A human iPS cell model for gene therapy vector safety evaluation

Michael Themis

Successful Trials – Gene Therapy

- *SCID-X1 (RV)
- ADA SCID (RV)
- *X-CDG (RV)
- *Wiskott-Aldrich syndrome (RV/LV)
- *β thalasaemia (LV)
- *X-linked adrenoleuko-dystrophy (X-ALD) (LV)
- Haemophilia (AAV)
- Cancer gene therapy (CAR T, Ad/CRADs)
- Blindness (AAV)
- New products: ‘Glybera’ (AAV) - Uniqure and Strimvelis (RV) - GSK

*** Clonal dominance**

Models of genotoxicity

In vivo:

Mouse

- Tumour prone Cdkn2-/- Ifnar1-/- mouse (–ve p53 and pRb) - RV>LV and vector LTR design (Montini)
- Serial transformation assay shows RV>LV causes clonal transformation (Sorentino)
- Fetal/neonatal mouse – liver oncogenesis by different LVs (Themis/Waddington)

Large animals - Canine, feline, non-human primates

- RV>LV CIS the MDS/EVI1 and clonal dominance in HSCs (Torentino)

In vitro

- Immortalisation assay - mHSc mutagenesis of Evi1 and Prdm16 genes (Modlich) RV>LV
- Jurkat/LMO-2 cell line tests effects of insertions in intron 1 of the LMO-2 (Sorentino)
- V79 HPRT (Themis), TK (Grosovski) knockout
- IL-3-independent mutagenesis murine cell line – gain of function (Takeuchi and Collins)

Genotoxicity models – Challenges

- Estimates of insertional mutagenesis frequency vary
- Endpoints may involve animal suffering, many animals used - 3Rs
- Non-representative genetic background/biased/ may restrict clinical progress
- Evidence limited to cell and vector type used
- Outcome is mostly oncogenesis based
- Costly and time consuming
- Genotoxicity outread - May not occur in humans!
- **Not standardized**

Genotoxicity factors

- Vector mediated gene activation/inactivation (promoter/enhancer)
- Chimeric vector/host transcripts (aberrant splicing/readthrough)
- Transgene effects
- Host genomic background and epigenetic response to the vector

CRACK-IT InMutaGene Challenge MRC NC3Rs Innovate UK – GSK/Novartis

- Human platform for genotoxicity
- 3Rs – Reduction, Refinement, Replacement
- Unbiased
- Predictive test for safe vectors – characterization and standardization
- **Competition – Phase I (6m) and II**

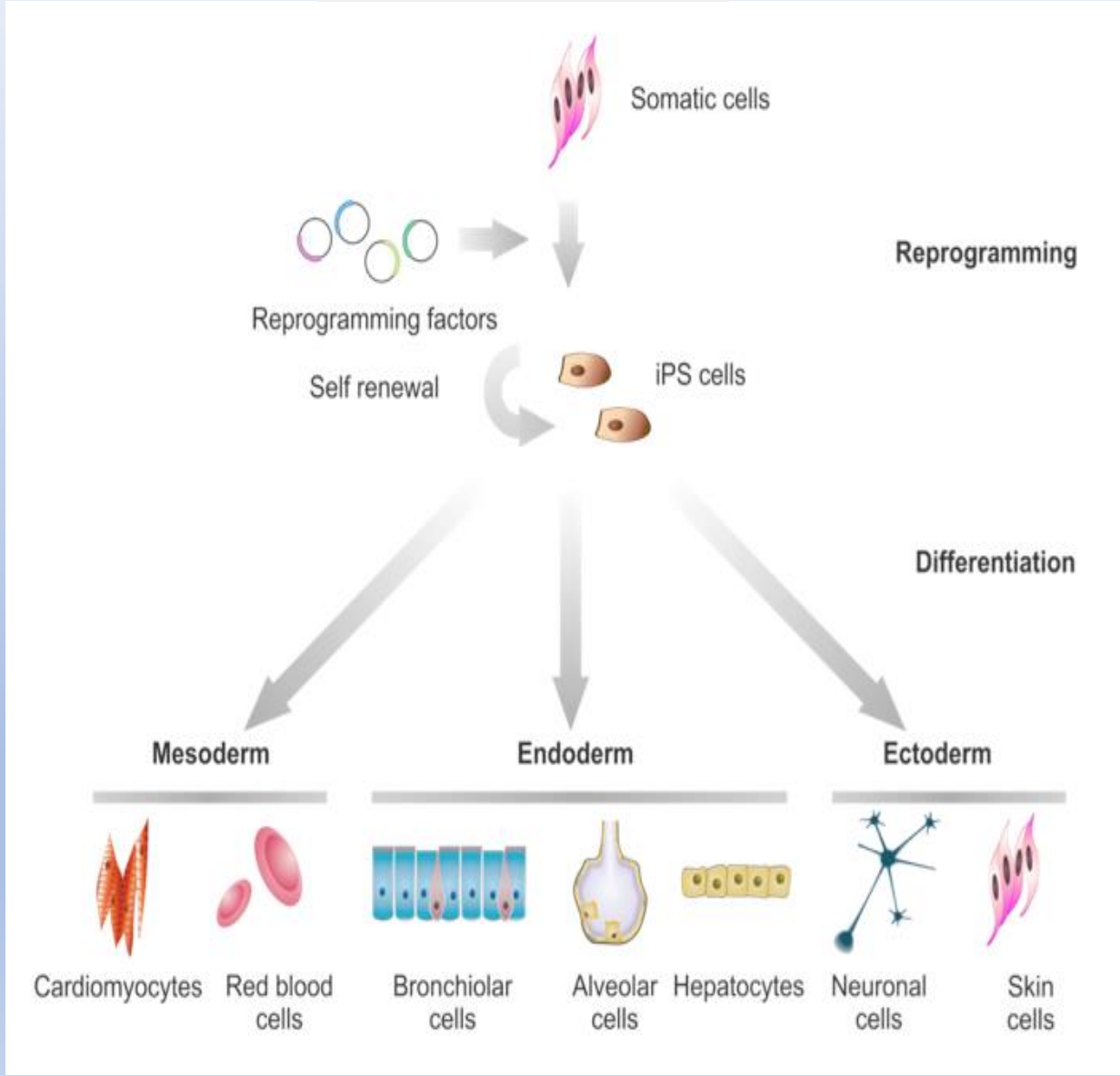
A human *in vitro* model for genotoxicity - Rationale

