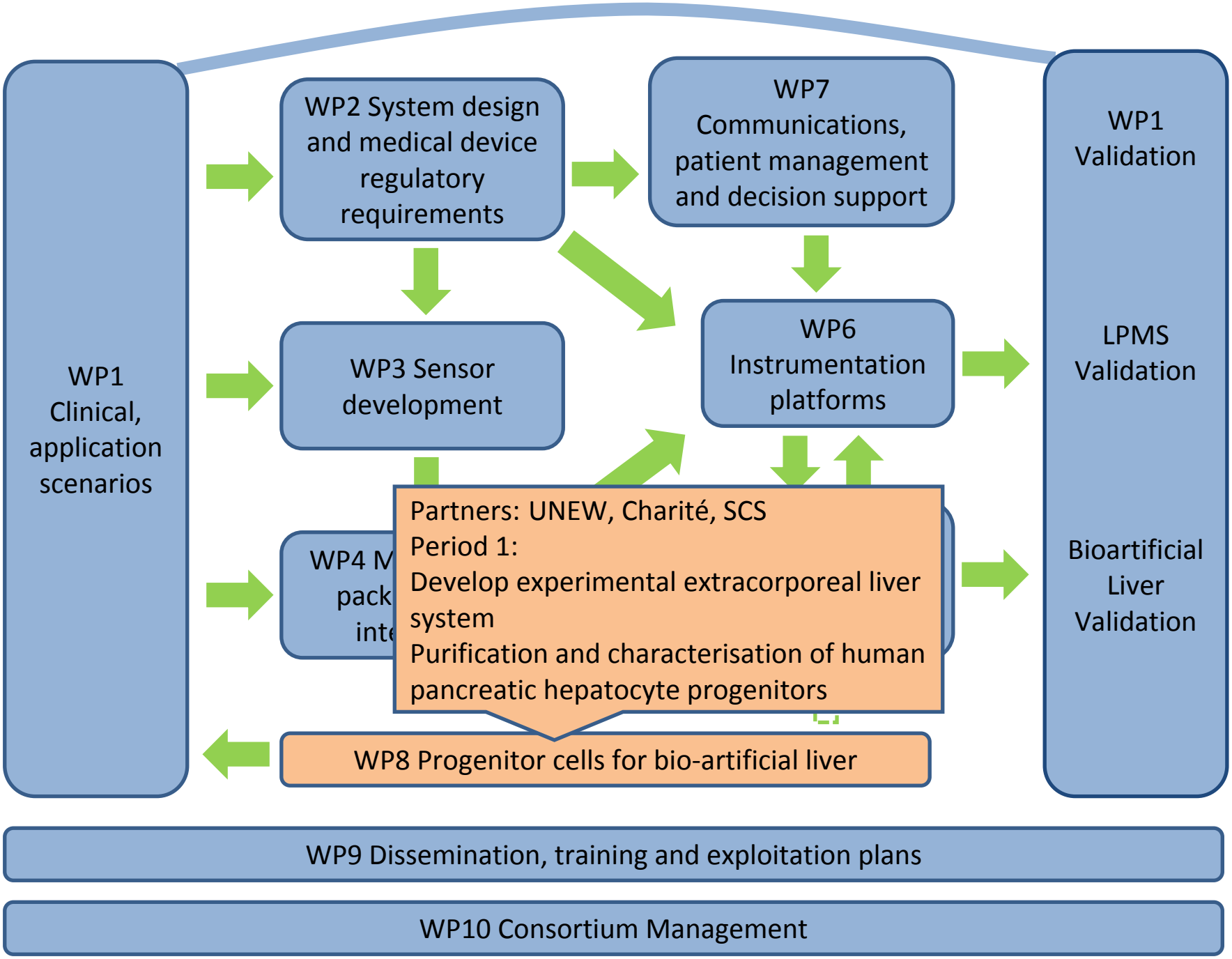
Relative cost of hepatocytes - WORKPACKAGE 8 PROGENITOR CELLS FOR BIO-ARTIFICIAL LIVER

