StemBANCC:

iPSC Models for Drug Discovery & Safety Assessment

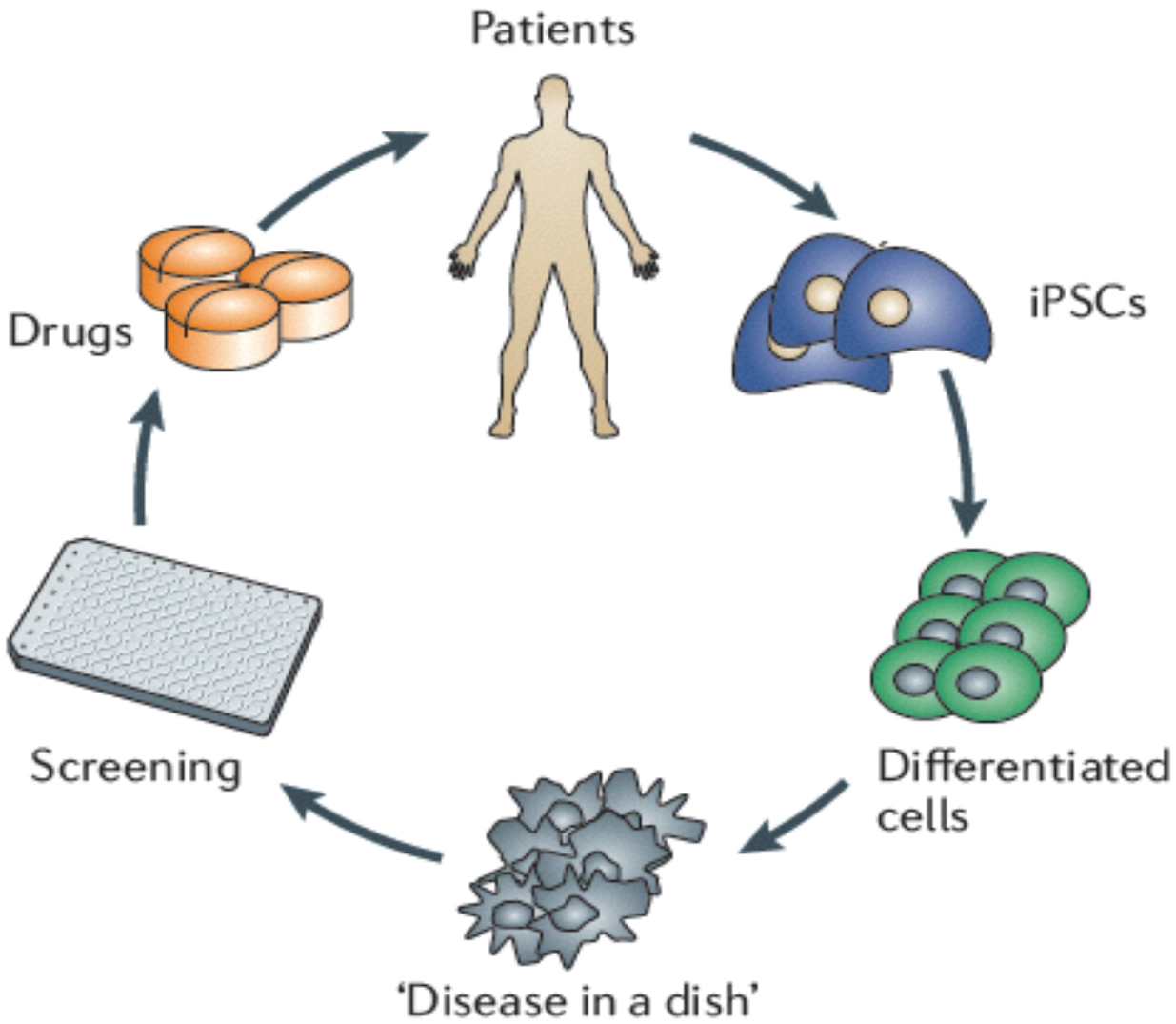
Jim Ross, University of Edinburgh

Overview of the Project - October, 2015

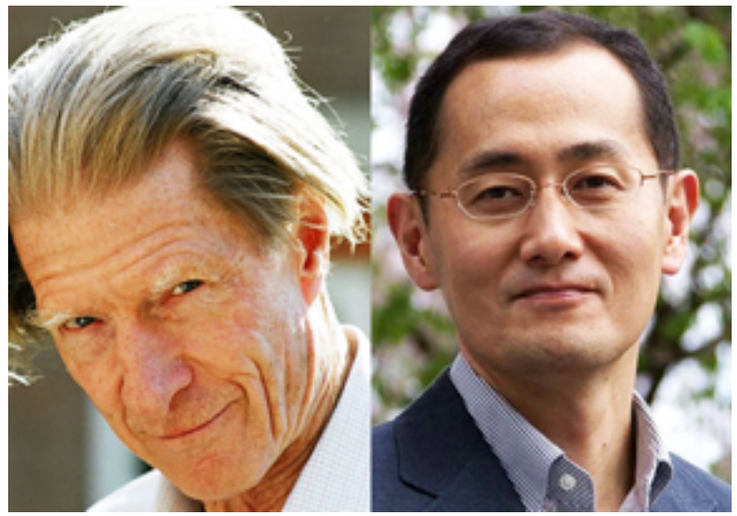
STEM cells for **B**iological **A**ssays of **N**ovel drugs and predi**C**tive toxi**C**ology

The project has received support from EFPIA companies and the European Union (IMI JU)

