Advances in Industrial-Scale Generation of Human Hepatocytes for Liver-Disease and Drug Development Studies

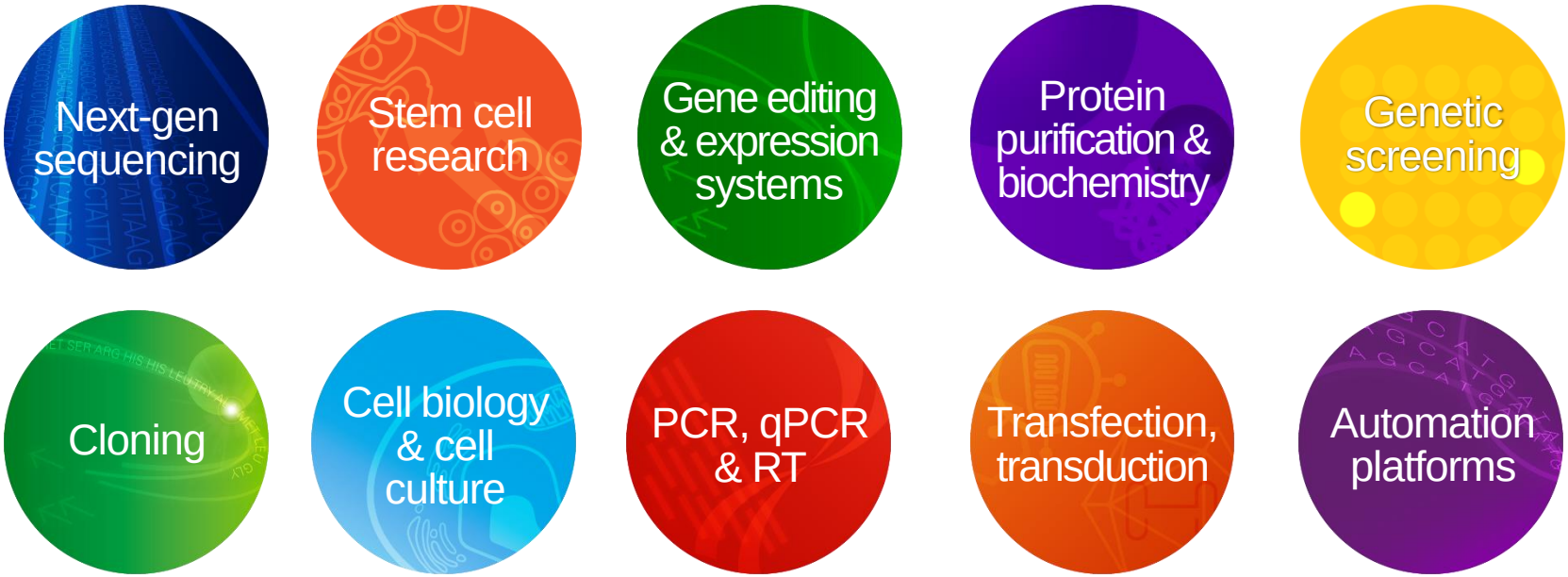
Elizabeth Quinn, PhD

February 5, 2019

that's GOOD science!®

Solutions for research challenges

Reagents, instrument systems, integrated solutions, and services



Core values

Best-in-class products

- Our dedicated and talented R&D team develops class-leading products

Expert support

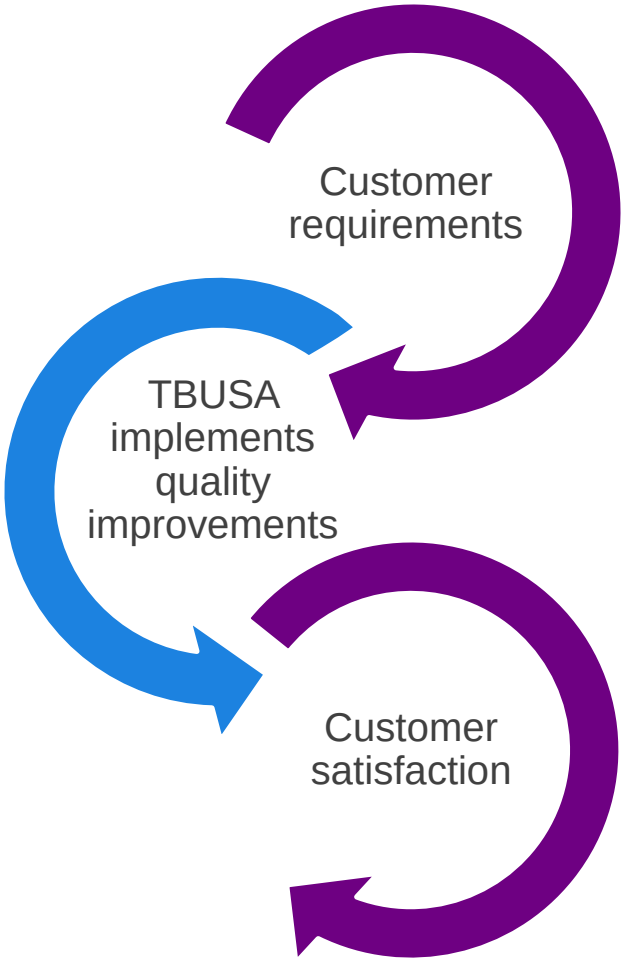
- Your scientific endeavors are supported by a knowledgeable team of technical support professionals

Superior value

- Our products offer great performance at a competitive price

…and that's good science.

