Stem Cells USA

& Regenerative Medicine Congress

Boston

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Successful Exploitation of Stem Cell Assays in Predictive Toxicology

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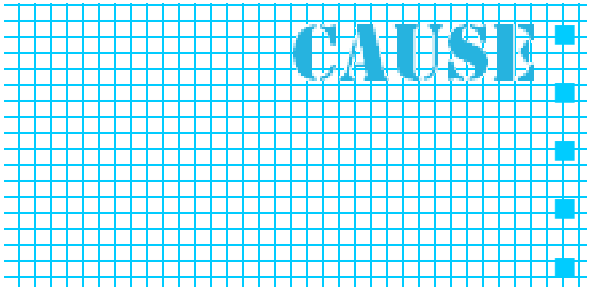
Stem Cells for Safer Medicines

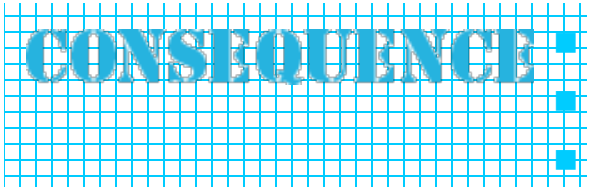
Outline

- What are the important issues challenging the pharmaceutical industry?
- Why do we need improved predictive toxicology assays in drug development?
- SC4SM Predictive Toxicology consortium: progress and plans
- What are the prerequisites for successful exploitation of stem cell assays?
- Emerging opportunities

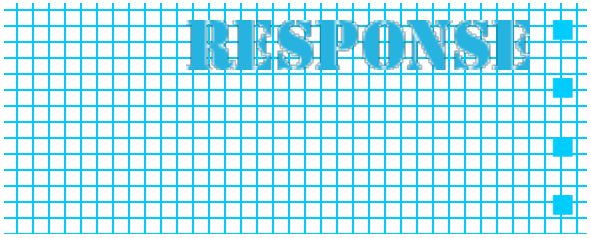
Pharmaceutical Industry Trends



- Generic erosion of products
- Drug attrition
- Product withdrawals
- Healthcare reforms
- Higher regulatory hurdles



- Decreased revenues
- Decreased profitability
- Decreased ROI



- Mergers, acquisitions and partnerships
- Rationalisation of R&D pipelines
- Reorganisation and job losses
- New business opportunities e.g. generics, new markets

TRANSFORMATION OF THE R&D PROCESS

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Possible saving in drug development

	Overall probability of success (probability in brackets)				
	20% decrease (0.172)	10% decrease (0.194)	Base Case (0.215)	10% increase (0.237)	20% increase (0.258)
Cost of an NCE ($ millions)	1023	909	802	744	682
% change in cost of NCE vs Base Case	28%	13%		-7%	-15%

Source: OHE calculations from Di Masi et al. (2003)

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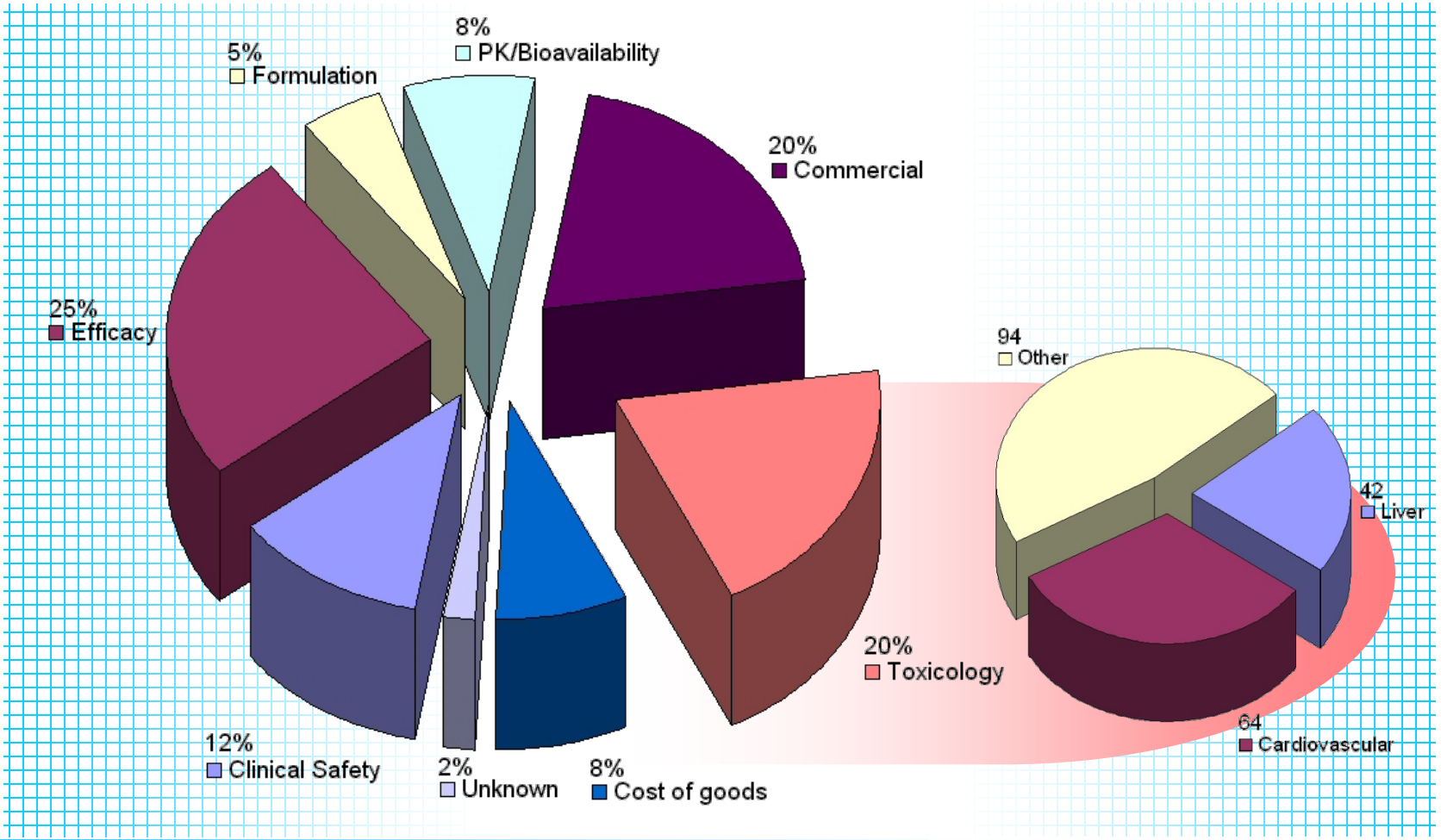
Overall Drug Attrition 1991 - 2000