Quantum Change:
Patient iPSCs* – disease modelling



Grskovic et al. Nature Reviews, Dec 2011



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

Oct 4
Sox2
Klf4
c-myc

reprogramming factors

***induced pluripotent stem cells**

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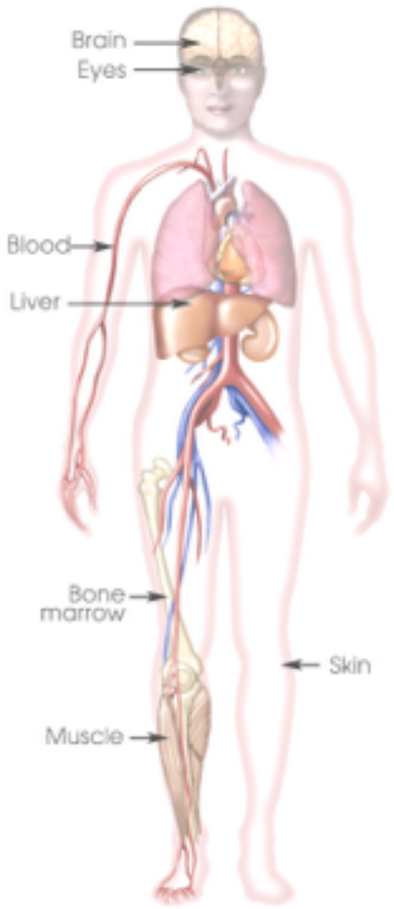
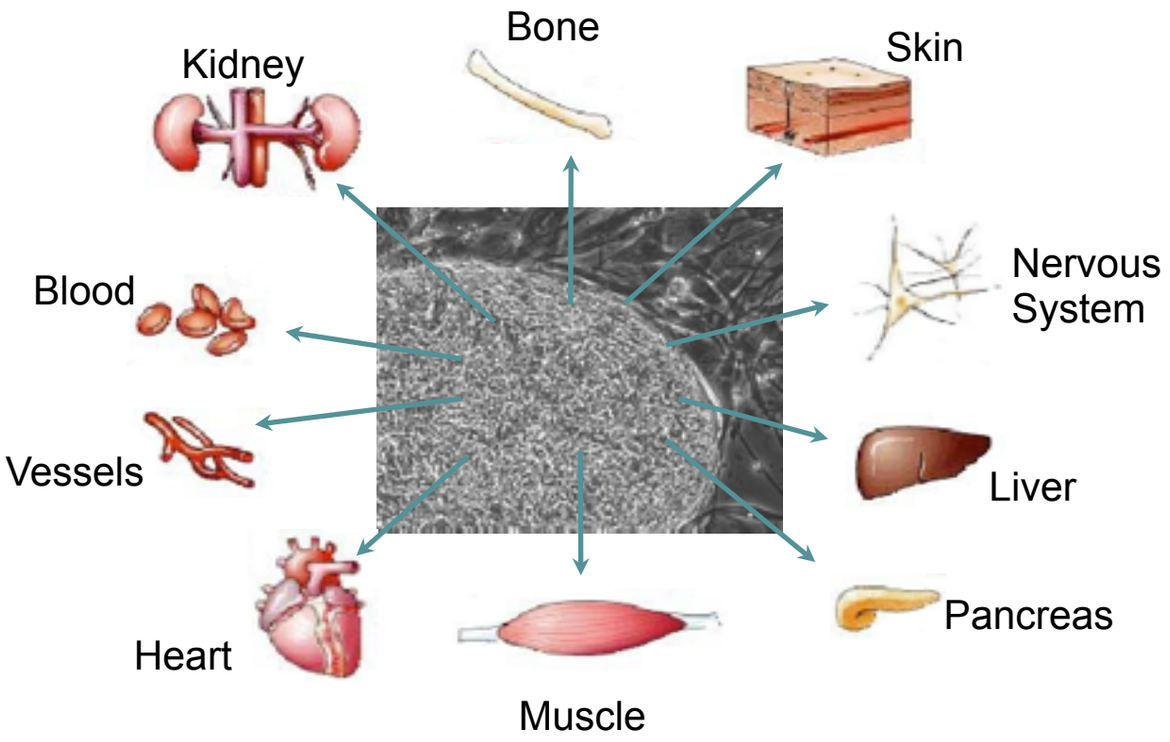
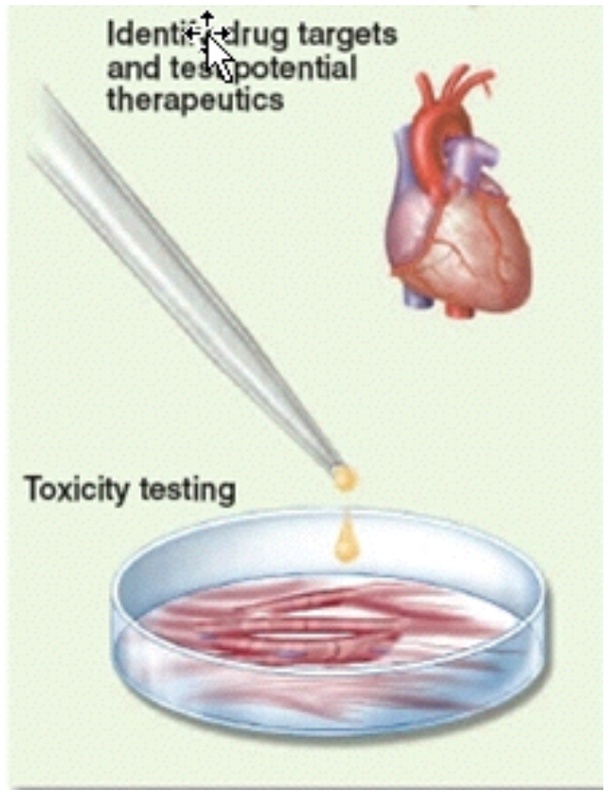
Stem cells in Discovery and Safety

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

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POC: iPS cells and disease

HEART	Long QT Type I Leopard Syndrome Pompe Disease Hypertrophy Timothy Syndrome		Moretti et al. (2010) Itzhaki, et al. (2011) Carvajal-Vergara et al. (2010) Raval et al. (2010) Foldes et al. (2010) Yazawa, et al. (2011)	
LIVER	Alpha I Antirtrypsin deficiency Familial Hypercholesteronemia Glycogen storage diesease type Ia		Rashid et al. (2010) Rashid et al. (2010) Rashid et al. (2010)	
NEURON	Spinal muscle atrophy Familial Dysautonomia Parkinson Disease Huntington‘s disease Rett Syndrome		Ebert et al. (2009) Lee et al. (2009) Schneider et al. (2007) Chan et al. (2010) Marchetto, et al. (2010)	
	Dyskeratosis (telomere shortening)		Agarwal et al. (2010)	