Induced pluripotent stem cells (iPSc) to Hepatocytes (hHE)

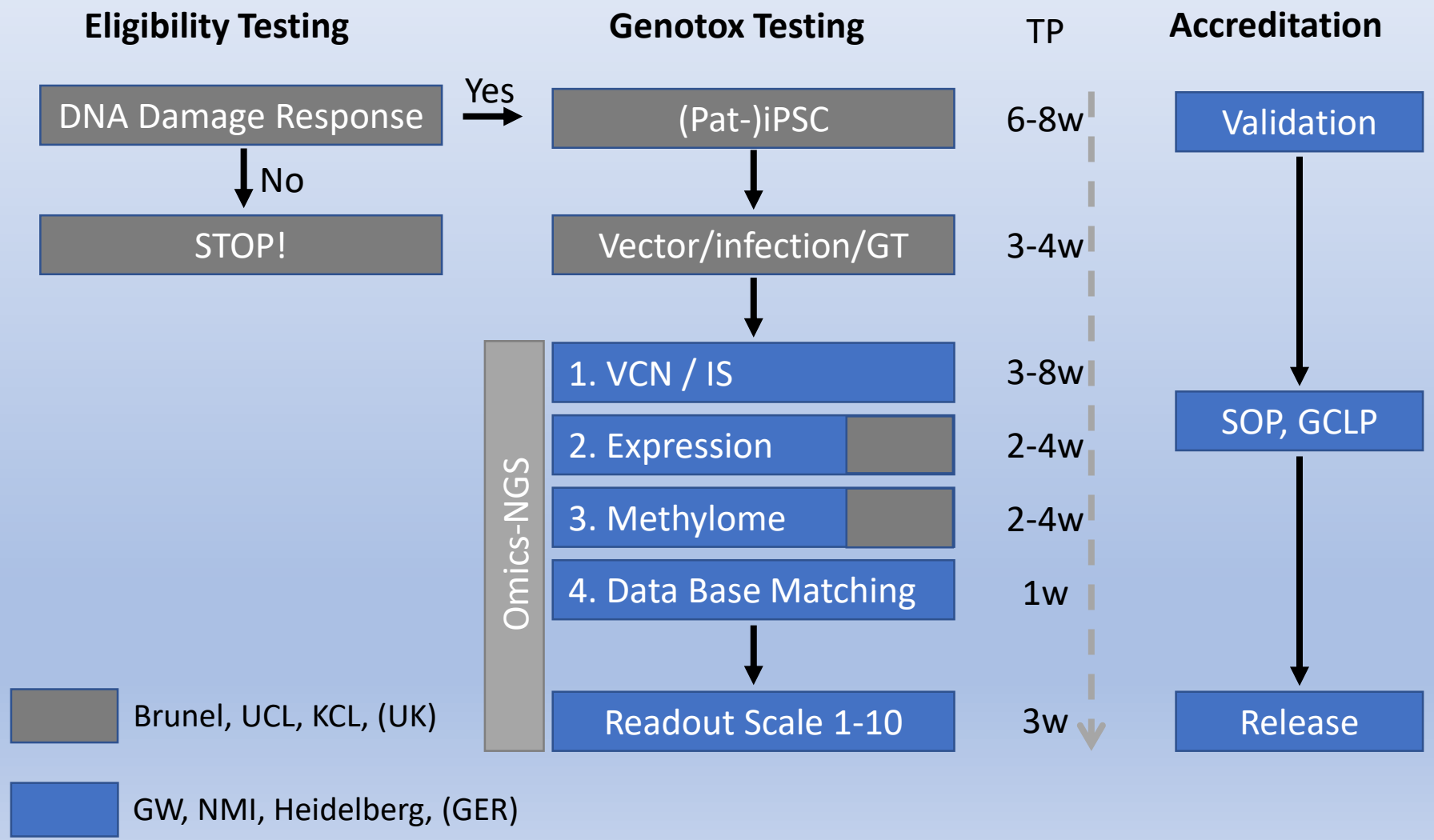
- Personalized genotoxicity approach in human cells
- Genetic background of host considered
- Reprogramming to many cell types intended for GT