Commitment to quality



- We are committed to understanding and meeting customers' quality needs
- We strive to provide quality, innovative products and services
- We meet our commitment to customer satisfaction through our comprehensive quality assurance system
- Every employee is responsible for continuous quality improvement

The Takara Bio USA, Inc. (TBUSA) quality management system is registered under ISO 13485:2016.

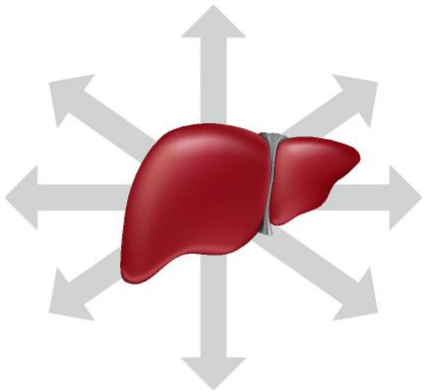
Outline

- Introduction to liver disease and hepatocyte models
- Human iPSC-derived hepatocytes for disease modeling
  - Key limitations with current models
  - iPS cell to hepatocyte differentiation
- Primary hepatocytes for drug development studies
  - Key limitations with current models
  - Long-term drug metabolism studies

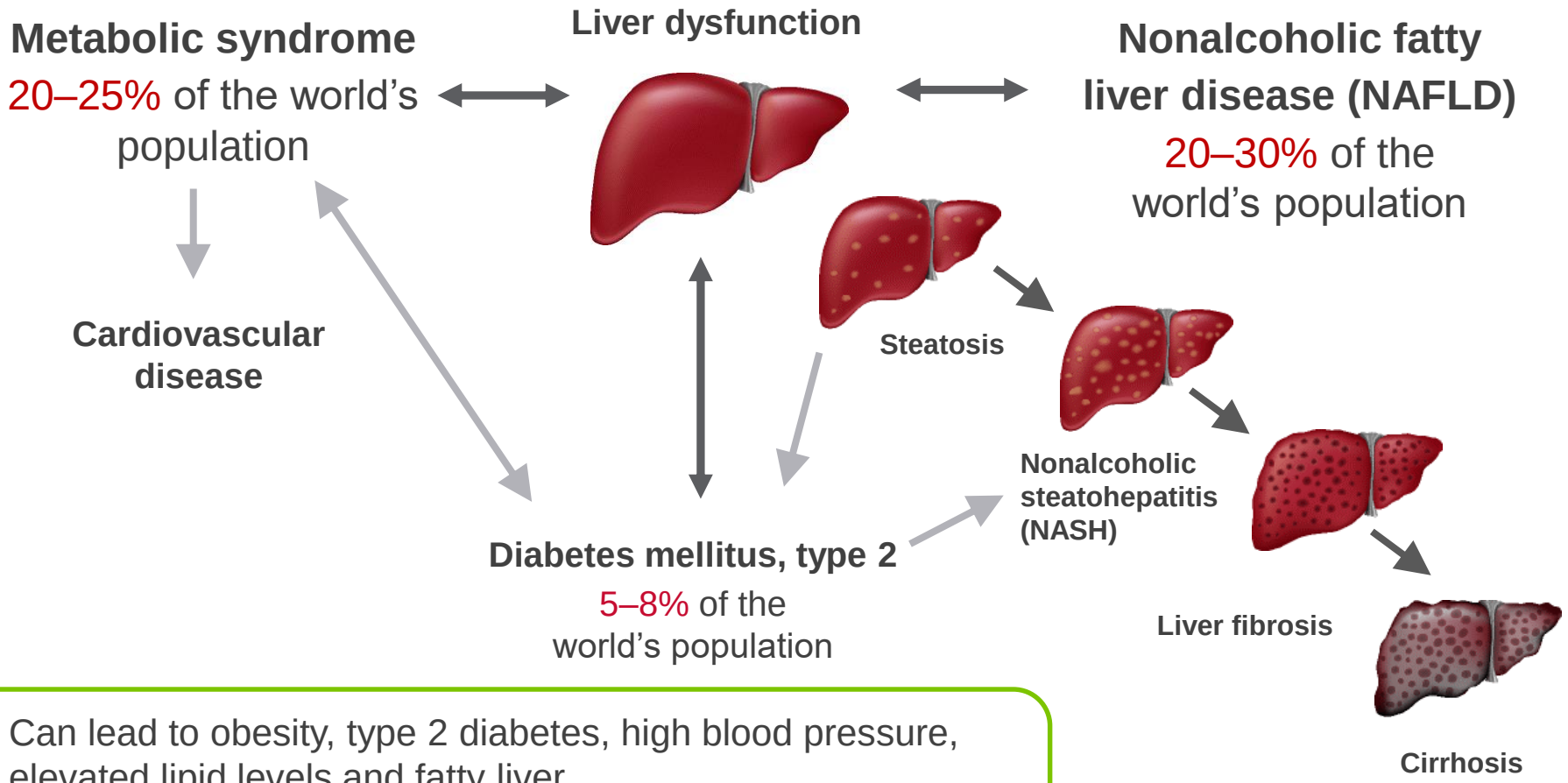
Liver function

The liver performs more than 500 vital functions, including:

- Blood coagulation
- Bile production for fat digestion
- Innate immune system
- Production of proteins and cholesterol
- Drug metabolism
- *De novo* generation of glucose
- Excretion of waste via bile
- Storage of excess glucose and glycogen

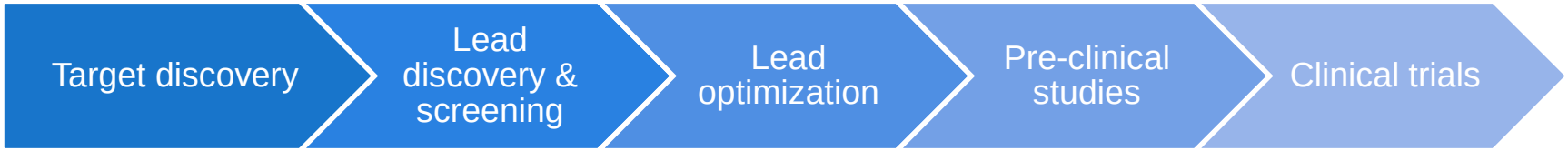


Metabolic disease & liver dysfunction



- Can lead to obesity, type 2 diabetes, high blood pressure, elevated lipid levels and fatty liver
- NASH and NAFLD pathogenesis not well understood → until recently, heavily dominated by diabetes treatments

Understanding and treating liver diseases



Disease modeling

- Model development for high-throughout screening

Drug metabolism and toxicity assessment

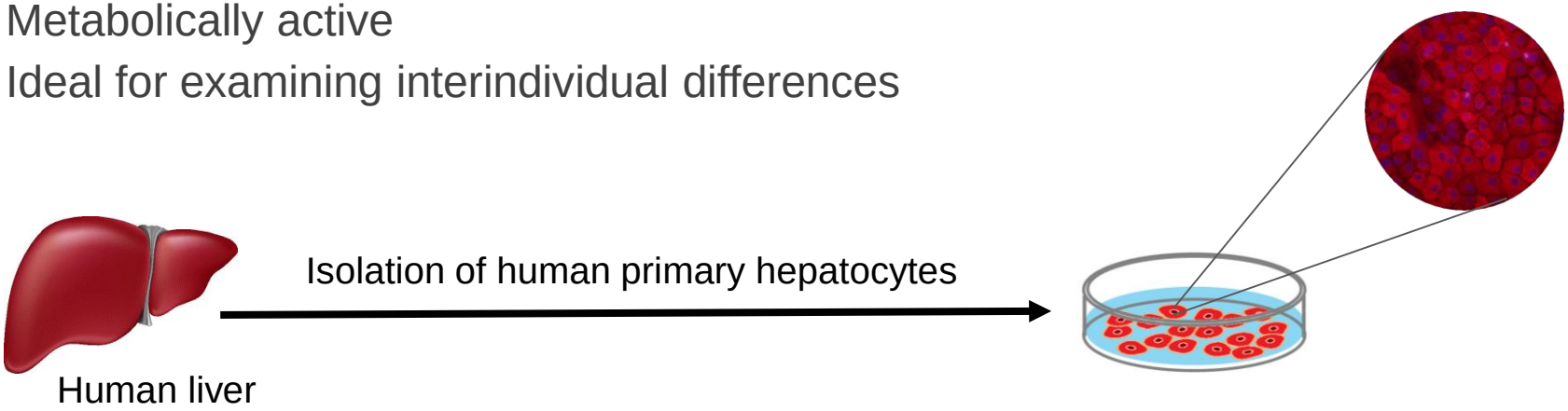
- Use *in vitro* cellular models to predict toxicity, metabolism, tissue perfusion, and biodistribution
- *In vitro* safety profiling

- **Disease modeling**: develop models to understand disease mechanisms, models can then be used for compound library screening
- **Drug metabolism assessment**: use *in vitro* models to predict *in vivo* safety

What are liver researchers currently using?

Primary hepatocytes are the gold standard for liver research

- Reflect the functionalities of the liver
- Metabolically active
- Ideal for examining interindividual differences



Major limitations:

- Can be difficult to obtain cells with desired background or mutation, which is not ideal for disease modeling
- Rapid loss of function and viability when cultured *in vitro*—not suitable for long-term drug metabolism studies

Image adapted from Katrin Zeilinger, Nora Freyer, Georg Damm, *et al.* Cell sources for *in vitro* human liver cell culture models. *Exp. Biol. Med.* **241**, 1684–1698 (2016).