Adapted from from Morrow & Holder, Drug Discovery World. 2010/2011

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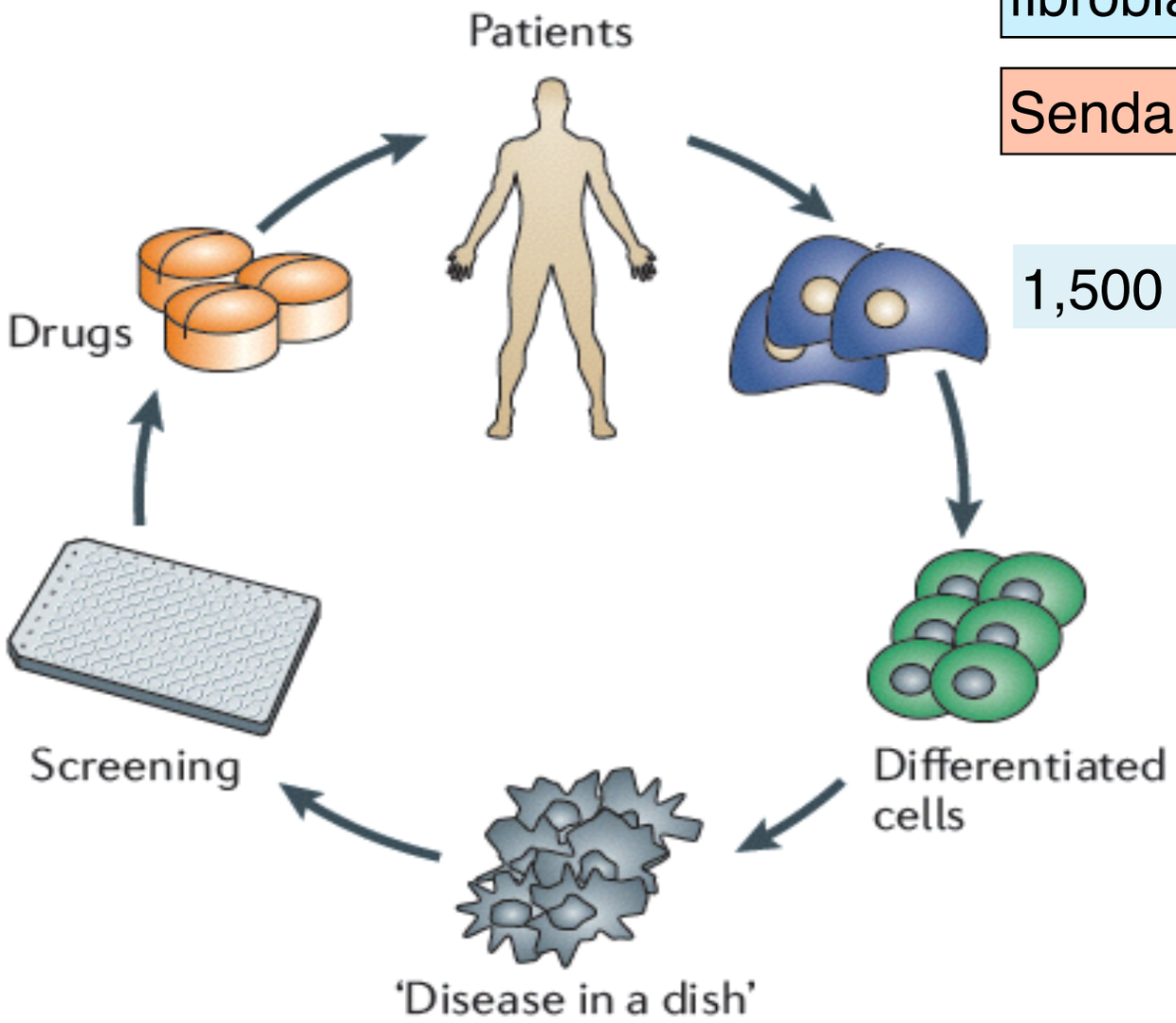
StemBANCC Project - Overview

500 Patients

fibroblasts

Sendai virus system

1,500 iPSC lines



Disease Areas
- Diabetes
- PNS
- CNS
- Toxicology

Identify phenotypes,
Develop differentiation protocols & assays . . .

Grskovic et al. Nature Reviews, Dec 2011

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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

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Partners

Company / Institution	IMI Acronym	Acad/EFPIA	Country
F. Hoffmann-La Roche	ROCHE	EFPIA	Switzerland
University of Oxford	UOXF	Academic	UK
concentris research management gmbh	concentris	SME	Germany
King's College London	KCL	Academic	UK
University College London	UCL	Academic	UK
Natural and Medical Sciences Intitute at the University of Tuebingen	NMI	Academic	Germany
Univercell-Biosloutions	UB	SME	France
Islensk Erfdagreining ehf	deCode	SME	Iceland
University of Edinburgh	UEDIN	Academic	UK
Region Hovedstaden	RegionH	NPRO/Public Body	Denmark
University of Birmingham	UoB	Academic	UK
Helmholtz Zentrum München	HMGU	NPRO/Public Body	Germany
Charité - Universitaetsmedizin Berlin	CHARITÉ	Academic	Germany
University of Luebeck	UniLuebeck	Academic	Germany
Newcastle University	UNEW	Academic	UK
Université de Lausanne	UNIL	Academic	Switzerland
Medizinische Universität Innsbruck	IMU	Academic	Austria
Université de Genève	UNIGE	Academic	Switzerland
INSERM	INSERM	NPRO/Public Body	France
University of Cambridge	UCAM	Academic	UK
Medizinische Hochschule Hannover	MHH	Academic	Germany
Tel Aviv University	TAU	Academic	Israel
Université de Technologie de Compiègne	UTC	Academic	France
Linköping University	LIU	Academic	Sweden
Abbott	ABT	EFPIA	Germany
Boehringer Ingelheim	BI	EFPIA	Germany
Janssen	JANSSEN	EFPIA	Belgium
Eli Lilly	Lilly	EFPIA	Switzerland
Merck Serono	Merck	EFPIA	Germany
Novo Nordisk	NN	EFPIA	Denmark
Orion Pharma	OP	EFPIA	Finland
Pfizer	Pfizer	EFPIA	UK
Sanofi-Aventis	SARD	EFPIA	France
Medical Research Council	MRC	NPRO/Public Body	UK
Hebrew University of Jerusalem	HUJI	Academic	Israel

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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 M'gment & Interpretaion

WP 9: Cell diff. DIABETES

WP 7: Cell diff. PNS

WP 8: Cell diff. CNS

WP 10: Cell diff. Toxicology

A) DIABETES | B) PNS | C) CNS | D) Tox

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	**35**
Alzheimer's: Monogenic (PS1, PS2, APP, MAPT, C90rf); Sporadic (ApoE4 homozygotes, ApoE4 heterozygotes, ApoE other, not yet genotyped, Others (e.g. TREM)	**70**
Parkinson's: Monogenic (SCNA, LRRK2, GBA, Gaucher, Parkin, PINK1); Sporadic (PD dementia, others)	**70**
Autism: Non-synaptic CNVs, high functioning	**40**
Schizophrenia: CNVs/GWAS, sporadic	**40**
Bipolar: Treatment responsive, treatment resistant	**40**
Migraine: Monogenic (FHM1 and FHM2), Familial MA/GWAS, Sporadic	**30**
Diabetes: Monogenic, Early onset familial T2D, Sporadic Typical T2D	**75**
Drug metabolism: Long QT, Brugada, DILI, others	**40**
Healthy Volunteers	**60**

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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

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Timeline Overview

	Year 1	Year 2	Year 3	Year 4	Year 5	
Project management						WP1
Establish ethics framework						WP2
Provision of biomaterials		500 subjects				
Provision of Clinical Phenotypes		500 subjects				
Establish reprogramming technology & QC						WP3
Reprogramming to generate 1500 iPSC lines		300 monogenic				
		1200 sporadic lines				
Differentiation protocols and standards						WP4
iPSC Upscaling						
iPSC Tools						
Genotyping/Exome sequencing of 500 subjects						WP5
Omics profiling of 200 iPSC lines						
Method development for WP5 and WP11						
Data warehousing						WP6
Data interpretation	Pilot Data	Monogenic disease				
			Polygenic Disease			
Differentiation for WP5		Monogenic lines				WP7-10
			Polygenic lines			
Cellular phenotyping	Method dvlpmnt	Monogenic lines				
	Method dvlpmnt		Polygenic lines			
Higher throughput phenotyping (e.g. HCS, MEA)	Method dvlpmnt					WP11
Translation of phenotype to drug screening assay						
Communication						WP12