Liver

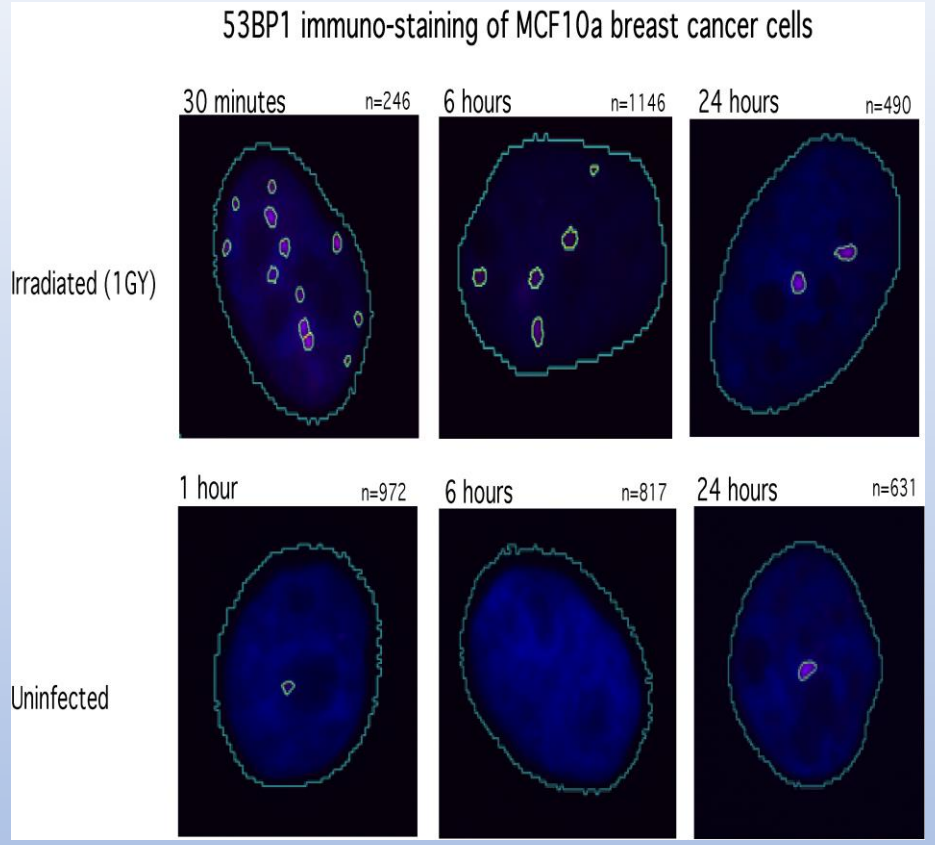
- ‘Gold Standard’ for drug screening/toxicity
- Show safe ‘IS’ clone isolation
- Offers toxicogenomics data for profiling
- BUT, may be too short-lived for toxicity testing



Individualized Genotoxicity (InGeTox) Assay – A Cross-Functional Team Initiative



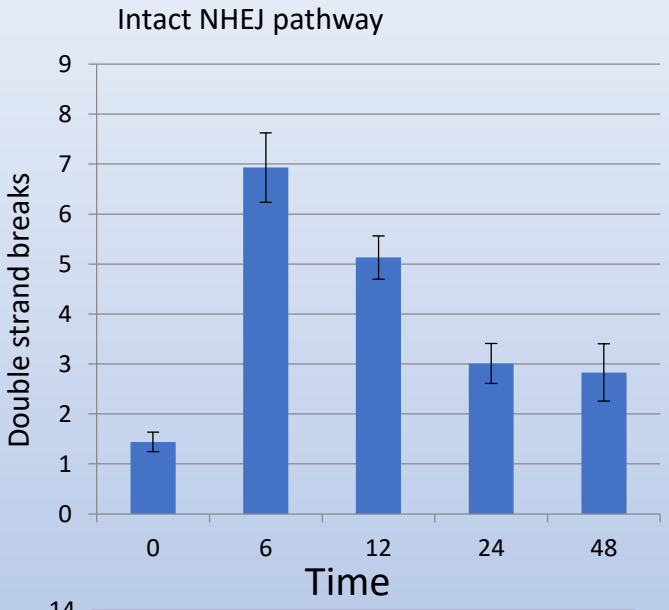
Predictive assay for DNA damage/repair following insertion

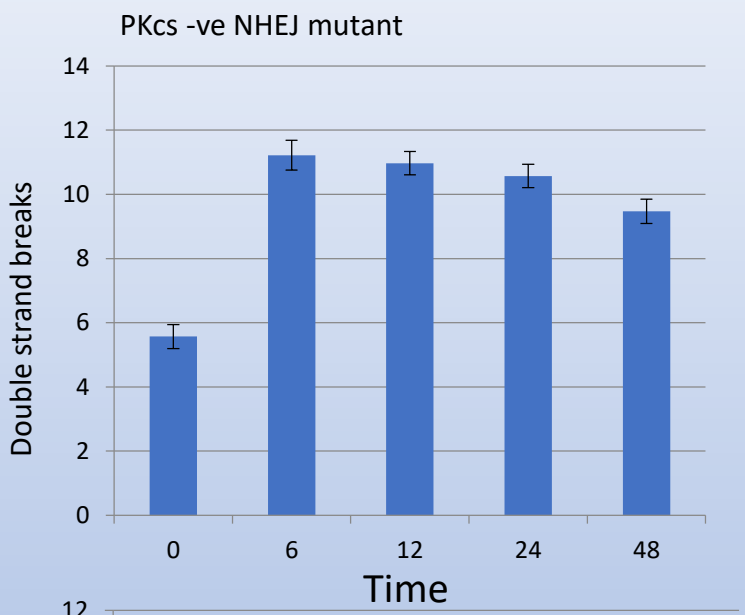


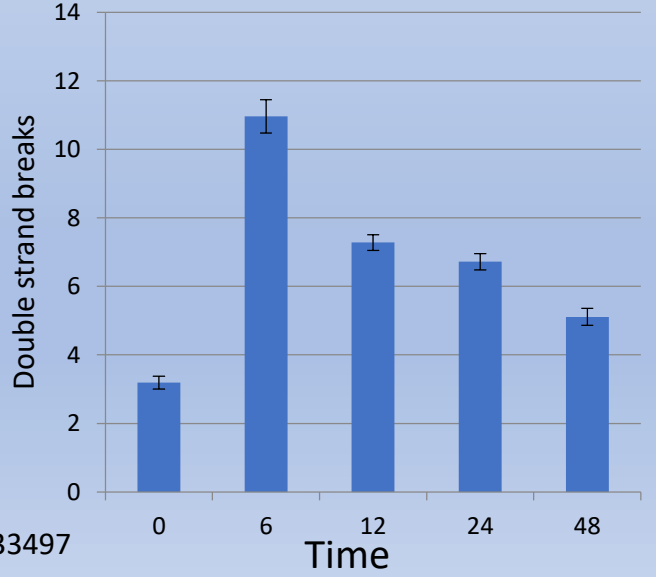
- Infected cells with RV or LV
- γH2AX/53BP1 immunostaining
- Follow DSB repair