Complete hepatocyte portfolio

iPSC-derived hepatocytes

DO IT YOURSELF

Cellartis iPS Cell to Hepatocyte Differentiation System

- Cellartis DEF-CS™ Culture System
- Cellartis Definitive Endoderm Differentiation Kit
- Cellartis Hepatocyte Differentiation Kit

Cellartis Hepatocyte Maintenance Medium

READY MADE

- Cellartis Human iPS Cell Lines
- Cellartis Definitive Endoderm Cells
- Cellartis Enhanced hiPS-HEP v2 Cells

CUSTOM MADE

Cellartis Human Pluripotent Stem Cell Services

Sourcing | Reprogramming | Cell Banking | Gene Editing | Differentiation

Primary hepatocytes

Cellartis® Power™ Primary HEP Medium

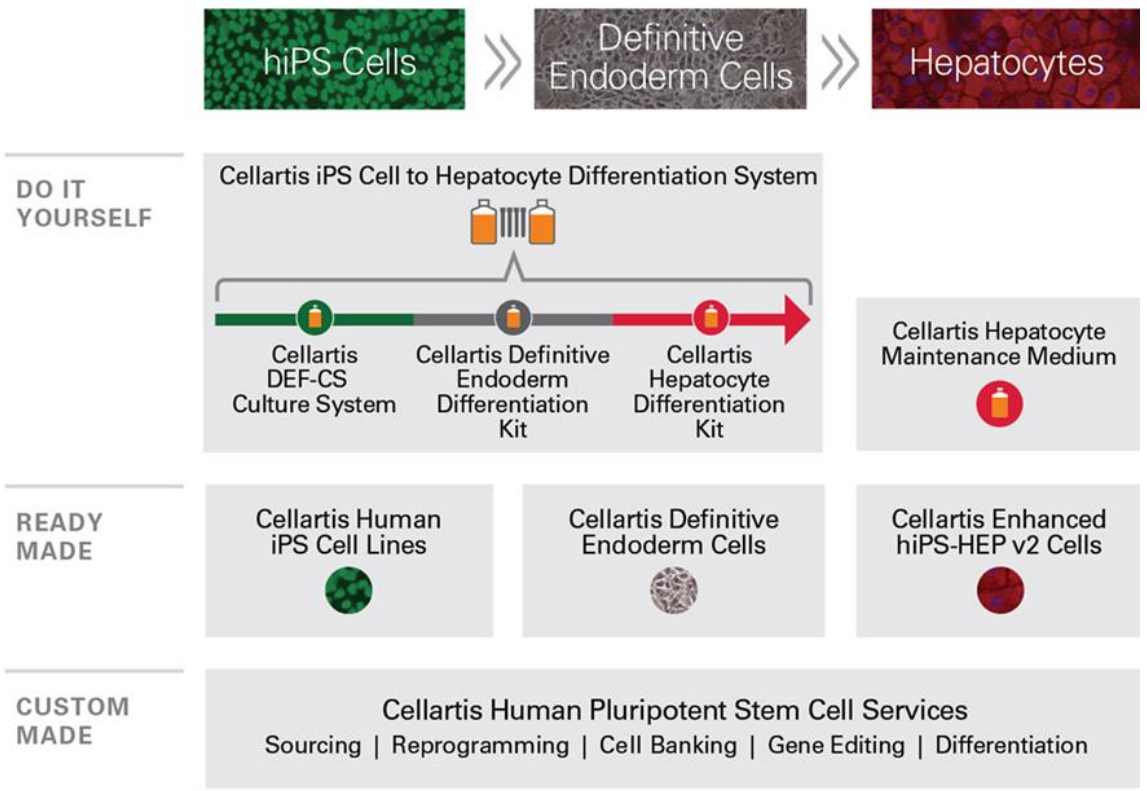
- Metabolically active phenotype for up to 4 weeks
- Minimizes variability
- No daily feedings or sandwich overlay
- Convenient, complete medium
- Enables accurate intrinsic clearance (CL_{int}) studies

iPSC-derived hepatocytes for disease modeling

Target discovery | **Lead discovery & screening** | Lead optimization | Pre-clinical studies | Clinical trials

Disease modeling
- Model development for high-throughout screening

- Generate disease models from patient/disease-specific cells
- Accurate hepatocyte model
- Unlimited supply of human hepatocytes



Clontech TaKaRa cellartis

Power medium for drug metabolism

Target discovery → Lead discovery & screening → **Lead optimization** → Pre-clinical studies → Clinical trials

Drug metabolism and toxicity assessment

- Use *in vitro* cellular models to predict toxicity, metabolism, tissue perfusion, and biodistribution
- *In vitro* safety profiling

Cellartis Power Primary HEP Medium

- Achieve a healthy, functional phenotype for up to 4 weeks in culture
- Maintains metabolically active hepatocytes
- Minimizes intra-assay donor variability
- Eliminates need for daily feedings; no sandwich overlay required
- Includes complete medium, frozen and premixed
- Enables accurate prediction of intrinsic clearance (CL_{int})

IPSC-DERIVED HEPATOCYTES FOR DISEASE MODELING

Generation & applications of disease models

- iPSC-derived cells now widely accepted for disease modeling
  - Access to normal or diseased donors
- Disease model researchers often want to do gene editing
  - Introduce or correct a mutation to study a disease mechanism
  - Knock out a gene to study gene function
  - Generate a reporter line for assays
- 22% of all research now uses iPSCs
  - Increasing adoption for advanced applications