Data from:
Kola & Landis, Nature Reviews Drug Disc., 2004;
ABPI Biomarker Working Group, 2007

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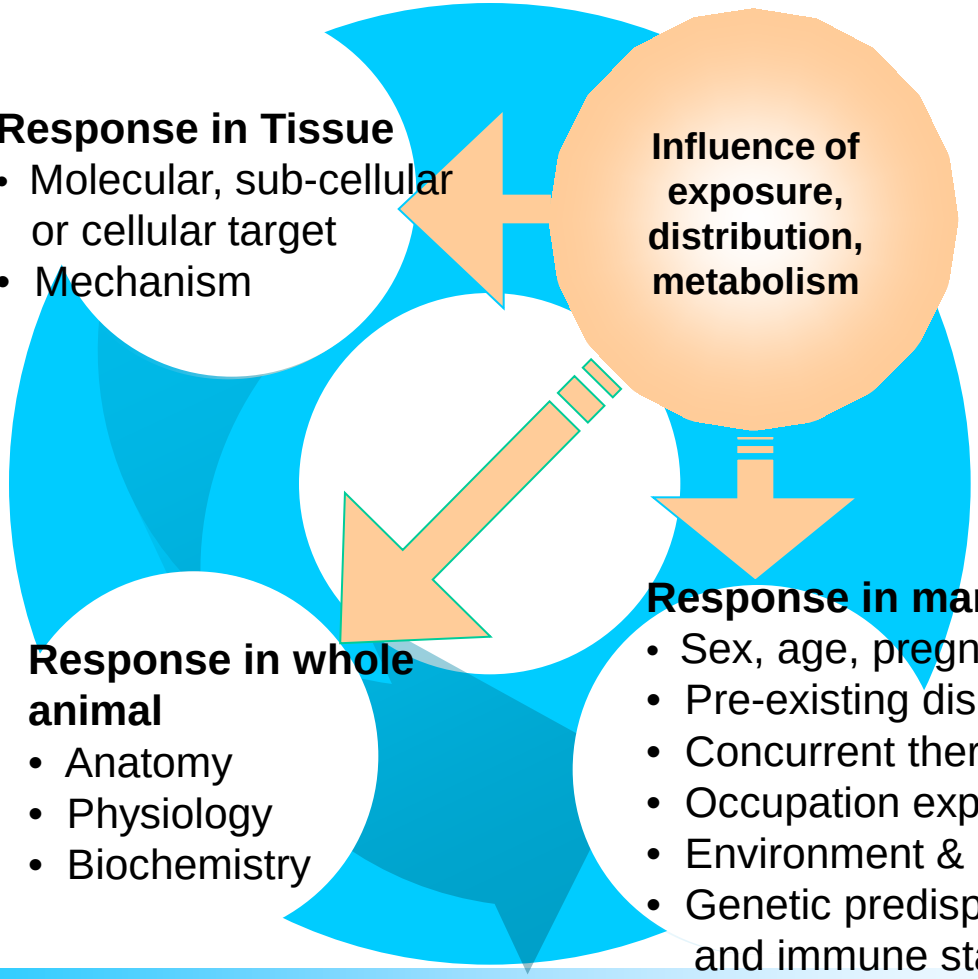
Hurdles in translational medicine



The Challenge:

Translation between species and different levels of biological organisation for prediction of risk for man

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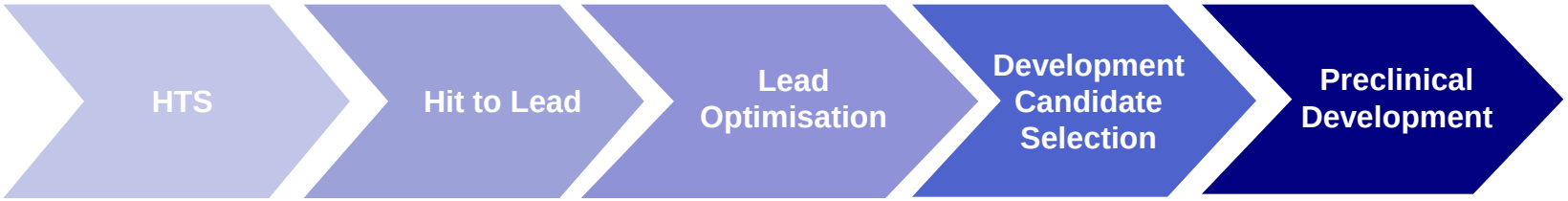
Typical screening cascade



In Silico
- SAR
- Prediction & simulation

In vitro
- Ames
- Greenscreen
- hERG

In vitro
- Cellular assays
- Hepatocytes
- HepG2, HepaRG

Stem Cells

In vivo
- Target organ models
- Chronic effects
- Carcinogenicity
- Reproductive toxicity

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- Report & Recommendations of the UK Stem Cell Initiative (Sir John Pattison Report, 2005)
  - The UK Government should establish a public-private partnership to develop predictive toxicology tools from stem cell lines
- The establishment of SC4SM recognised the strength of stem cell science in the UK and a political imperative to foster innovation and technology development
- At the same time, there was a recognition of the increasing demands on the pharmaceutical industry to improve the productivity of the R&D process
- The Company is a not for profit organisation and operates as a pre-competitive consortium of industrial (AstraZeneca, GSK, Roche and UCB) and academic partners
- SC4SM has committed up-front funding to support academic research directed towards the needs of the industrial membership