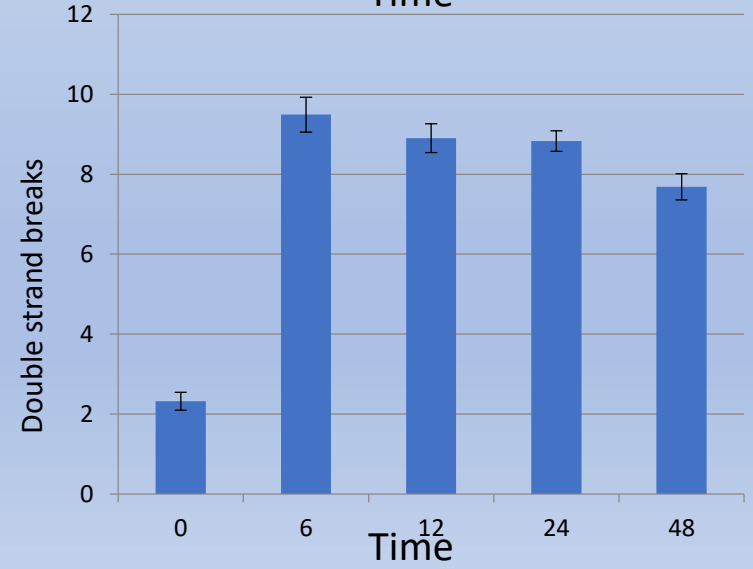
RV

LV



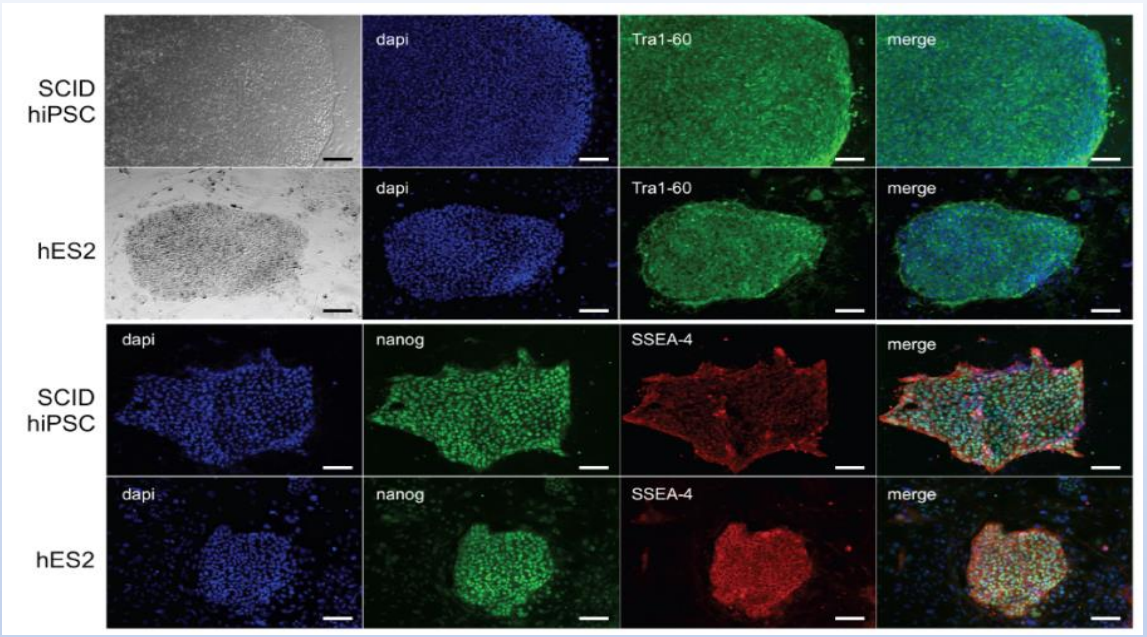




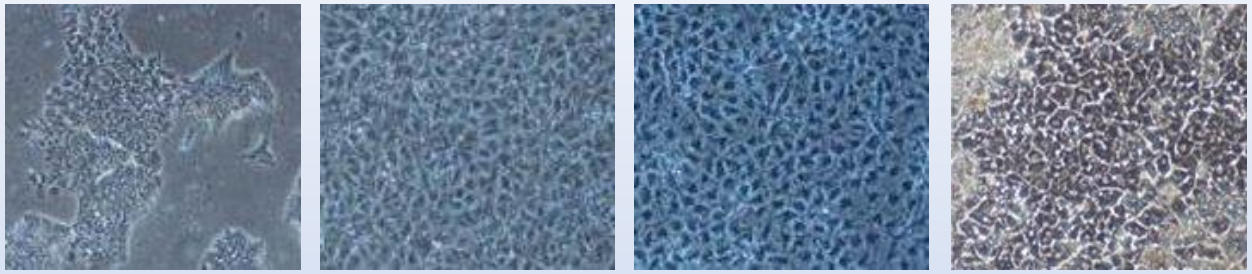


Method of Predicting the Likelihood of Success of Gene Therapy – P- KE/RON/N33497