Figure 3.1 – General timeline of StemBANCC

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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

definition of fully differentiated cell is difficult

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

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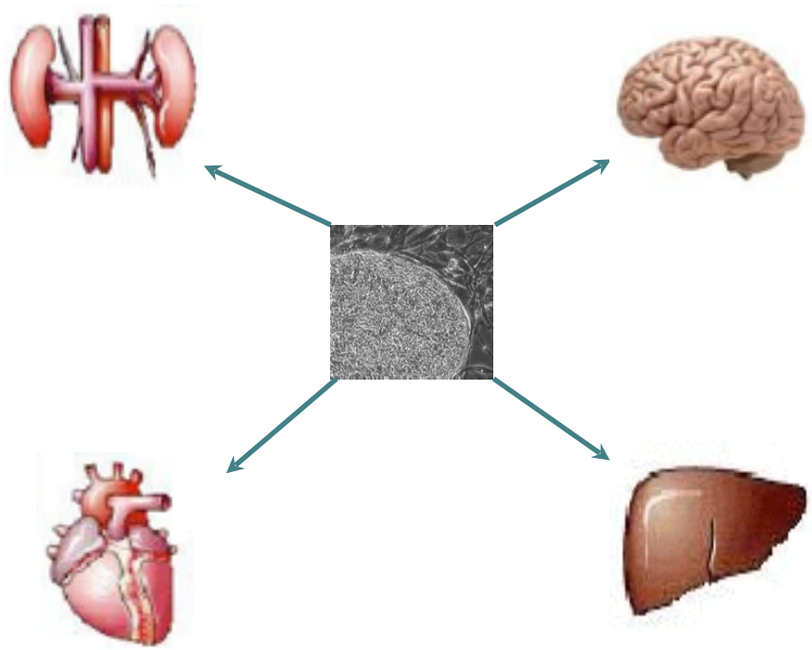
WP 10 - Toxicology

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.

renal cells

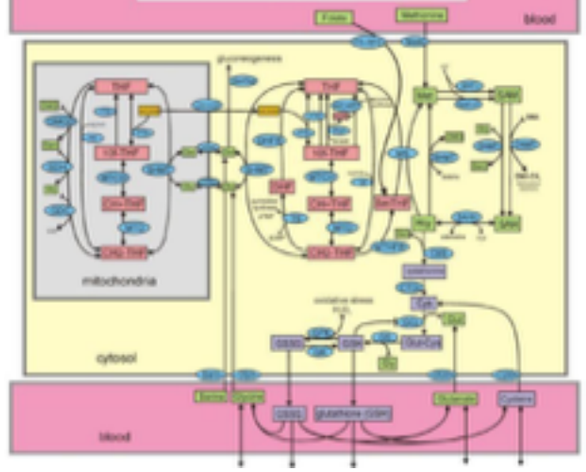
brain aggregates



cardiomyocytes

hepatocytes

Toxicity pathway analysis



Jim Ross - University of Edinburgh
Nicole Clemann - Roche

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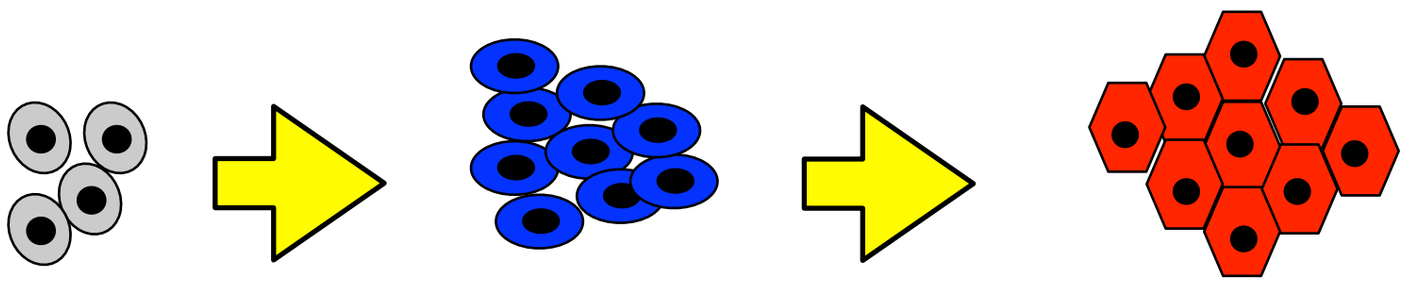
iPS cells and toxicology

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)

Induced pluripotent stem cells



Endoderm

Liver cells (hepatocyte-like cells)

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

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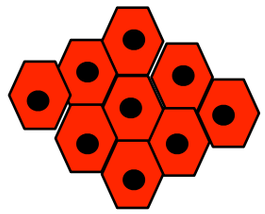
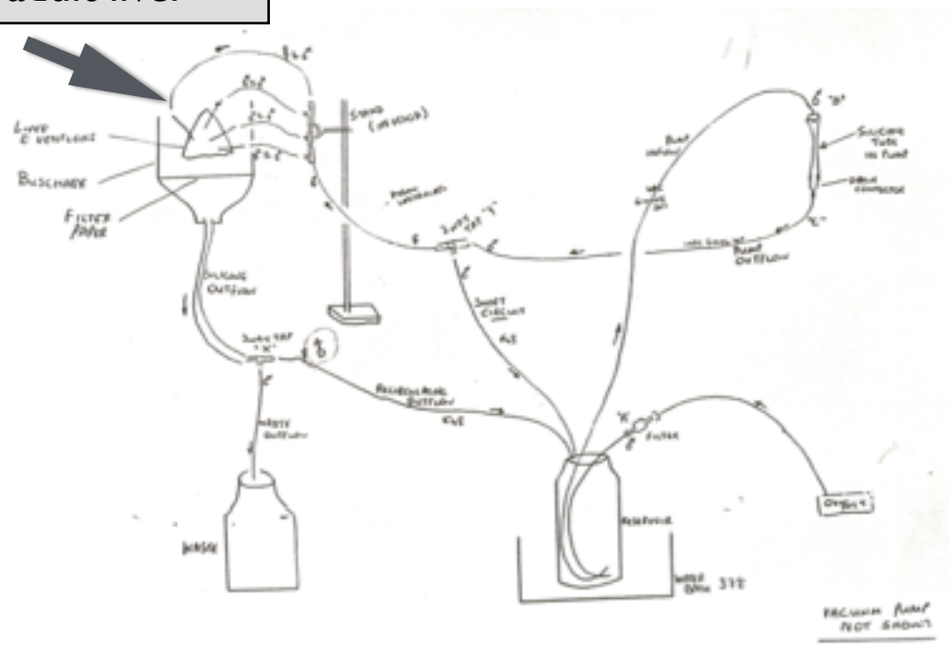
Benchmarking for hepatocytes informed by studies on adult human hepatocytes