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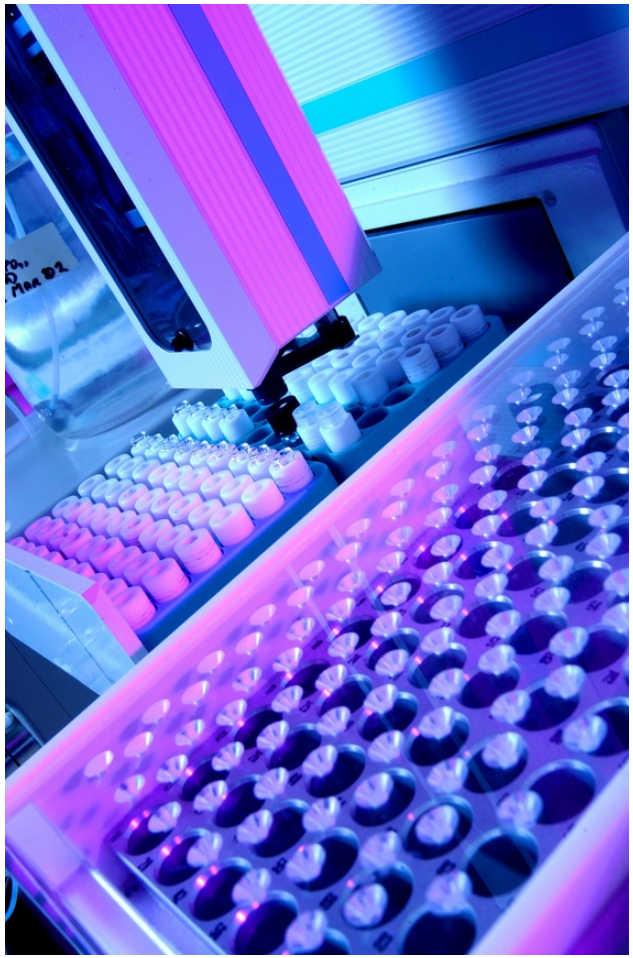
SC4SM Goal

- To generate optimised protocols to enable the consistent differentiation of stable, homogeneous populations of particular cell types with defined functional characteristics
- To develop medium to high throughput screens for early predictive toxicology to reduce risk in clinical development which can be scaled up, automated and integrated into current screening technology platforms
  - focused on hepatotoxicity (and cardiotoxicity)
  - range of cell lines with key genotypes and ‘fit for purpose’ functionality
  - validated using standardised compound library of positive and negative controls



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Hepatocyte projects: outline

Differentiation

Outline Plan:
To evaluate established methods and novel approaches to define the conditions required to promote differentiation towards definitive endoderm (DE) and hepatocyte-like cells (HLC's)

Characterisation

Outline Plan:
To generate a comprehensive and validated panel of screens for a pre-determined set of hepatic phenotypic and functional characteristics in order to assess cell health and evaluate response to drugs

Phase 2 Programme

Testing & Validation

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Acknowledgment:

- Bath University: Principal Investigators
  - *Melanie Welham & David Tosh*
- Manchester University: Principal Investigator
  - *Neil Hanley*
- Edinburgh University: Principal Investigators
  - *David Hay & Josh Brickman*
- Liverpool University: Principal Investigators
  - *Chris Goldring*

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Phase 1 summary of progress: differentiation

Ability to differentiate a variety of hESC lines towards definitive endoderm and hepatocyte-like cells using a number of different protocols has been successfully demonstrated

Bath University

Using a defined media and feeder-free system designed to manipulate Wnt signaling, including use of a novel GSK-3 inhibitor

Manchester University

Using an optimised monolayer-based protocol to compare the ability of a range of hESC lines to differentiate under a variety of defined conditions

Edinburgh University

Using a variety of feeder-free systems including Wnt and Activin to promote differentiation followed by FACS sorting to purify cell populations

Phase 2 Programme structure

Differentiation

Outline Plan:
To continue to optimise and refine protocols in order to improve yield, functionality and scalability for the production of hepatocyte-like cells for subsequent evaluation of response to drug treatment

Characterisation, testing and validation

Outline Plan:
To confirm 'fit for purpose' functionality of derived cells, design integrated assays including a wide variety of toxicity endpoints, perform validation of responsiveness against a comprehensive library of test compounds and benchmarked against current existing cellular models

Scale-up, manufacture and technology transfer

Outline Plan:
To define the conditions for scale-up, including quality control measures in order to facilitate the manufacture of cells, automation of assay procedures and technology transfer to industrial partners for incorporation into screening platforms

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Prerequisites for success

- Well defined need for improvement
- Optimised differentiation protocols
- ‘Fit for purpose’ functional characteristics
- Comparable or better than existing models
- Incorporating wide range of toxicity endpoints
- Validated response predicting risk for man
- Amenable to scale up and manufacture
- Amenable to automation and technology transfer

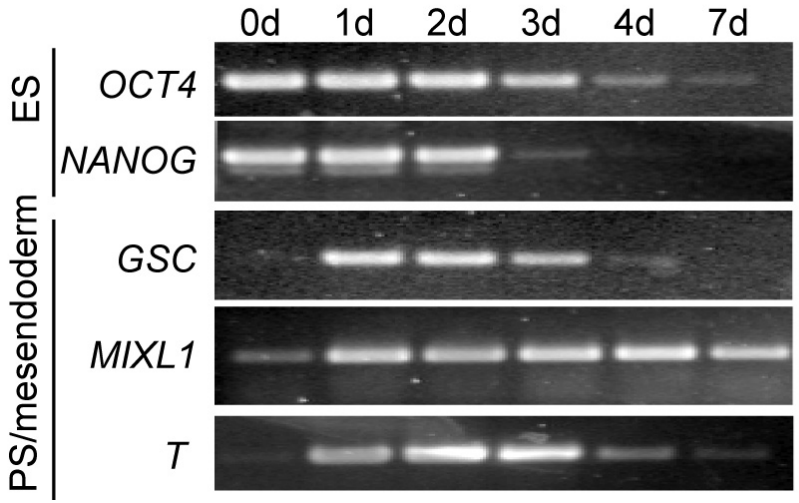
Well defined need for improvement

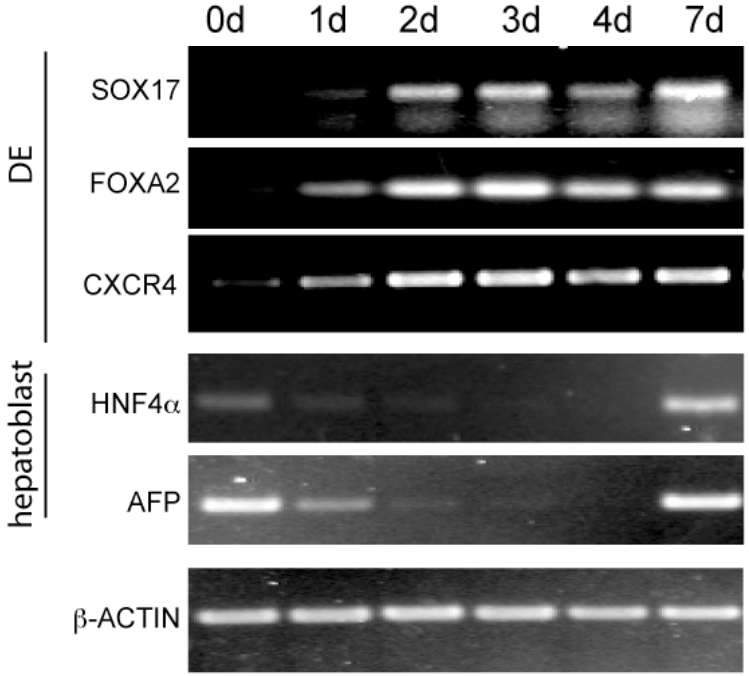
- The drug discovery and development process is in need of re-engineering to improve productivity
- There is an opportunity to incorporate safety testing models earlier into the process to reduce late stage attrition
  - Candidate selection should be less reliant upon biological potency and specificity but also consider safety (ADMET) characteristics
- Conventional safety testing paradigms are constraining
  - Time, cost, compound supply, use of animals etc.
- We need to develop and validate more innovative models that focus upon:
  - Early identification of potential target organ effects
  - Practicability (robust, reproducible, feasible etc.)
  - Higher throughput and increased predictiveness