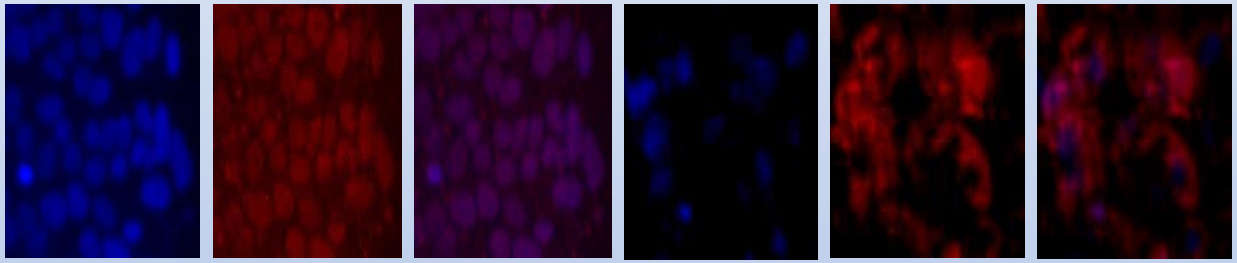
iPSc characterization and differentiation to hepatocytes



iPS – day 0 | DE – day 3 | HB – day 8 | HC– day 12

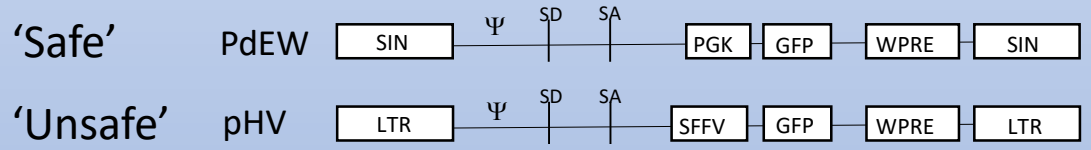


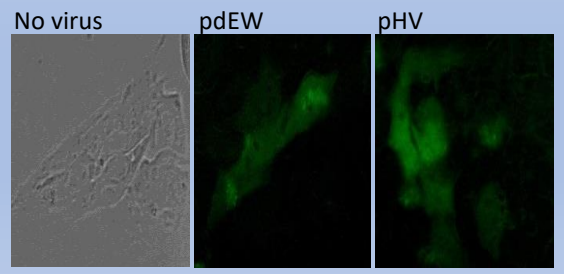
DAPI | Oct4 | Merge | DAPI | Albumin | Merge