resected piece of adult liver



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

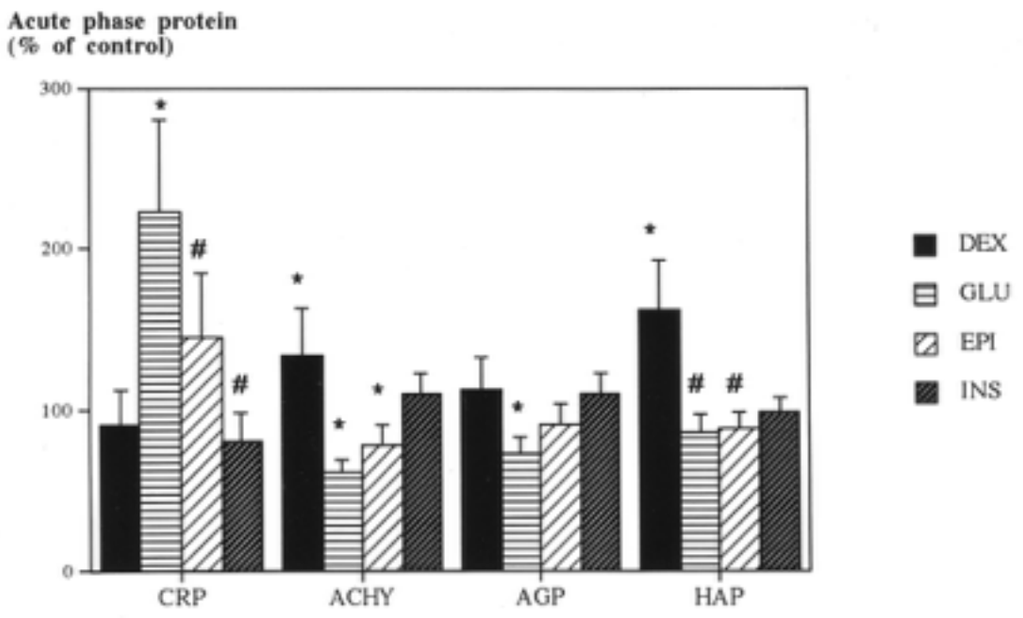
MICHAEL G. O'RIORDAIN, JAMES A. ROSS, KENNETH C. H. FEARON, JEAN MAINGAY, MARWAN FAROUK, O. JAMES GARDEN, AND DAVID C. CARTER
University Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW, United Kingdom

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



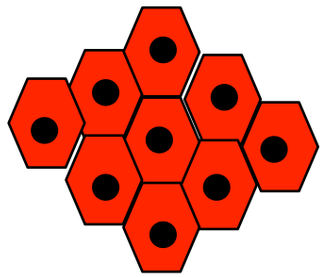
Proteolysis-inducing factor regulates hepatic gene expression via the transcription factors NF-κB and STAT3

FASEB J 2001

T. M. WATCHORN,[2] I. WADDELL,[*,2] N. DOWIDAR, AND J. A. ROSS[2]
Molecular Immunology Group, Department of Clinical and Surgical Sciences, Edinburgh University, U.K.; and *Cardiovascular and Gastrointestinal Discovery Department, Astrazeneca, Macclesfield, U.K.

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?

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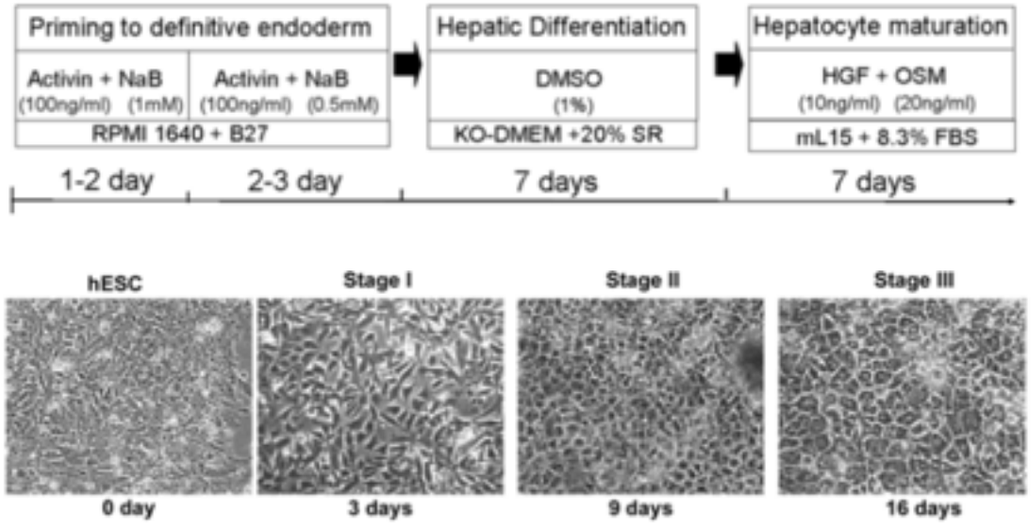
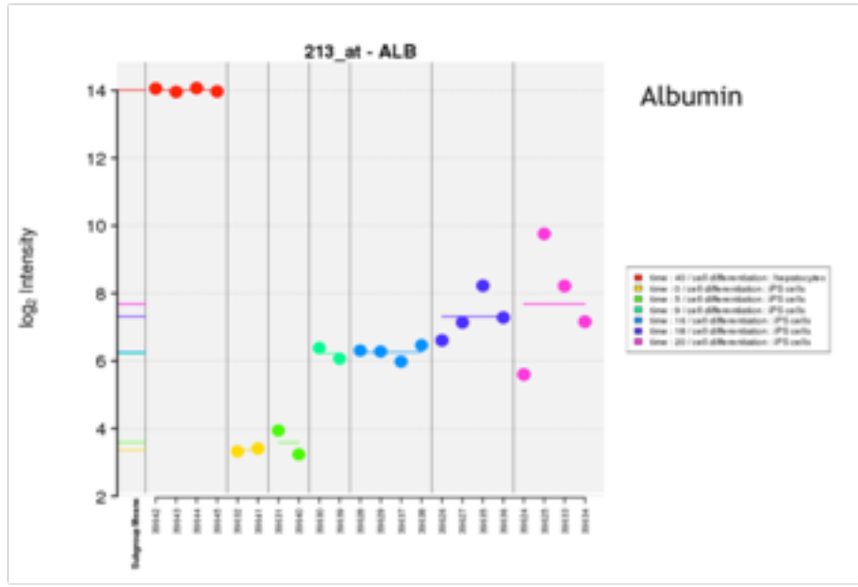
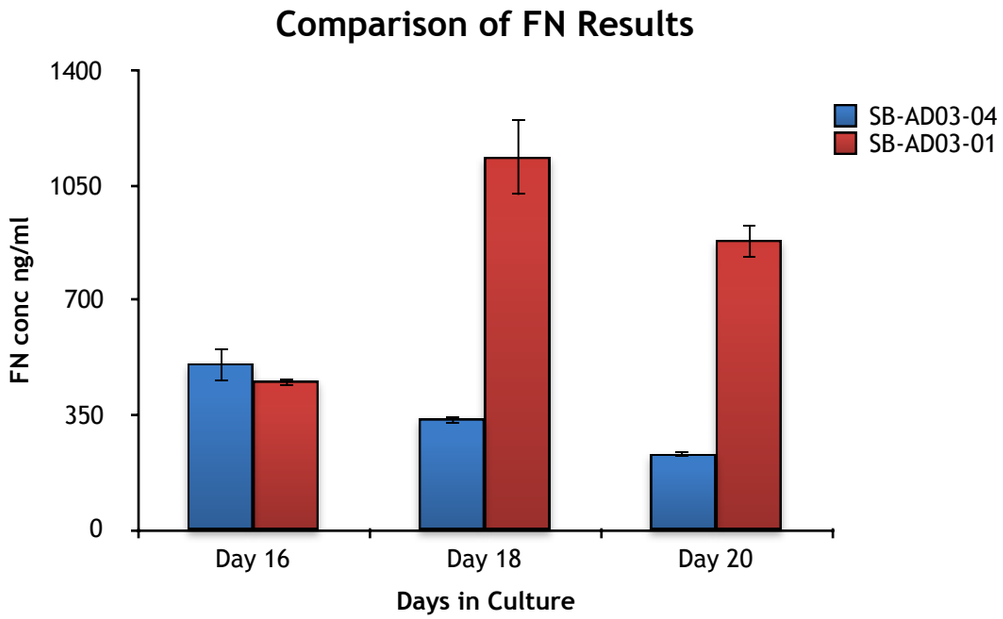
Progress to date - Hepatocytes