Optimised differentiation protocols

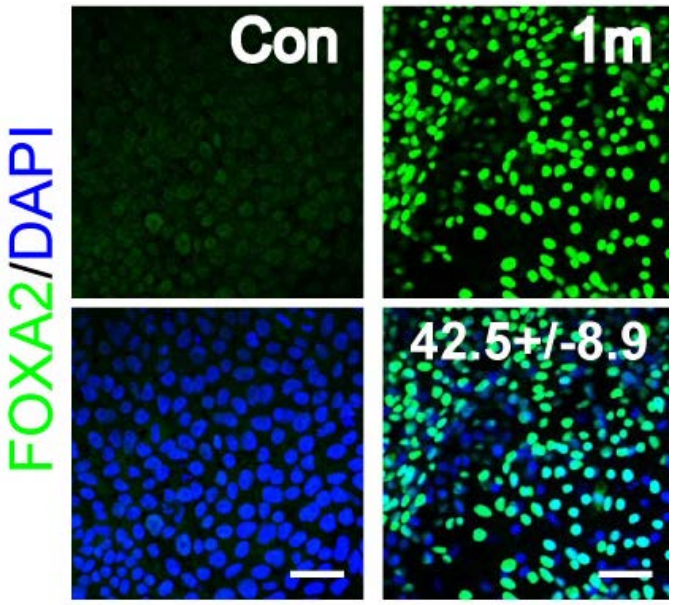
- Currently, there is no one definitive and robust protocol that efficiently generates hepatocyte-like cells form hESC's
- The promotion of differentiation involves multiple signaling pathways and growth factors which are not fully understood
  - Wnt signaling proteins, TGFβ and Activin receptors, GSK-3 inhibitors etc.
- Different hESC lines exhibit varying capacities to undergo differentiation towards definitive endoderm under similar culture environments
- The use of extracellular matrices can enhance the generation of definitive endoderm
  - Variety of synthetic polymers known to moderate Pi3 kinase signaling
- Ongoing effort to refine and simplify experimental conditions (e.g. feeder-free culture)

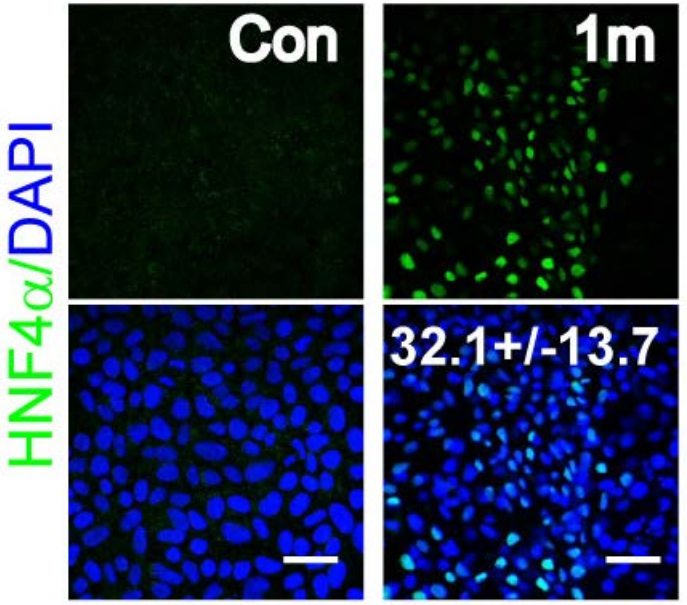
Inhibition of GSK-3 induces differentiation of hESCs to definitive endoderm





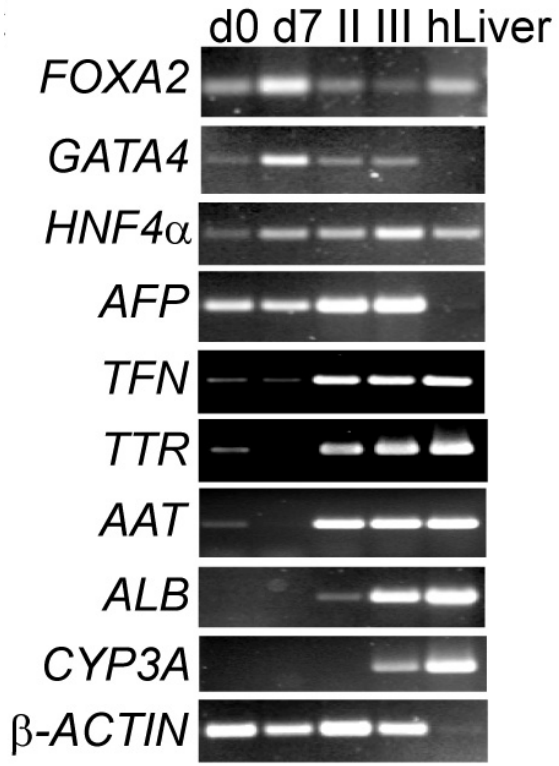
DE generated by GSK-3 inhibition expresses FOXA2 and HNF4a



