (GA n°115439); financial contributions from FP7/2007-2013 and EFPIA in kind contributions.
Key challenges to be addressed

Reprogramming primary cells to pluripotency with minimal ‘off-target’ effects

Producing enough differentiated cells for medium to high-throughput screening

Well-defined meaningful disease groups for iPSC generation

Useful assays and endpoints to characterise and test the iPSCs

Consistent and standardised protocols to achieve fully differentiated mature cells

Identify disease-relevant phenotypes in cell lines

Establish robust ethical and research governance framework to enable future industry-academic collaborations
WP 10 - Toxicology

Objective

• to generate **functionally mature** target cells of toxicological interest from human induced pluripotent (hiPS) cells, in a robust and scalable manner
• to test these lines in toxicological assays.

Toxicity pathway analysis

Jim Ross - University of Edinburgh
Nicole Clemann - Roche

renal cells  brain aggregates

cardiomyocytes  hepatocytes
iPS cells and toxicology

Target for creation of iPS cells is 1500 lines - from 500 individuals (control & disease)

Target for differentiation of hepatocyte-like cells is 100 lines using best current differentiation protocol

Analysis of differentiation using e.g. microarray analysis and RNAseq techniques (WP5)

Are stem cell-derived hepatocytes sufficiently mature to use them mechanistically and/or predictively?
Benchmarking for hepatocytes informed by studies on adult human hepatocytes

Insulin and counterregulatory hormones influence acute-phase protein production in human hepatocytes

Michael G. O’Riordan, James A. Ross, Kenneth C. H. Fearon, Jean Maingay, Marwan Farouk, O. James Garden, and David C. Carter

University Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW, United Kingdom

AJP 1995

Interleukin-8 can mediate acute-phase protein production by isolated human hepatocytes

Stephen J. Wigmore, Kenneth C. H. Fearon, Jean P. Maingay, Paul B. S. Lai, and James A. Ross

University Department of Surgery, Royal Infirmary of Edinburgh, Edinburgh EH3 9YW, United Kingdom

AJP 1997

Proteolysis-inducing factor regulates hepatic gene expression via the transcription factors NF-κB and STAT3

T. M. Watchorn, I. Waddele, N. Dowidar, and J. A. Ross

Molecular Immunology Group, Department of Clinical and Surgical Sciences, Edinburgh University, U.K.; and Cardiovascular and Gastrointestinal Discovery Department, AstraZeneca, Macclesfield, U.K.

FASEB J 2001

Development of assays to examine hepatocyte function
Benchmarking for hepatocytes

1. Hepatic export proteins by ELISA:
alpha-fetoprotein, albumin, pre-albumin (transthyretin), alpha-2-macroglobulin, fibrinogen, haptoglobin

2. Gene expression by PCR:
AFP [alpha-fetoprotein]; ALB [albumin]; TO [tryptophan dioxygenase]; HNF4 alpha; OCT4; CYP3A4 [cytochrome p450 3A4]; TAT [tyrosine amino transferase]; APOF [apolipoprotein F]; CYP7A1 [cytochrome p450 7A1]

3. Cytochrome p450 function:
CYP3A4, CYP1A2, CYP2C9, CYP2C19, CYP2D6 activities assessed using the p450-Glo kits from Promega.

4. Expression of membrane transporters:
BCRP (ABCG2), MRP2, MDR1 (by PCR, immunohistochemistry)

5. Ureagenesis
(by enzymatic assay)

Problems with current hepatocyte protocols:
differentiated hepatocytes, like freshly isolated adult human hepatocytes, have a very short life in culture - improve longevity?
do differentiated hepatocytes achieve mature function - improve function?
The project has received support from EFPIA companies and the European Union (IMI JU)

→ Priority is to improve the protocol

→ Comparison with ‘real’ hepatocytes

Comparison of FN Results

Comparison of FIB Results

comparison between lines & between clones

10.01 milestone completed M24
Benchmarking for hepatocytes

Training compounds - CYP450 inducing cocktail

<table>
<thead>
<tr>
<th>CYP</th>
<th>compound</th>
<th>uM (final)</th>
<th>stock (mM)</th>
<th>MW</th>
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<tbody>
<tr>
<td>2C19</td>
<td>S-Mephenytoin</td>
<td>25-100</td>
<td>125-500</td>
<td>218.252</td>
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<tr>
<td>2C9</td>
<td>Diclofenac</td>
<td>2-25</td>
<td>10-125</td>
<td>319.14</td>
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<tr>
<td>3A4</td>
<td>Midazolam</td>
<td>0.6-2</td>
<td>3-10</td>
<td>362.14</td>
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<tr>
<td>2D6</td>
<td>Dextromethorphan</td>
<td>2-10</td>
<td>10-50</td>
<td>370.33</td>
</tr>
</tbody>
</table>