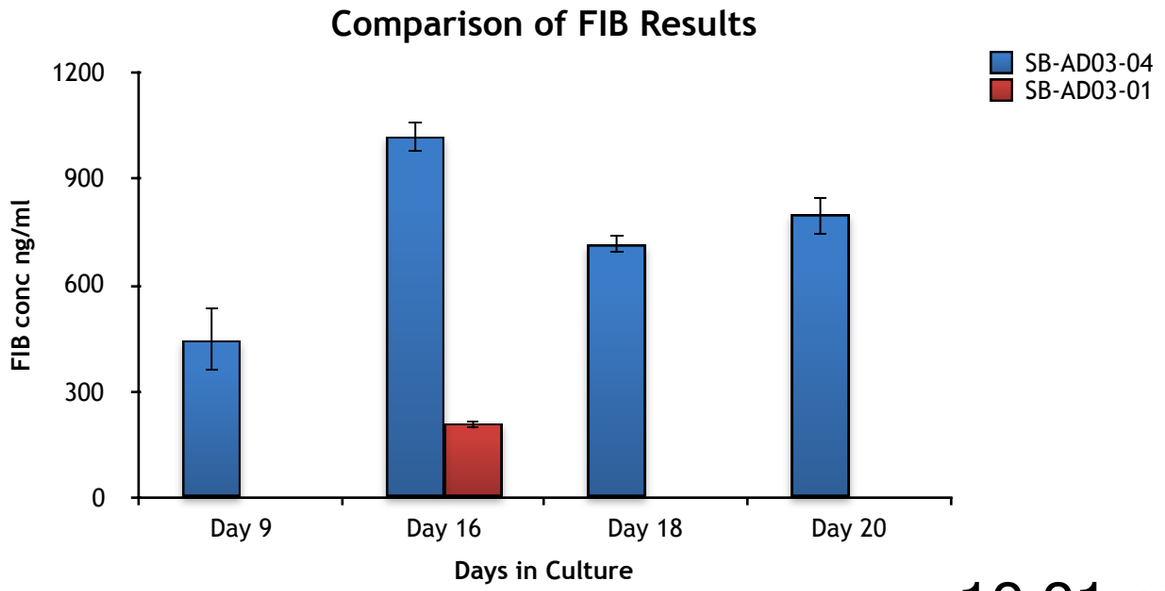




→ **Priority is to improve the protocol**

→ **Comparison with 'real' hepatocytes**





comparison between lines & between clones

10.01 milestone completed M24

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Benchmarking for hepatocytes

Training compounds - CYP450 inducing cocktail

CYP	compound	uM (final)	stock (mM)	MW
2C19	S-Mephenytoin	25-100	125-500	218.252
2C9	Diclofenac	2-25	10-125	319.14
3A4	Midazolam	0.6-2	3-10	362.14
2D6	Dextromethorphan	2-10	10-50	370.33

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?

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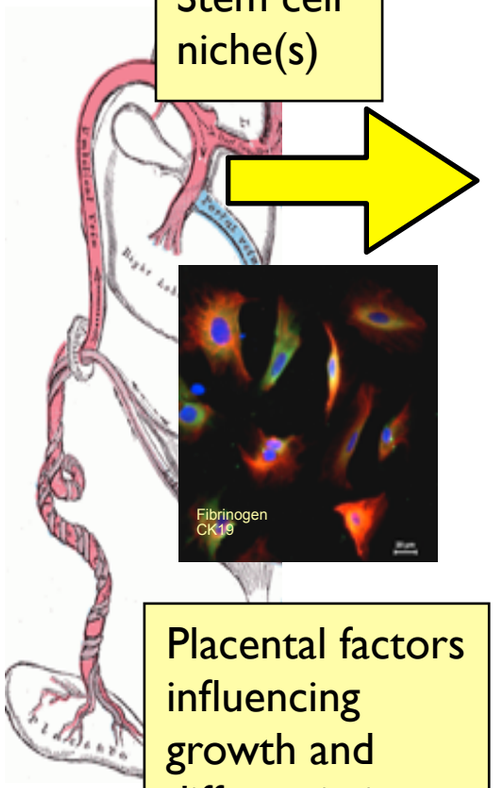
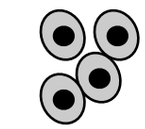
Improving function and longevity of differentiated hepatocyte-like cells informed by studies on human liver development

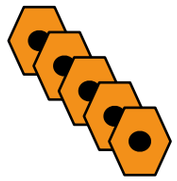


Stem cell niche(s)