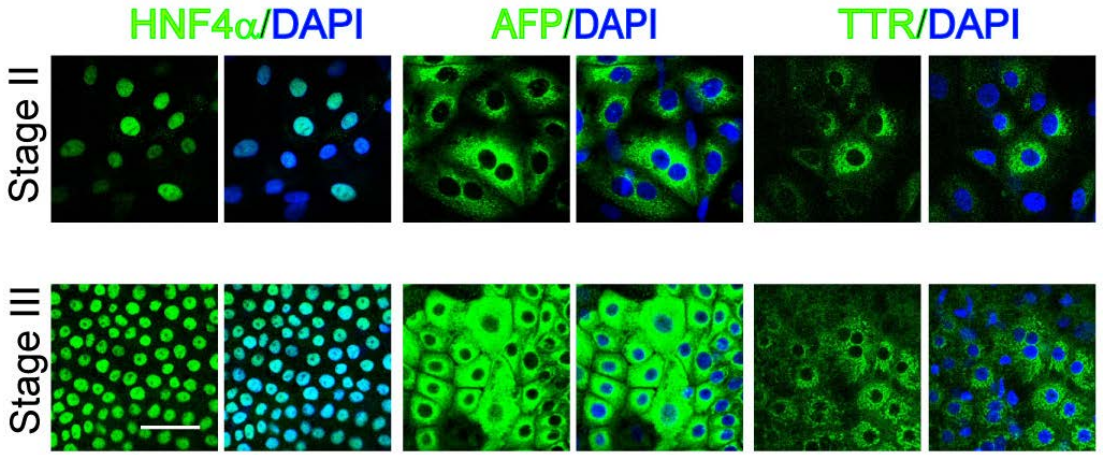


Hepatocyte-like cells generated by GSK-3i-induced DE express mature phenotypic markers

PCR



Immunostaining



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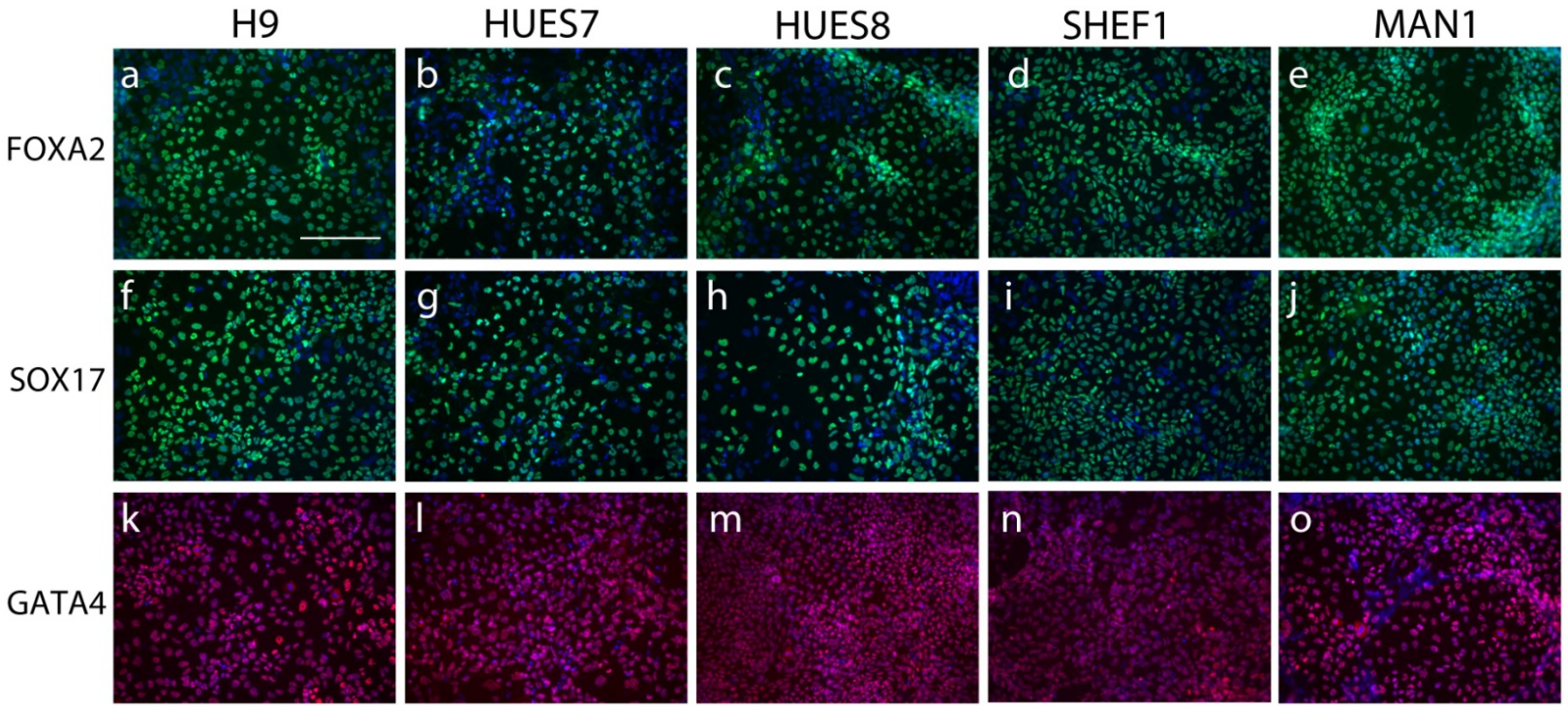
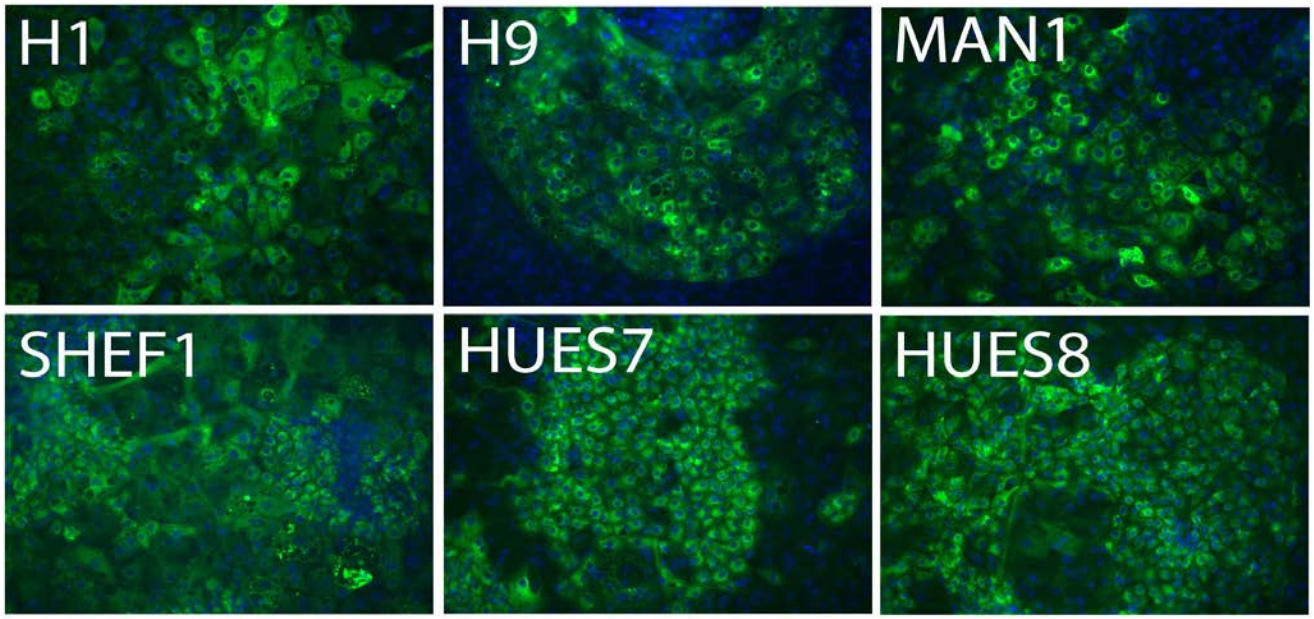
HLCs generated from different hESC lines express DE markers