UNEW, Charité, SCS

Newcastle University

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

StemCellSystems


WP1
Clinical,
application
scenarios
WP2 System design and medical device regulatory requirements
WP7
Communications,
patient management
and decision support
WP1
Validation
WP3 Sensor development
WP6
Instrumentation
platforms
LPMS
Validation
WP4 M
pack
inte
Partners: UNEW, Charité, SCS
Period 1:
Develop experimental extracorporeal liver system
Purification and characterisation of human pancreatic hepatocyte progenitors
Bioartificial
Liver
Validation
WP8 Progenitor cells for bio-artificial liver
WP9 Dissemination, training and exploitation plans
WP10 Consortium Management

Workpackage progress - Introduction

- Primary human hepatocytes
- iPS / ESC-derived hepatocytes
  - difficult to genetically manipulate
  - 10 years
  - EXPENSE
  - B-13 - serendipitous rat hepatocyte progenitor

Current cost of iPSC-derived hepatocytes

- In the absence of sufficient human hepatocytes from donor livers, the main approach examined for generating human hepatocytes is through differentiation of human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). Despite significant progress over the last 12 years, it has not been possible to generate hepatocytes with function quantitatively similar to adult human hepatocytes. It is widely reported that hepatocytes remain in a foetal state and cannot progress further unless transplanted in vivo. **Since normal hepatocytes de-differentiate into a foetal state in vitro (even when present within culture tissue slices), it may be not be surprising that this barrier exists in stem cell-derived hepatocyte culture [1].** In addition, a major hurdle to the use of ESC/iPS-derived hepatocyte is their high cost of generation. When a high level of functionality is required (e.g. if the proposed use is in an extracorporeal device), then this significantly impacts on cost.

- As part of the EC funded d-Liver project, the cost of hepatocytes derived from iPSCs was calculated, taking into consideration the most recent research, which compared drug metabolism activity in iPSC-derived hepatocytes with human hepatocytes [2]. In order to obtain the hepatocytes, the iPSCs in this publication required a four-stage differentiation protocol with a variety of recombinant growth factors. In addition, the cells were infected with adenovirus directed to over-express 2 transcription factors. Considering growth factors alone (without taking into account the cost of culture media, virus production, culture ware and the cost of failures), it was calculated that the cost to generate enough hepatocytes sufficient to populate a bioreactor capable of impacting on a patient with decompensating liver disease would be approximately **£150,000** each time. With current technologies, it is unlikely that iPSC-derived hepatocytes will be a practical solution in extracorporeal liver devices. Widespread cost-effective use in other technologies - such as in routine in vitro toxicity testing – also remains a distant possibility.

- 1. Wallace K, et al, Wright MC. AR42J-B-13 cell: an expandable progenitor to generate an unlimited supply of functional hepatocytes. Toxicology 2010;278:277-87.
- 2. Takayama K, et al. Generation of metabolically functioning hepatocytes from human pluripotent stem cells by FOXA2 and HNF1α transduction. J Hepatol. 2012;57:628-36.

And what about the cost of hepatocytes from stem cells (sufficient to provide sufficient function)

		ng/ml conc required	ng total required/2days	cost £	amount in ng	cost £/ng	cost for 2 days of medium in £	number of 2 day treatments	total cost in £	
STAGE 1	activin a	100	750000	158	5000	0.0316	23700	3	71100	
	bFGF	10	75000	169	25000	0.00676	507	3	1521	72621
STAGE 2	BMP4	30	225000	89	2000	0.0445	10012.5	2	20025	
	FGF4	20	150000	237	25000	0.00948	1422	2	2844	22869
STAGE 3	HGF	10	75000	279	5000	0.0558	4185	1.5	6277.5	
	FGF1	10	75000	159	25000	0.00636	477	1.5	715.5	
	FGF4	10	75000	237	25000	0.00948	711	1.5	1066.5	
	FGF10	10	75000	239	25000	0.00956	717	1.5	1075.5	9135
STAGE 4	HGF	20	150000	279	5000	0.0558	8370	4	33480	
	OSM	20	150000	219	10000	0.0219	3285	4	13140	46620
								TOTAL		151245

if it was just dexamethasone		pmoles/ml	pmoles	cost (sigma)	amount in pmoles	cost £/pmole				
	DEX	10	75000	135	2544529000	0.000000053	0.004	7	0.03	
								B-13 TOTAL		0.03