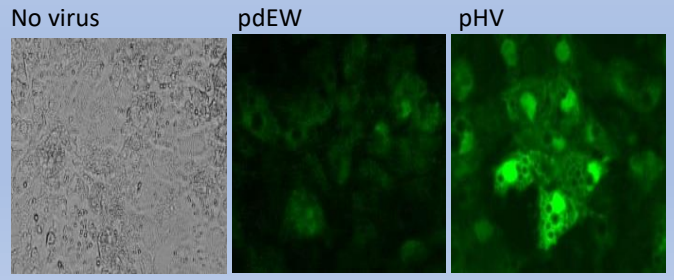
iPSc | hHE

Vector standards



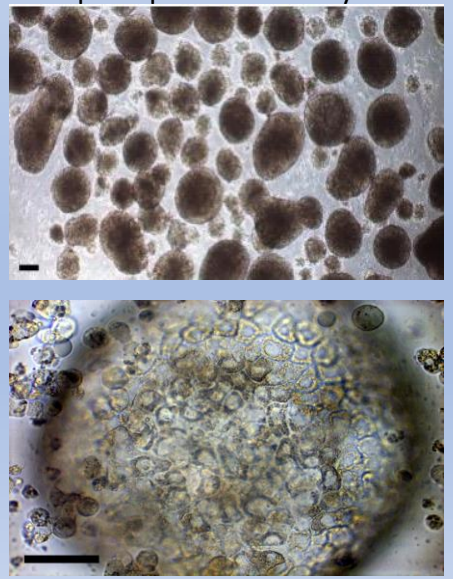


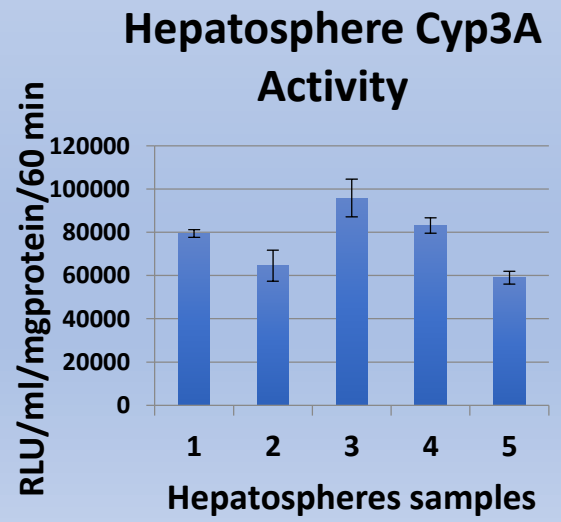
hiPSc



hHE

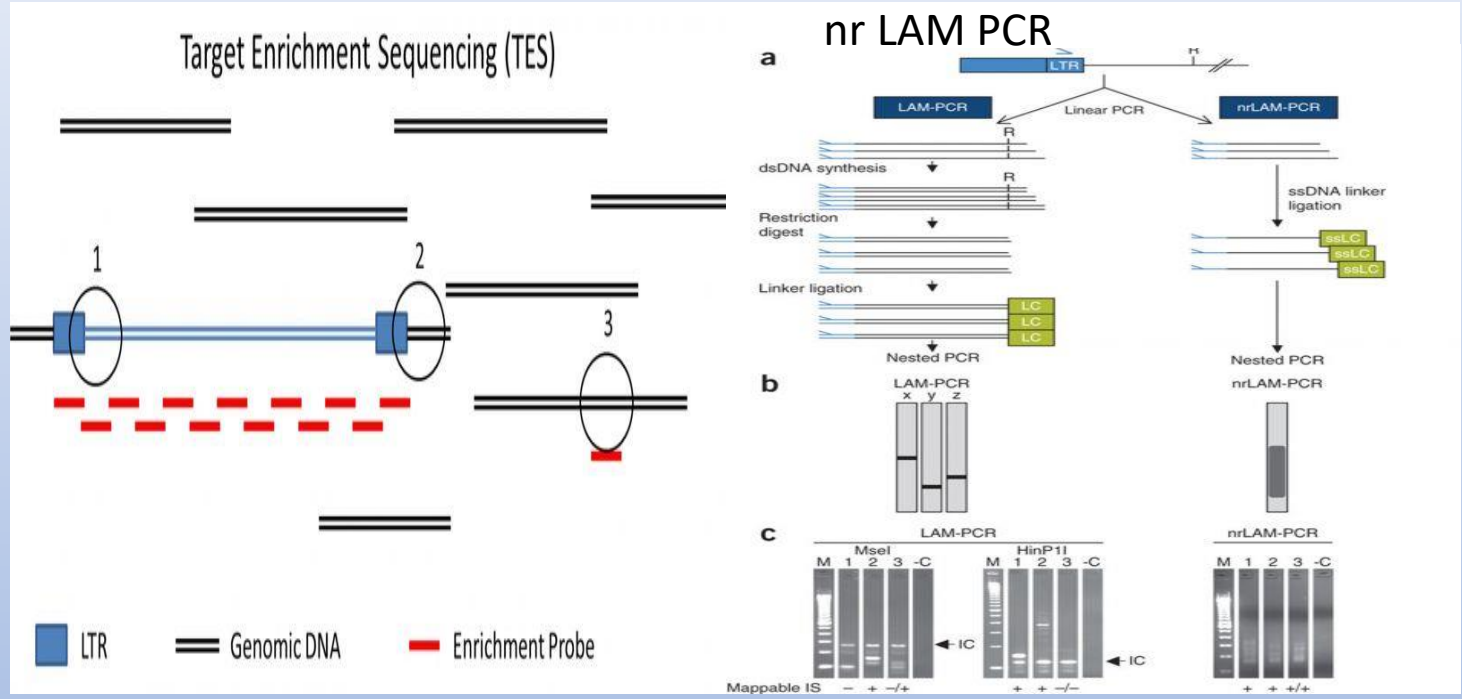
3D Hepatospheres – 60 days





Insertion site analysis

- Uses Next Gen Seq/TES/nRLAMPCR
  - Qualitative
  - Quantitative
  - Clonal contribution
  - Vector stability
  - VCN