HLCs generated from 6 hESC lines express albumin and AAT



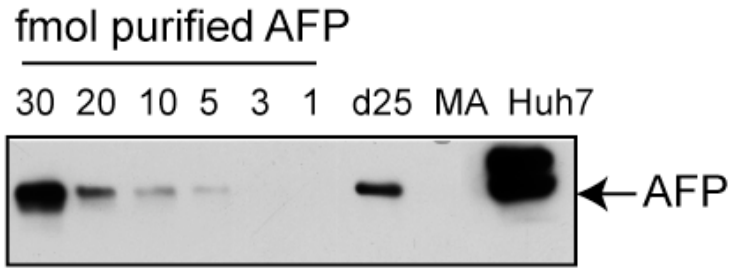
	H1	H9	MAN1	SHEF1	HUES7	HUES8
ALBUMIN-positive (%)	87	69	54	86	75	59
AAT-positive (%)	40	14	30	29	42	34

Fit for purpose functional characteristics

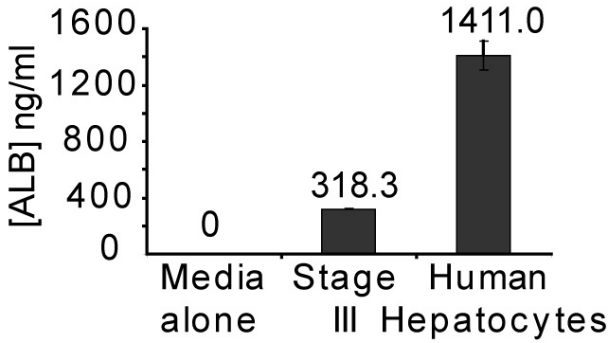
- Maturity of the derived cell?
  - HLC's tend to display foetal phenotypic characteristics
- Needs to display multiple indices of intermediary metabolism characteristic of the specific cell type
  - Protein synthesis, lipid metabolism, urea synthesis, steroid metabolism, fibrinogen synthesis etc.
- Exhibit capacity (inducible) for exogenous metabolism of drugs and chemicals
  - Battery of factors associated with activation/deactivation of xenobiotics including nuclear receptors (PXR, CAR, AHR etc.), CYP P450 subfamilies (esp. 3A, 2D etc.), phase 2 enzymes (conjugation reactions etc.), transporters (OATP etc.)
- Need to understand the advantages and disadvantages inherent with co-culture (e.g. presence of non-parenchymal cells)
- Need to demonstrate phenotypic stability

Hepatocyte-like cells derived from GSK-3i-induced DE have functional activity

α-fetoprotein secretion

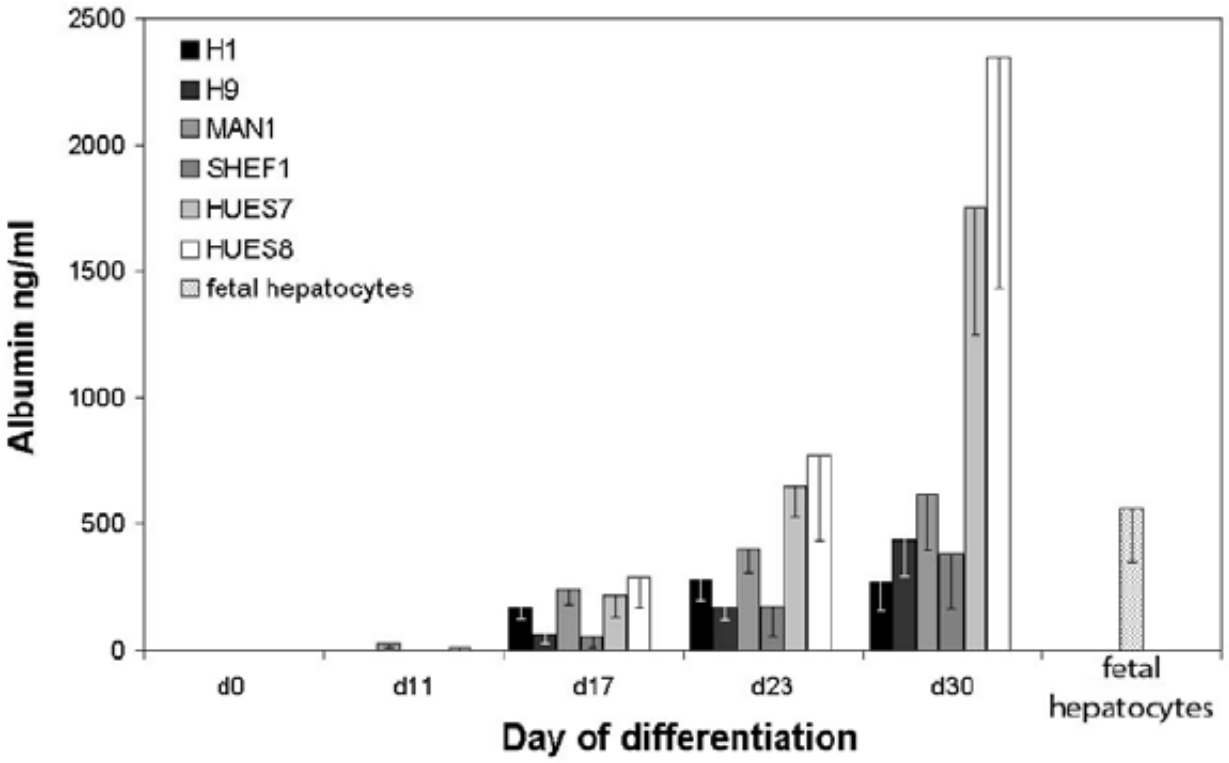


albumin secretion



- GSK-3i induces differentiation to DE and progression to hepatoblasts
- GSK-3i-induced DE has hepatic potential, HLCs express mature markers and show functional activity
- Successfully developed novel, robust, efficient and scalable monolayer-based protocol using chemically defined conditions