Placental factors influencing growth and differentiation of hepatocytes

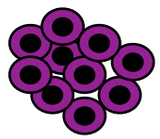


side-population cells



bi-potential cells [hepatoblasts]

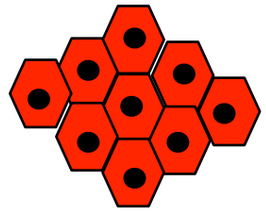
mesenchymal stem cells



haematopoietic stem cells

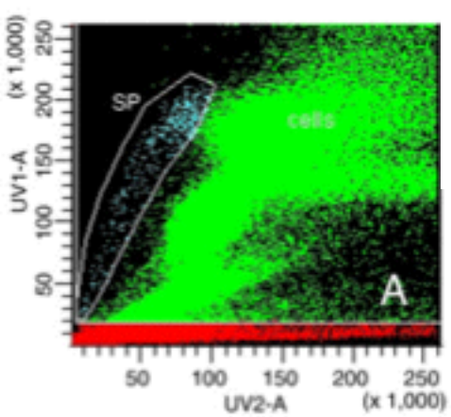
endothelial progenitors

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?



Research Article

Side population cells in developing human liver are primarily haematopoietic progenitor cells

John D. Terrace, David C. Hay, Kay Samuel, Catherine Payne, Richard A. Anderson, Ian S. Currie, Rowan W. Parks, Stuart J. Forbes, James A. Ross*

2009

Portal venous endothelium in developing human liver contains haematopoietic and epithelial progenitor cells

John D. Terrace[a], David C. Hay[a], Kay Samuel[a], Richard A. Anderson[b], Ian S. Currie[a], Rowan W. Parks[a], Stuart J. Forbes[a], James A. Ross[a,]*

2010

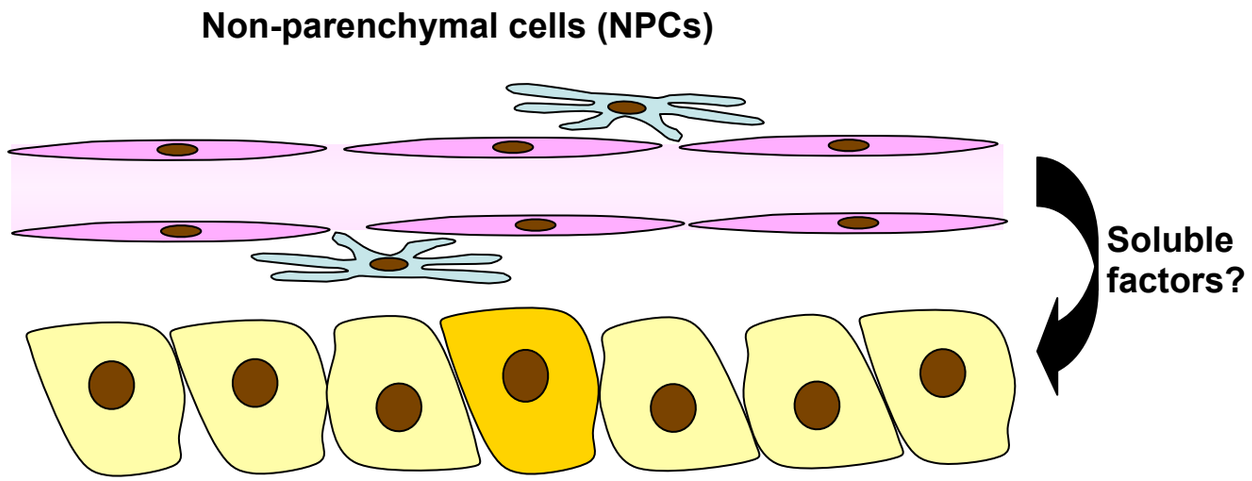
Hepatic progenitor cells in human fetal liver express the oval cell marker Thy-1

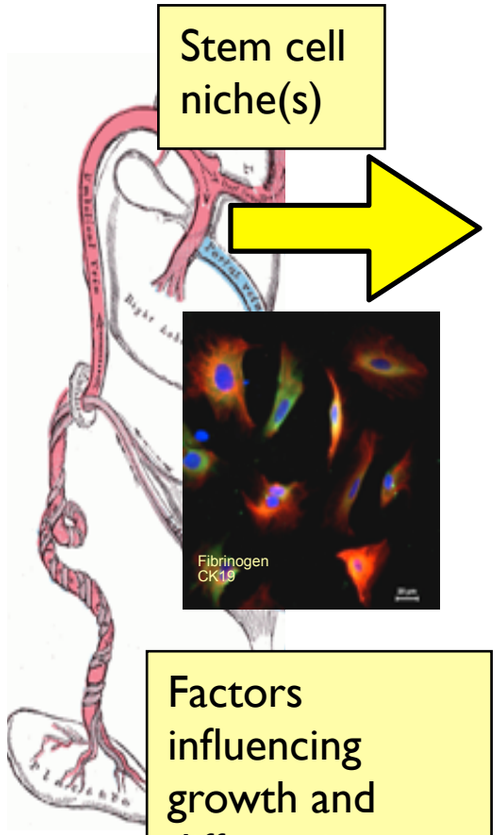
2006

Neil M. Masson, Ian S. Currie, John D. Terrace, O. James Garden, Rowan W. Parks, and James A. Ross

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





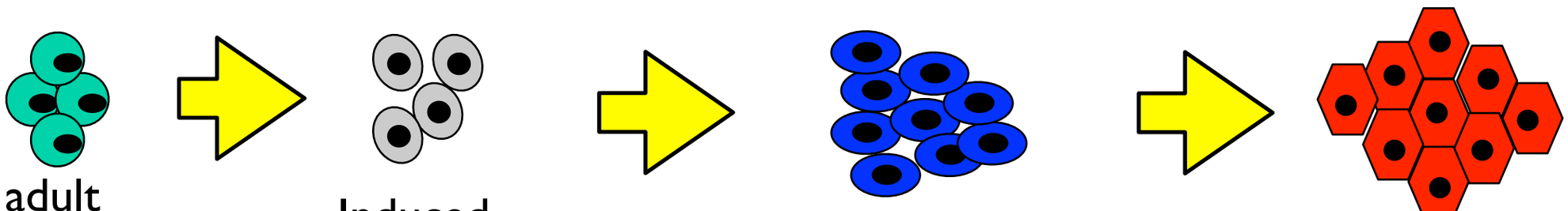
Now have good evidence that hepatocyte/endothelial cell co-culture improves hepatocyte function in the developing liver.