CYP activities assessed using the p450-Glo kits from Promega. Improved and high throughput assays being developed with WP11

Are there specific toxicological endpoints and through-points that we should use for stem cell-derived hepatocytes? Which translatable biomarkers might help assess the physiological relevance of the cells and the relevance of their response to chemicals? Would a consensus panel of test chemicals (including concentrations-with relevance to PK, Cmax, AUC in animal models and time-courses) allow proper comparisons to be made across different stem cell projects? What should these chemicals be?
Placental factors influencing growth and differentiation of hepatocytes

- Stem cell niche(s)
- Side-population cells
- Bi-potential cells [hepatoblasts]
- Mesenchymal stem cells
- Haematopoietic stem cells
- Endothelial progenitors

Liver cells (hepatocytes)

Are stem cell-derived hepatocytes sufficiently mature to use them mechanistically and/or predictively?

And would co-culture with other liver cells/3D culture help to achieve this?
Cell interactions and soluble factors may be important

**hepatocytes**: short life in culture - cell interactions may be important both for longevity of mature hepatocytes and for improved function

Now have good evidence that hepatocyte/endothelial cell co-culture improves hepatocyte function in the developing liver.

Factors influencing growth and differentiation of hepatocytes

Now have good evidence that certain developmental factors can improve hepatocyte function and differentiation.
WP 10 - Toxicology
Hepatocytes - improve protocol

Generation of hepatocytes from iPSCs derived from healthy controls and specific diseases

- Adult cells
- Induced pluripotent stem cells
- Endoderm
- Liver cells (hepatocytes)

Co-culture approaches (e.g. endothelial cell) novel substrates/matrices (e.g. polymers) survival factors small molecules

http://www.stembancc.org
3D bioreactors for hepatic differentiation of hiPSCs

• Hollow-fibre capillaries for medium and gas perfusion
• Cells are cultured in the extra-capillary space
• Different sizes available from 0.5 mL to 800 mL

Studies underway to compare 2D, 3D and microspheroid culture

Hepatic differentiation of hiPSC in 2 mL bioreactors vs. 2D cultures (n=3)

Expression of hepatic export proteins and CYP enzymes increased in 3D culture

The project has received support from EFPIA companies and the European Union (IMI JU)
Progress to date - Cardiomyocytes

On-going activities:

• Maturation of cardiac progenitors in cardiomyocytes without feeders
• Derivation of hiPS cells from different cardiac pathologies in progress
• Efficient cardiogenesis and scale-up of process to 100ml bioreactors

→ Priority is to improve the protocol & scale-up

10.02 milestone completed (M24)
Progress to date - renal lineages

Protocols to generate podocytes and proximal tubular-like cells developed

- PT-like cells express claudin 2 and cadherin 16, demonstrate organic cation transport
- Podocyte-like cells express the typical markers podocin and synaptopodin

→ Further work required to increase target cell purity and increase temporal phenotypic stability

10.04 milestone completed M36
Progress to date - cell differentiation into 3D brain aggregates

Main markers of neurons, astrocytes and oligodendrocytes expressed

On-going activities:
• assessment of cellular maturation and critical morphogenic events such as synaptogenesis and myelination

→ optimisation of differentiation protocol needed

Neuronal markers e.g. SYP
Astrocytic markers e.g. GFAP
Oligodendrocytic markers e.g. MBP

10.03 milestone completed M36

The project has received support from EFPIA companies and the European Union (IMI JU)
WP 10 – Next steps

• Work on improving protocols to provide more mature and functionally competent hepatocytes & cardiomyocytes
• Continue to develop protocols for renal cells and brain aggregates
• Expand proteomic, metabolic and RNA-seq studies with WP5
• Continue assay development & lab-on-a-chip studies with WP11
• Implementation of High content imaging
• Start comparative toxicity testing in renal and hepatic cells - pathway analysis
• Recruiting patients at UEDIN (adverse drug responders, Alport’s, cardiomyopathies)
WP 10 – Patient Recruitment

Patients being referred by Edinburgh clinicians.

Blood sampling and minimal dataset collected in Clinical Research Facility.

Samples processed in UEDIN (Ross lab).

Reprogramming in Oxford as priority 1 & 2.

16 Additional WP10 individuals to be identified from ‘healthy’ population (CYP polymorphisms, transporter polymorphisms, etc.) to bring total to n=40

Collaboration with WP2, WP3, WP6

LQT1 - 4 patients
LQT2 – 4 patients
LQT3 - 4 patients
Brugada – 4 patients
Alports syndrome – 4 patients
Drug Induced Liver Injury – 4 patients
(more if we want to include paracetamol)
StemBANCC

Steering Committee and StemBANCC Team leaders (Oct 2012)