HLCs generated from hESCs secrete albumin



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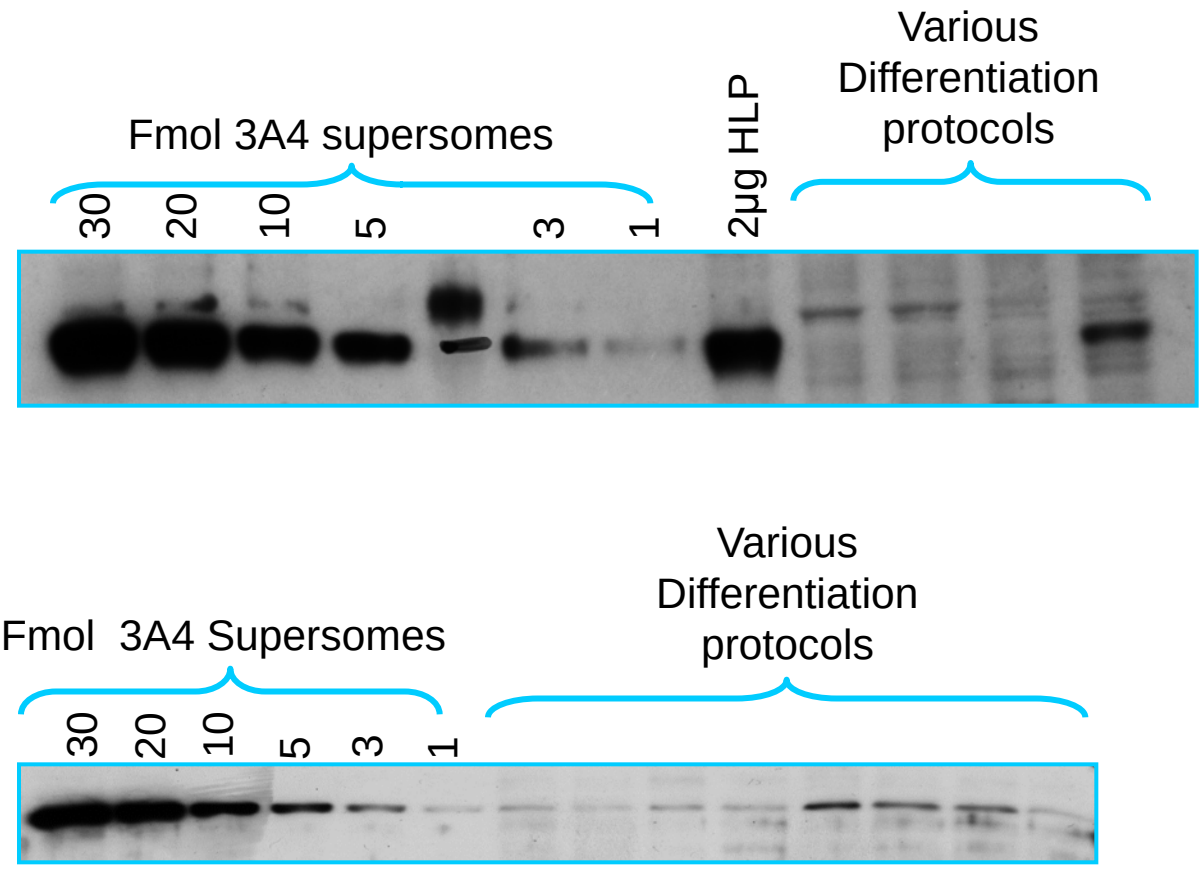
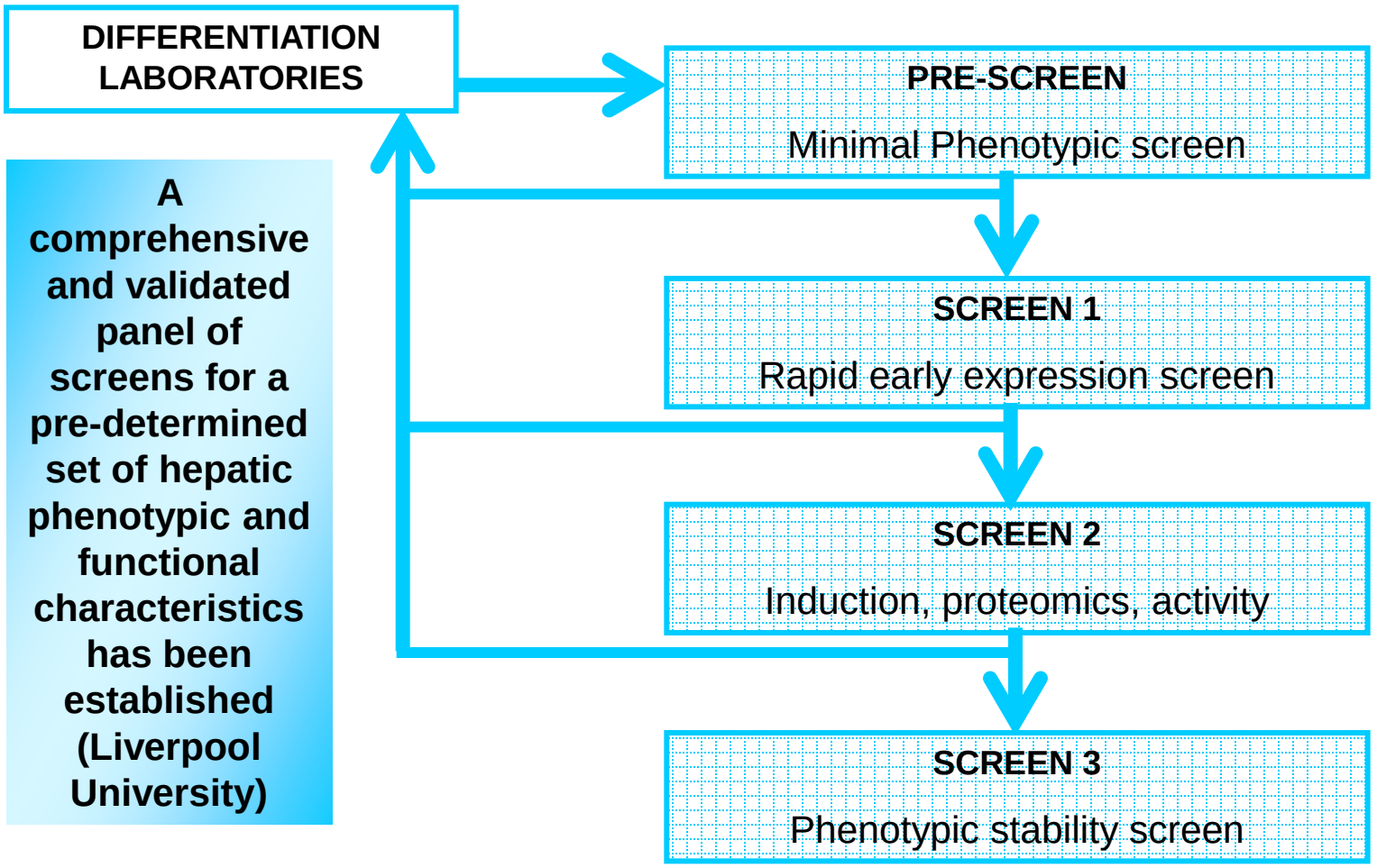
Western blot assay for CYP3A Protein in hepatic endoderm