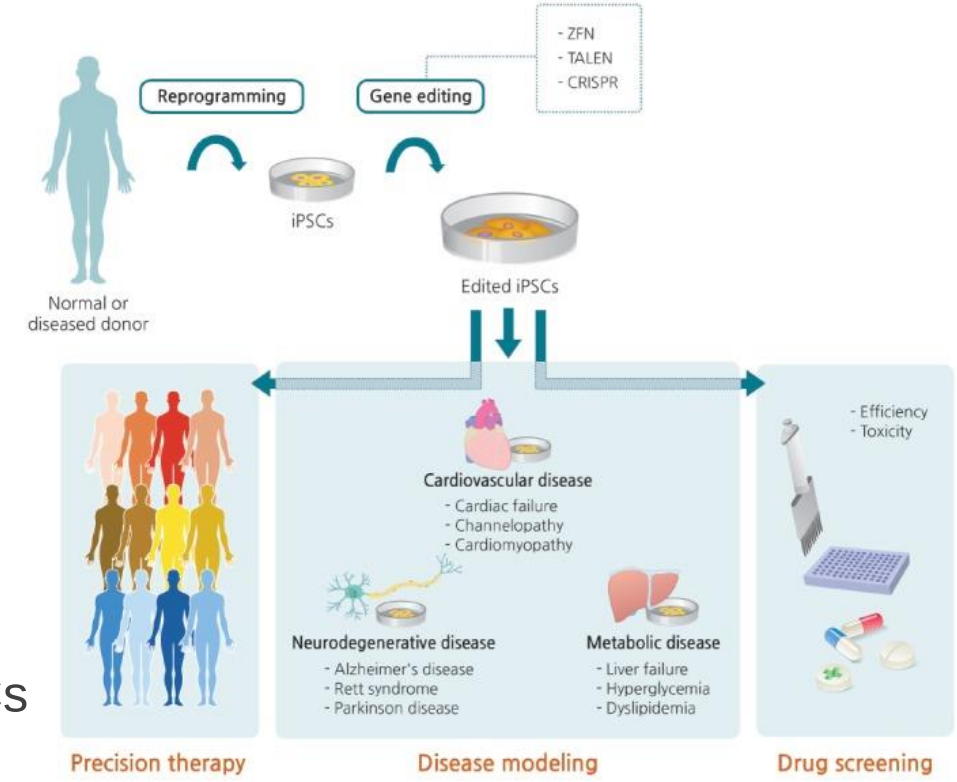


Figure from Kim, Kang & Ju 2017. Copyright © 2017 The Korean Association of Internal Medicine.

The future is in iPSC-derived, donor-specific cells that can be used for accurate disease modeling

Limitations of primary hepatocytes for disease modeling

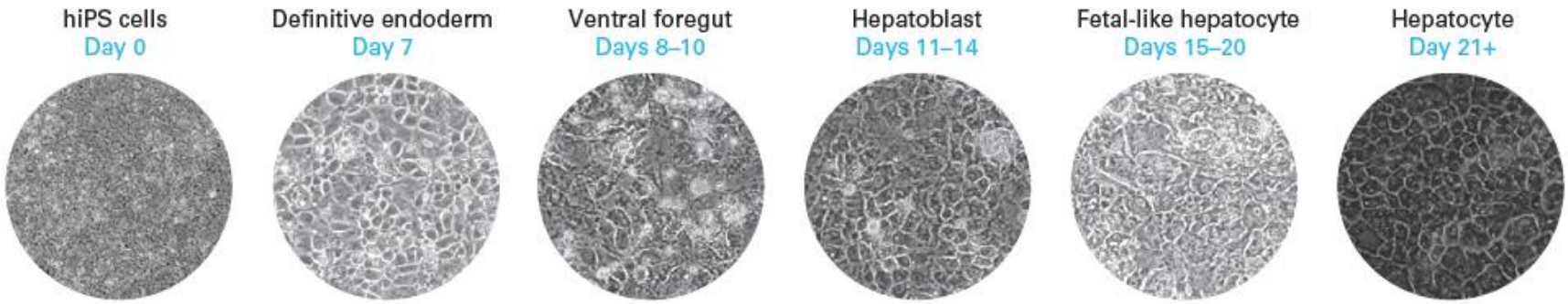
While primary hepatocytes are the gold standard for liver research, there are several challenges to using primary hepatocytes for disease modeling:

- Donor variability confounds results
- Can be difficult to find cells with desired genetic background
- Difficult to obtain large quantities
  - Limited source
  - Rapidly lose functionality
  - Dedifferentiation
  - Variable proliferation

Takara Bio's solution: hiPSC-derived hepatocytes with reduced variability, extended viability and functionality

Hepatocyte-directed differentiation

- Standardized serum- and feeder-free protocol
- Recapitulation of liver development *in vivo*
- Yields a pure population of functional and mature hepatocytes
- Provides a renewable source of hepatocytes