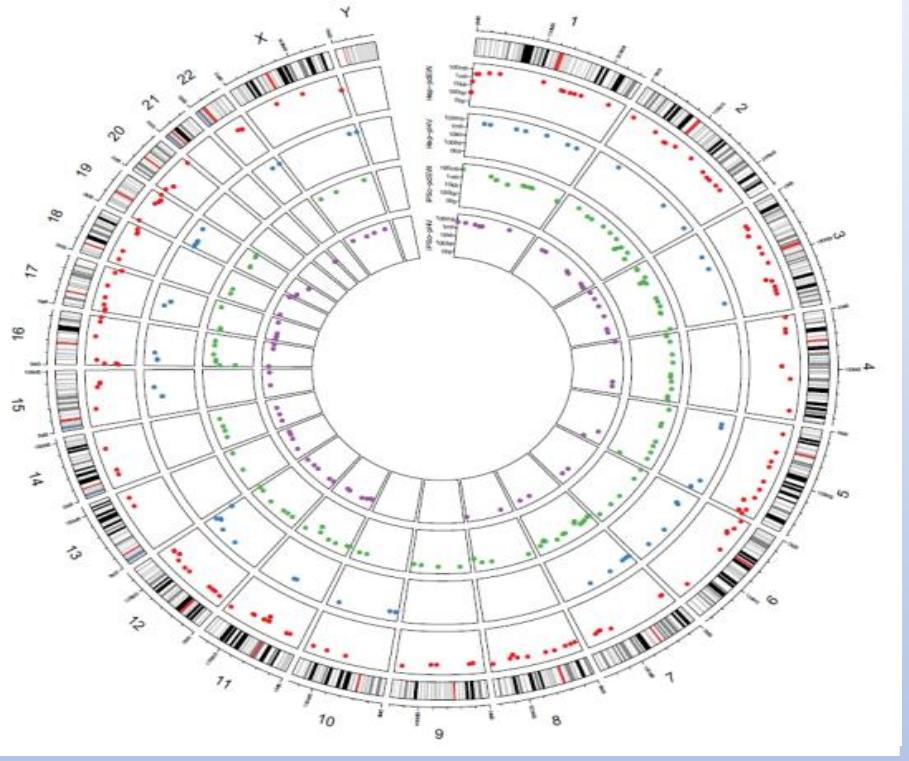


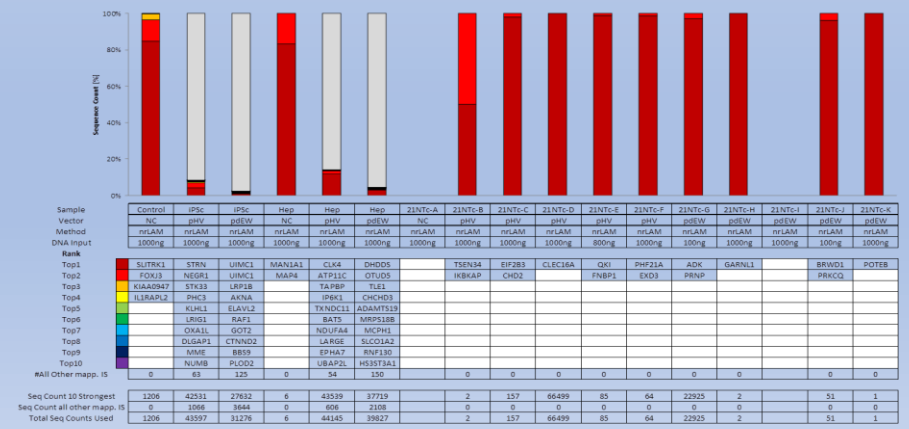
Transcriptomics

- Quant studio custom array for Q-RTPCR
- RNASeq
  - Read-through
  - Aberrant splicing

Genotox platform - GeneWerk

Integration genome wide

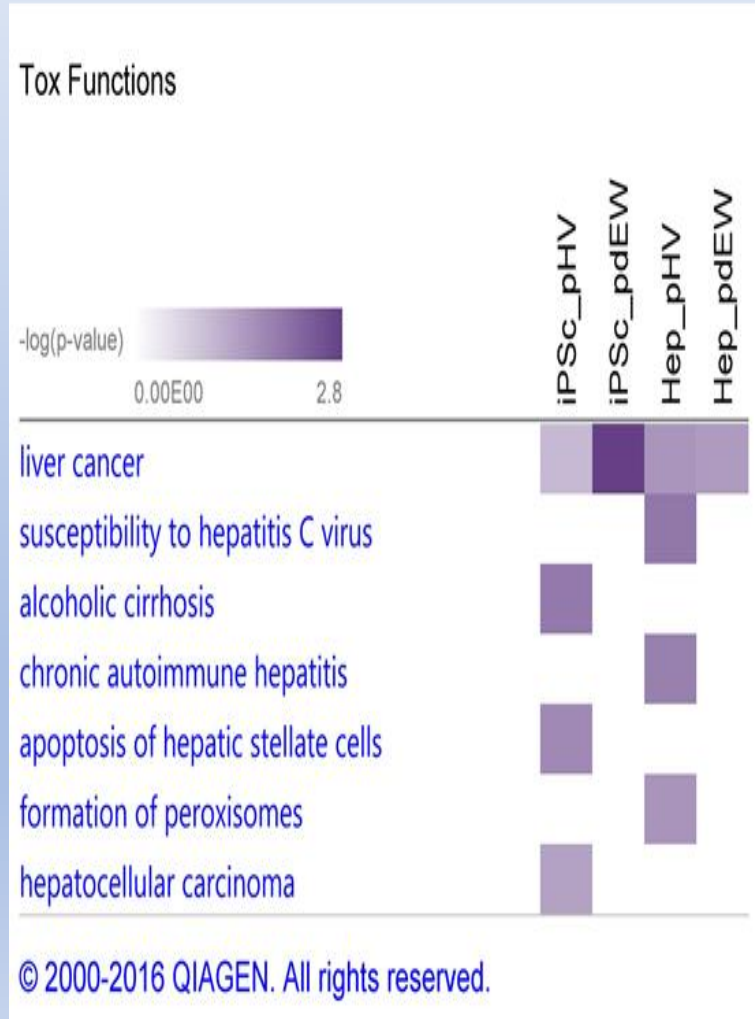




Sample	Control	iPSc	iPSc	Hep	Hep	Hep	21NTc-A	21NTc-B	21NTc-C	21NTc-D	21NTc-E	21NTc-F	21NTc-G	21NTc-H	21NTc-I	21NTc-J	21NTc-K
Vector	NC	pHV	pdEW	NC	pHV	pdEW	NC	pHV	pHV	pHV	pHV	pHV	pdEW	pdEW	pdEW	pdEW	pdEW
Method	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM	nrLAM
DNA Input	1000ng	1000ng	1000ng	1000ng	1000ng	1000ng	1000ng	1000ng	1000ng	1000ng	800ng	1000ng	100ng	1000ng	1000ng	100ng	1000ng
Rank																	
Top1	SLITRK1	STRN	UIMC1	MAN1A1	CLK4	DHDDS		TSEN34	EIF2B3	CLEC16A	QKI	PHF21A	ADK	GARNL1		BRWD1	POTEB
Top2	FOXJ3	NEGR1	UIMC1	MAP4	ATP11C	OTUD5		IKBKAP	CHD2		FNBP1	EXD3	PRNP			PRKCQ	
Top3	KIAA0947	STK33	LRP1B		TAPBP	TLE1											
Top4	IL1RAPL2	PHC3	AKNA		IPMK1	CHCHD3											
Top5		KLHL1	ELAVL2		TXNDC11	ADAMTS19											
Top6		LRIG1	RAF1		BAT5	MRPS18B											
Top7		OXA1L	GOT2		NDUFA4	MCPH1											
Top8		DUSAP1	CTNND2		LARGE	SLCO1A2											
Top9		MME	BBS9		EPHA7	RNF130											
Top10		NUMB	PLOD2		UBAP2L	HS3ST3A1											
#All Other mapp. IS	0	63	125	0	54	150		0	0	0	0	0	0	0		0	0
Seq Count 10 Strongest	1206	42531	27632	6	43539	37719		2	157	66499	85	64	22925	2		51	1
Seq Count all other mapp. IS	0	1066	3644	0	606	2108		0	0	0	0	0	0	0		0	0
Total Seq Counts Used	1206	43597	31276	6	44145	39827		2	157	66499	85	64	22925	2		51	1