Phase 1 summary of progress: characterisation



A comprehensive and validated panel of screens for a pre-determined set of hepatic phenotypic and functional characteristics has been established (Liverpool University)

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Comparison with existing models

- Primary human hepatocytes represent the gold standard model for drug screening
  - Limited supply, genetic and epigenetic diversity (variability), limited yield, inconsistencies in preparation, limited viability etc.
- Immortalised human cell lines such as HepG2 are routinely used
  - Relatively well differentiated but growth and functional characteristics are not normal
  - Minimal capacity for exogenous metabolism
- Improved Immortalised cell lines are becoming available
  - HepaRG may be more typical of primary human hepatocytes and exhibits expression of nuclear receptors, CYP sub-families etc.
- Comparison with other species used in drug development
  - Helpful to integrate response across the range of species used in discovery and development including rat, dog (mouse, sub-human primate)

Incorporation of toxicity endpoints

- Structural integrity
  - Membrane function and disruption
  - Membrane bound transporters, ion-channel receptors etc.
- Multiple endpoints reflecting diverse mechanisms of toxicity
  - Oxidative stress
  - Mitochondrial toxicity
  - Cell proliferation
  - Apoptosis and necrosis
  - Phospholipidosis
  - Inflammatory processes
- Organ specific effects
  - Toxicities associated with specific cell types within an organ
  - Toxicities associated with specific organ functionality (e.g. cardiac electrophysiology
- Model both acute and chronic toxicities

Validated response

- Need a standardised (inter-laboratory) evaluation of response
  - Consistent experimental protocols
  - Range of different chemical classes
  - Range of pharmacological activities
  - Represent diverse mechanisms of pathogenesis
- Demonstration of dose-response relationships
  - Sensitivity, threshold effects etc.
- Comparison across species
  - Need to understand species difference in response in order to translate to a predicted human response
- Integration of data to model risk for man
  - Opportunity to develop expert systems which integrate data from multiple models (in vitro, non-clinical in vivo, human) in order to predict risk

Scale-up and manufacture

- The overall objective is to manipulate culture conditions to ensure differentiation towards the desired cell lineage
  - quality and quantity
  - Uniform phenotype and predictable behaviour
- Processes to drive differentiation do not yield homogeneous cell populations
  - Need to be able to characterise cells within a heterogeneous population and monitor for spontaneous differentiation
- Enrichment and purification techniques (e.g. flow cytometry, cell surface markers etc.) are important strategies to improve yield and quality
- Need to maintain karyotypic integrity
- Need to incorporate processes to ensure viability during storage, transport and utility

Automation and technology transfer

- The overall objective is to adapt bench scale assays into high-throughput and automated format
- High content screening techniques are well developed
  - Incorporates multi-well plate format (96 well or higher)
  - Uses a combination of techniques such as high resolution digital microscopy, flow cytometry, image analysis, robotics and sample handling
  - Exploits fluorescent antibody methods (activation of cell surface and other markers) to monitor multiple biochemical pathways and morphological characteristics in order to evaluate cellular changes as a result of exposure to drugs and chemicals
- Commercially available platforms (Cellomics, GE Healthcare etc.) are undergoing constant improvement and refinement

Future opportunities: iPS cells

- **The development of iPS cells derived from re-programmed somatic cells presents novel opportunities in regenerative medicine and for drug screening and understanding drug action**
- **Circumvents ethical issues associated with the use of human embryonic stem cells**
- **Opportunities in drug screening include:**
  - Model diseases which have complex genetic basis
  - Novel target identification for drug therapy
  - Drug screening in specific genotypes which may be indicative of idiosyncratic toxicity
  - Develop panels of iPS cell lines which are more representative of the diversity of genetic backgrounds (disease predisposition, ethnicity etc.)
- **Recent evidence that cell re-programming can be associated with inherent DNA damage**

Future opportunities: 3-D culture

- **There is increasing evidence that 3-D culture techniques may produce cellular environments that more closely reflect in vivo behaviour**
  - Conventional monolayer culture does not adequately facilitate the complex intercellular connections that are required for ‘normal’ function (e.g. gap junctions)
  - 3-D culture techniques rely upon a range of support systems including scaffolds and suspension methods
  - Potential benefits include:
    - Improved cell viability
    - Enhanced architecture and morphology
    - Cell polarity and actin formation
    - Increased maintenance of intermediary metabolic function
  - Ongoing development of bioreactor (micro-bioreactor) technology including continuous perfusion systems for optimum transfer of nutrients and removal of waste products

Summary and outlook

- **There is a clear need to improve the productivity of the drug R&D process**
  - Profitability of the industry is significantly challenged
  - Too many drugs fail at late stages of development
- **Stem cell assays may provide novel and improved screening tools**
  - Higher throughput assays need to be incorporated earlier into the R&D process
  - Potential for unlimited supply, improved human relevance, wide range of functional endpoints etc.
- **SC4SM is public-private partnership with the goal of delivering validated assays for drug screening to predict risk for man**
  - Aim to develop novel cellular models with superior functionality and utility compared to currently available systems
- **The development and refinement of stem cell assays is an ongoing process**
  - Future opportunities include the application of iPS cells and 3-D culture techniques which could expand applications and enhance functionality

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  - University of Manchester (Neil Hanley)
  - University of Liverpool (Chris Goldring)
  - Imperial College (Sian Harding)
  - University of Nottingham (Chris Denning)
  - University of Glasgow (Andrew Baker)

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