Takara Bio's iPSC-derived hepatocytes

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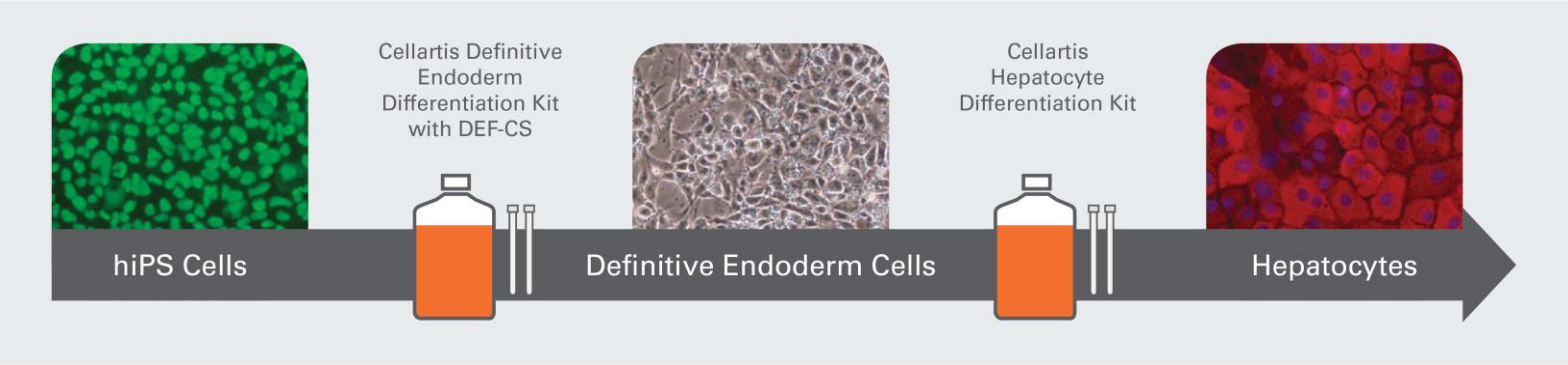
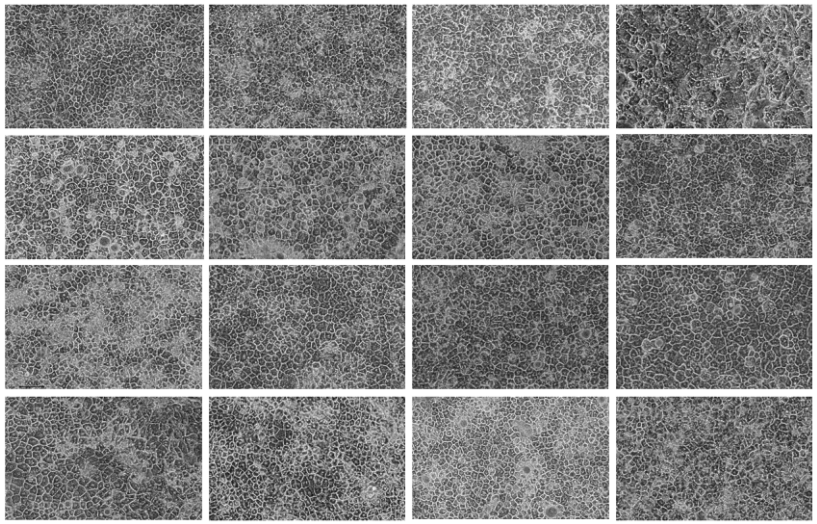
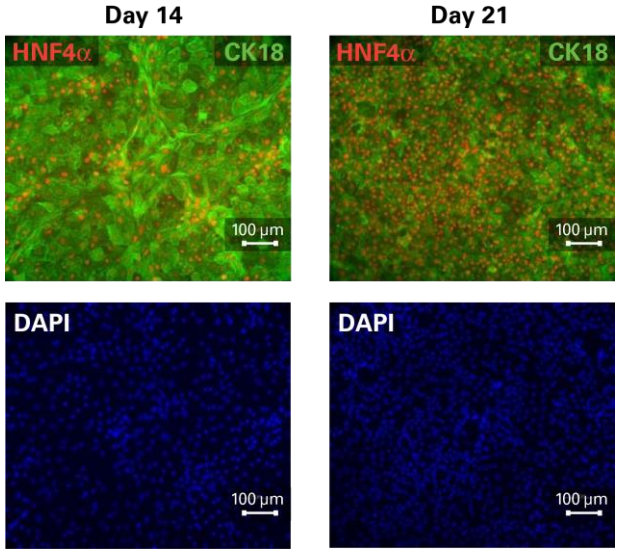
Sourcing | Reprogramming | Cell Banking | Gene Editing | Differentiation