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



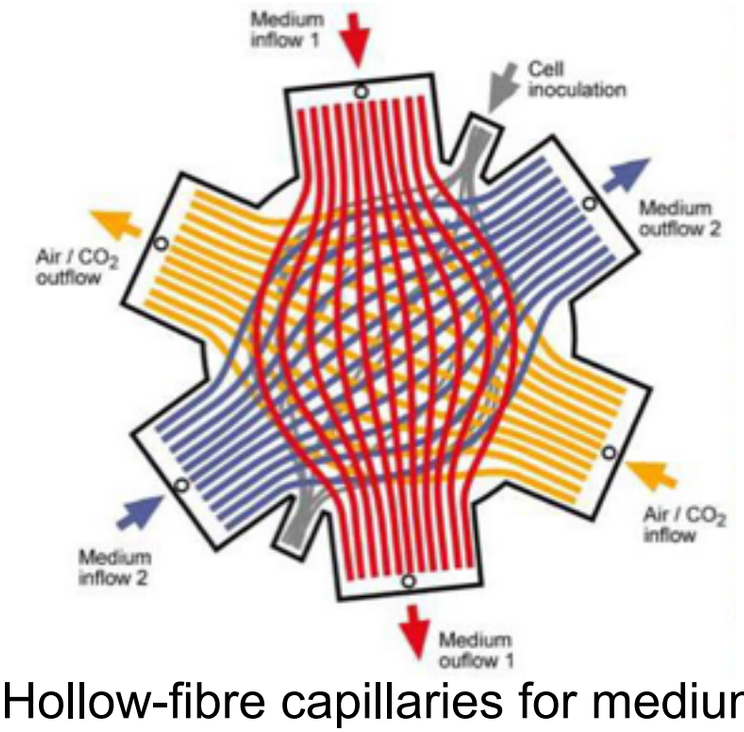
co-culture approaches (e.g. endothelial cell)
novel substrates/matrices (e.g. polymers)
survival factors
small molecules

→ improve function and longevity

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

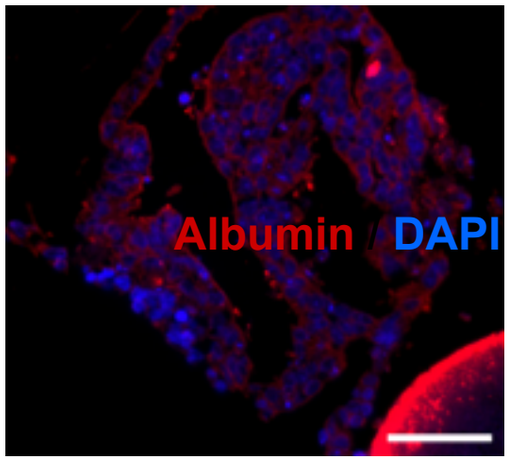
3D bioreactors for hepatic differentiation of hiPSCs

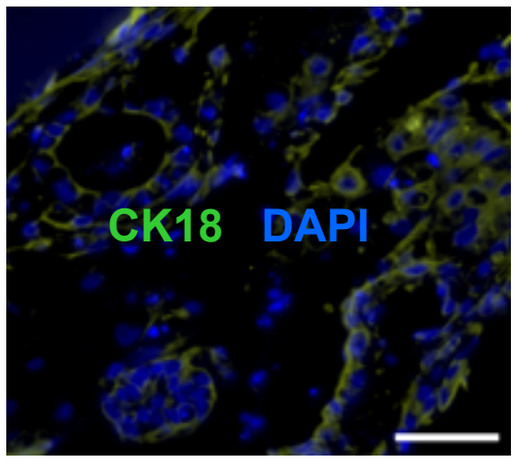


- Hollow-fibre capillaries for medium and gas perfusion
- Cells are cultured in the extra-capillary space
- Different sizes available from 0.5 mL to 800 mL

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

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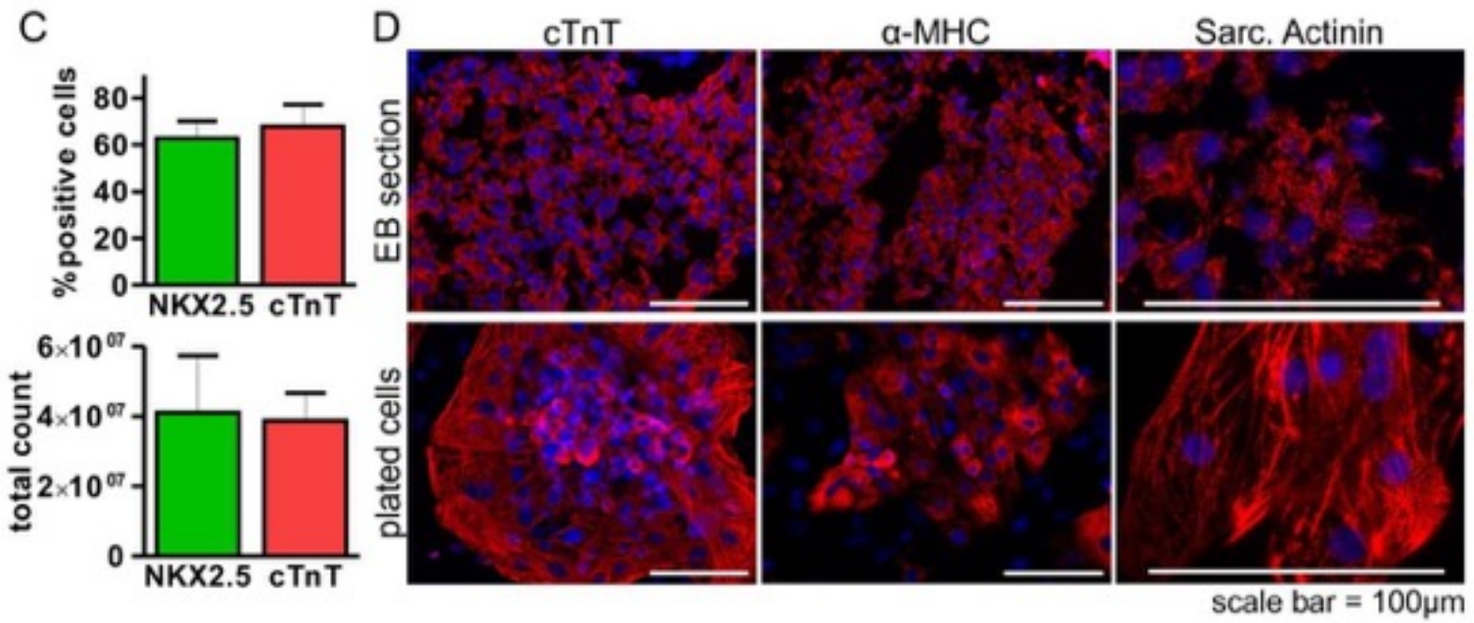
Progress to date - Cardiomyocytes

Differentiation



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)

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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

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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

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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)

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WP 10 – Patient Recruitment

UEDIN

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)

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

The project has received support from EFPIA companies and the European Union (IMI JU)

Steering Committee and StemBANCC Team leaders (Oct 2012)

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