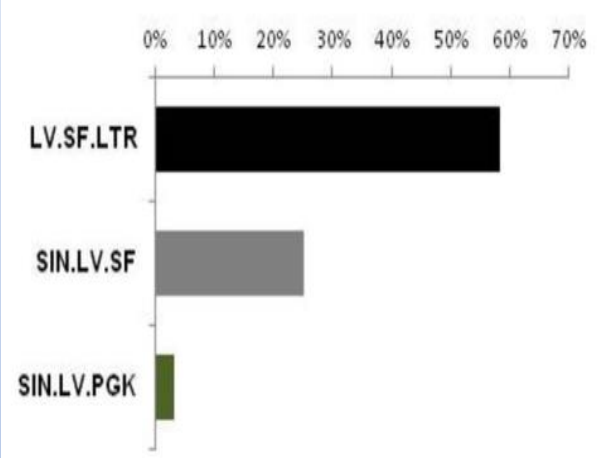
Toxicogenomics/IPA

http://www.ingenuity.com/products/ipa/toxicogenomics

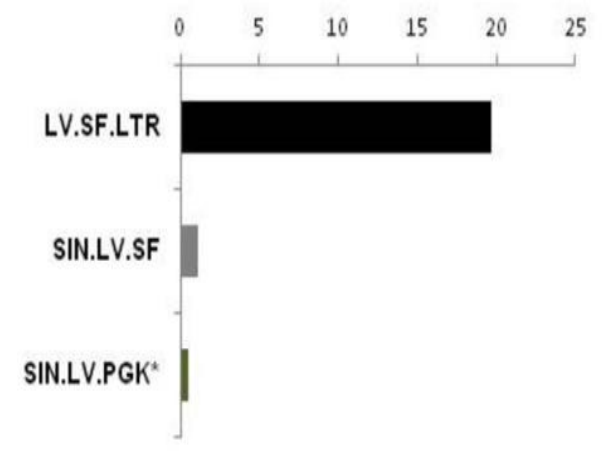


Functional analyses

Fusion transcript generating integrants

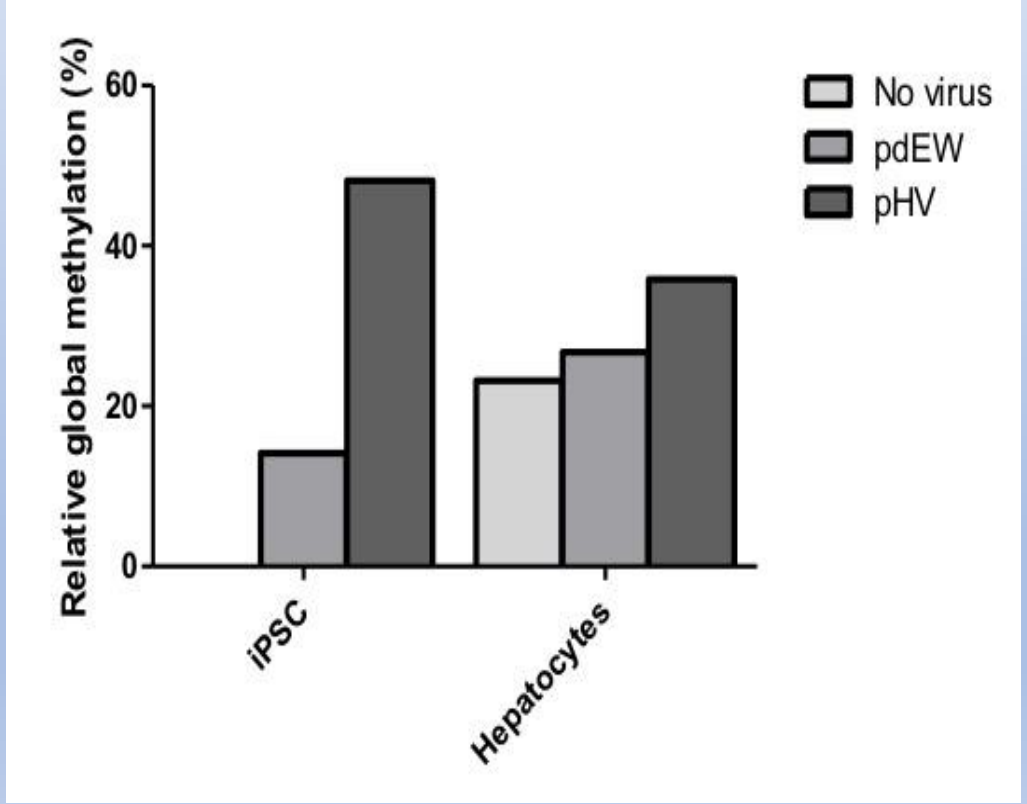


Clonal outgrowth



Methylomics

- Global methylation /ELISA
- Bisulfite mapping/RNASeq (ongoing)



Current position

- GeneWerk/Brunel, validation/set-up
- iPSc differentiation and clinical vectors
- Online marketing – CRACK-IT InGeTox Webpage linked with NC3Rs
- Leading Edge article
- NC3Rs/Concordat – Openness on Animal Research alternative to animal models
- BSGCT 2017 – Cardiff
- Exhibitions, Sponsoring – flyers, stands at ASGCT (2017) and ESGCT (2016)
- Customer newsletter – alerts and information

Nationales Zentrum fur Tumorerkrankungen (NCT), Heidelburg, Germany
Dr Manfred Schmidt CEO**, GeneWerk**
Wei Wang, **GeneWerk**

NMI Tubingen, Germany

Dr Frank Weise

University College London, UK
Yasuhiro Takeuchi
Mary Collins

King's College London
Els Henkaerts

MRC Centre for Regenerative Medicine, Edinburgh University

Dr David Hay