Industrialized and commercialized

Cellartis iPS Cell to Hepatocyte Differentiation System

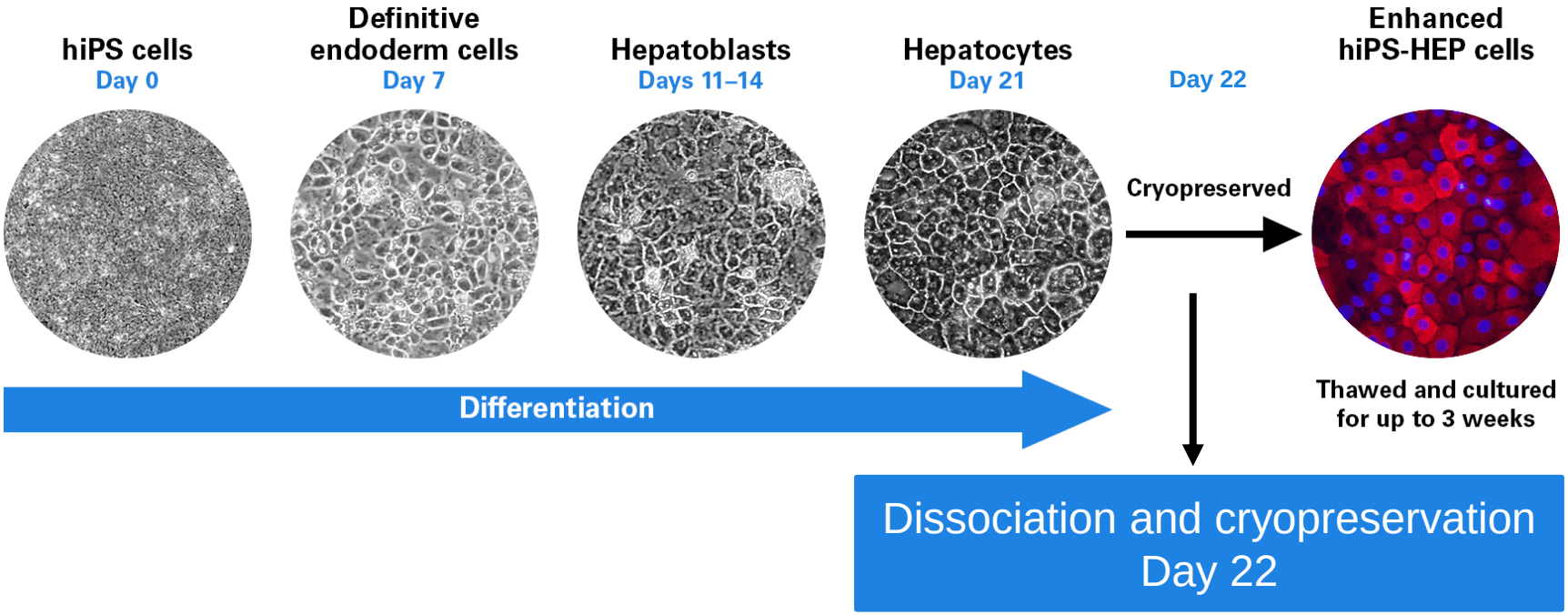




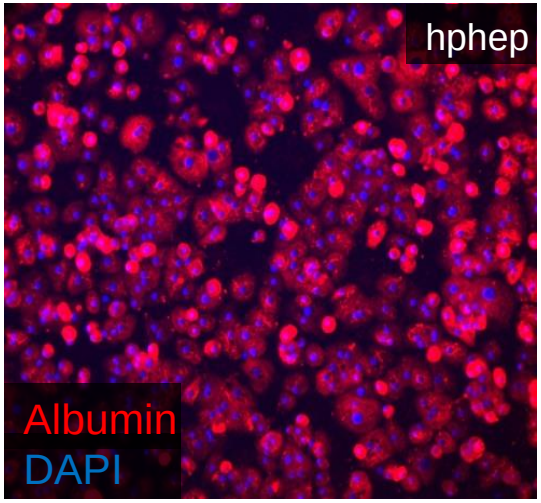


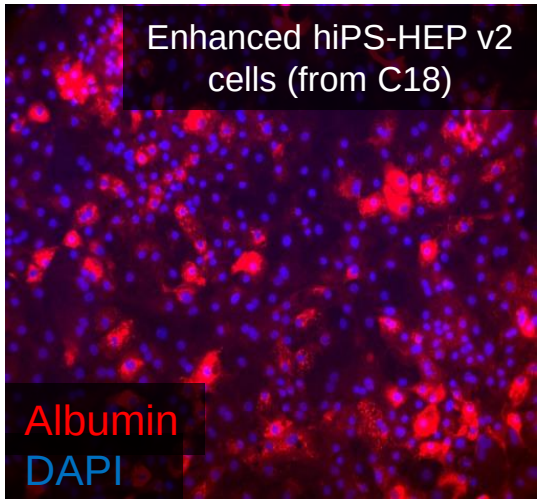
Cryopreserved enhanced hiPS-HEPs

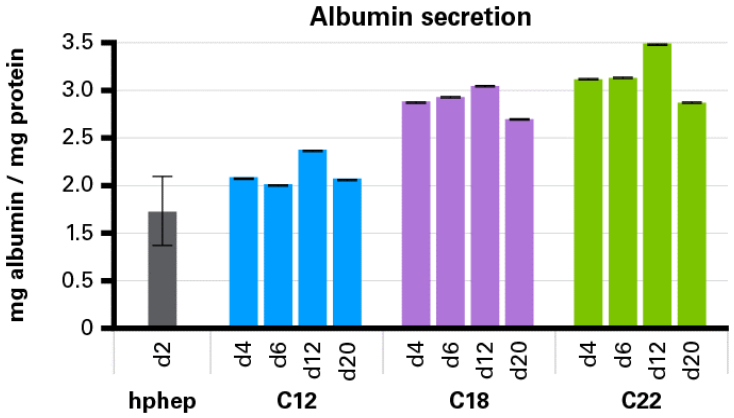
Frozen, iPSC-derived hepatocytes from healthy donors