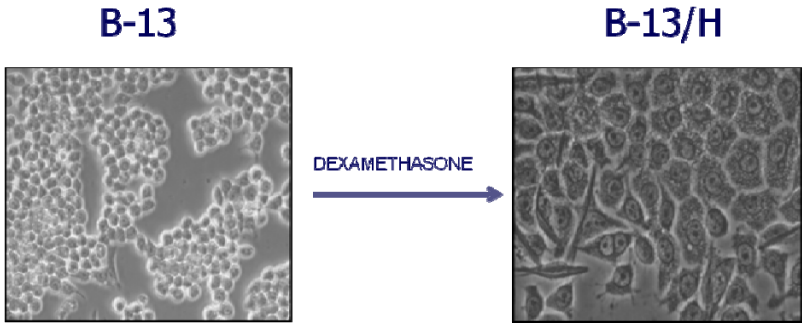
Current cost of iPSC-derived hepatocytes

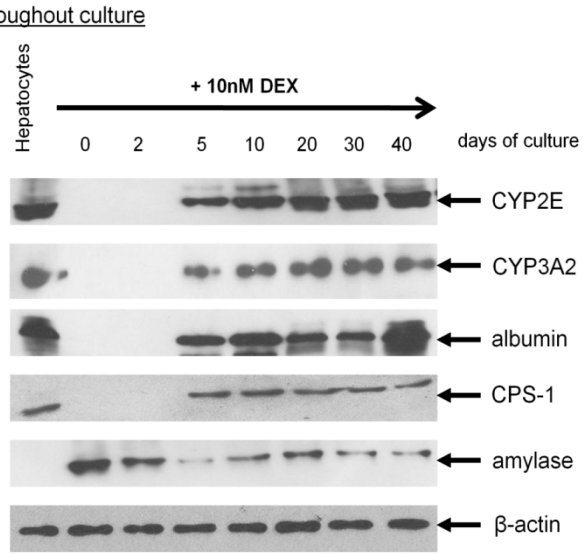
- A human "B13-like" cell could offer a potential route to delivering a cost-effective, simple solution to the production of functional hepatocytes in vitro. The B-13 cell is a rat pancreatic acinar-like cell line that is readily expandable in simple culture medium and in response to one simple glucocorticoid hormone, differentiates into non-proliferative hepatocyte-like (B-13/H) cells. As part of a program to develop an human equivalent, this laboratory has investigated the biology of the B-13 cell. B-13 cells model a pathophysiological response of rodent and human acinar tissue to differentiate into hepatocytes both in vitro and in vivo on exposure to high levels of glucocorticoid [3,4]. The cells retain a degree of biological stability in that they have maintained a normal karyotype in terms of the number of chromosomes per cell (although with some cytogenetic abnormalities); retain a requirement for substratum attachment for growth; and an ability to selectively engraft into the pancreas and liver [5]. Within the liver, the B-13 cells differentiate into hepatocytes [5]. The intrinsic value of this type of cell line is that it offers an unlimited and reproducible supply of hepatocytes in vitro, without the requirement for tissue donors. In the context of generating enough hepatocytes sufficient to populate a bioreactor capable of impacting on a patient with decompensating liver disease, the cost with respect to hormonal addition (using dexamethasone) is estimated to be **£0.03** each time. On this basis, the cost of generating hepatocytes via a B-13 type approach (based on hormonal requirements alone, many of the other costs would be similar) is approx. **5 million times cheaper than using iPSCs**. Given both the simplicity and costs of a B-13 approach to hepatocyte generation, a human B-13 equivalent would have huge potential in both experimental studies (e.g. toxicity screening) and clinical applications (e.g. extracorporeal liver support).
- 3. Wallace et al, **Wright MC**. Disrupted pancreatic exocrine differentiation and malabsorption in response to chronic elevated systemic glucocorticoid. Am J Pathol 2010;177:1225-32.
- 4. Fairhall EA, et al **Wright MC**. Adult human exocrine pancreas differentiation to hepatocytes – potential source of a human hepatocyte progenitor for use in toxicology research. Toxicology Research 2013;2:80–87.
- 5. Fairhall EA et al **Wright MC**. The "3Rs" hepatocyte progenitor B-13 cell resists pluripotency induction and differentiation to non-hepatocyte cells. Toxicol Res – submitted.

Workpackage progress – B-13s

B-13s

- expandable
- simple differentiation (1 cheap hormone)
- reliable
- CHEAP
- Advantages - understanding some of the mechanisms





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