Albumin secretion shows maturity and functionality

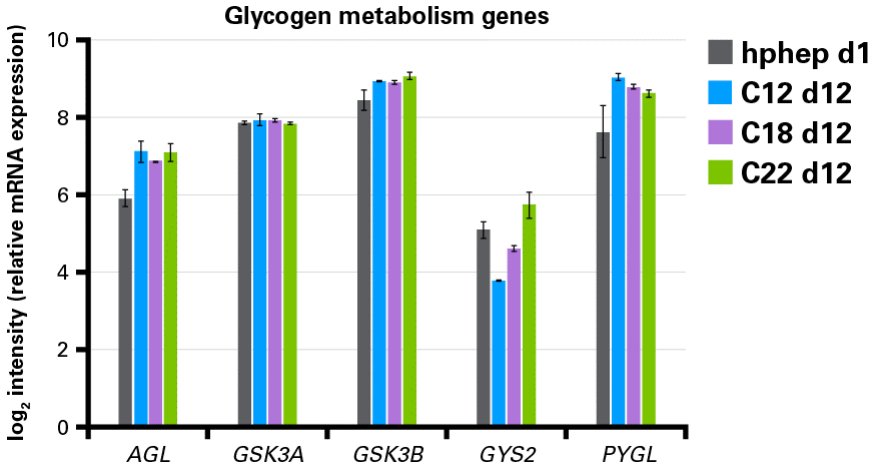


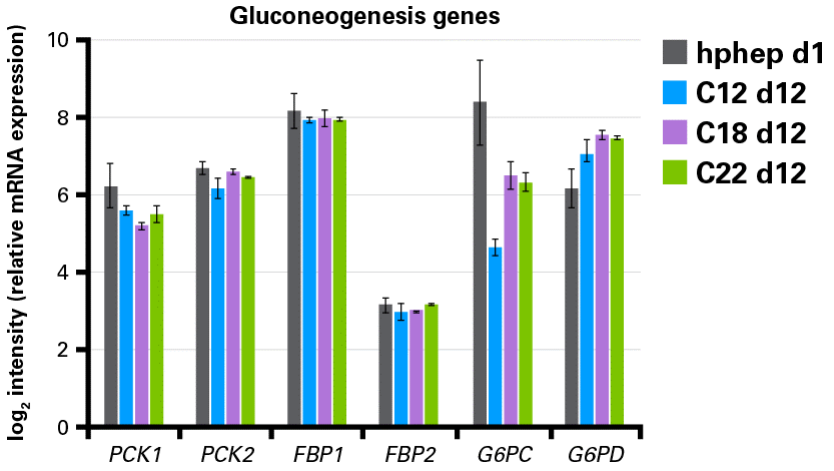




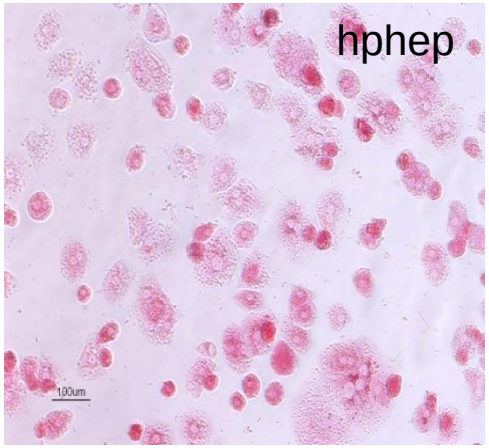
Albumin secretion and expression similar to primary hepatocytes

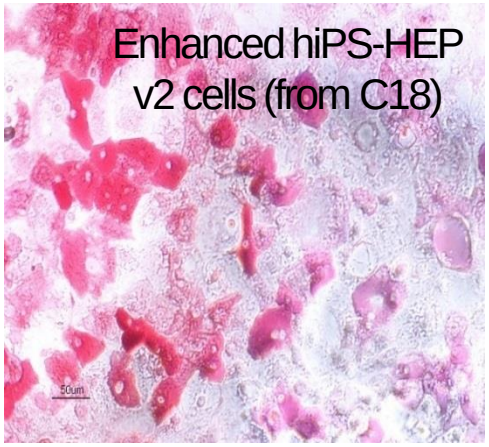
Enhanced hiPS-HEP v2 cells express key features of hepatic glucose metabolism





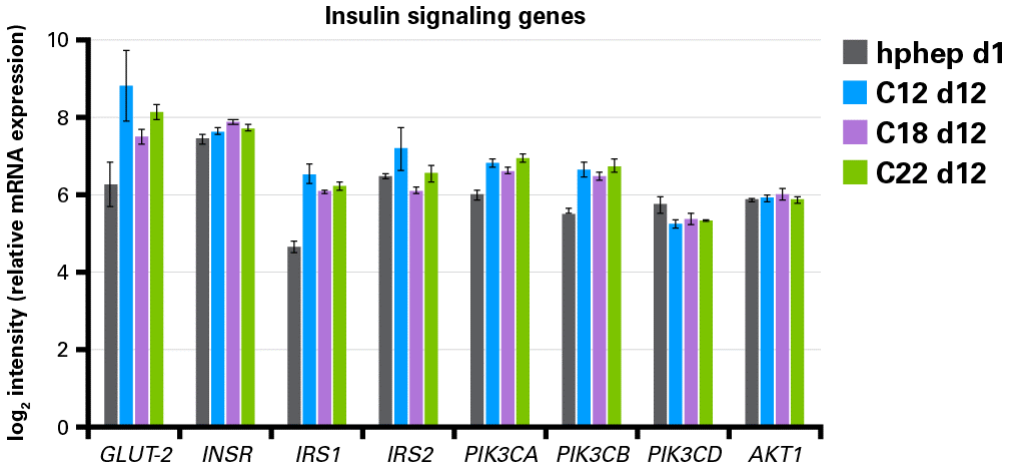
- Expression of genes for glycogen metabolism and gluconeogenesis comparable to primary hepatocytes
- Glycogen storage in subpopulation of cells

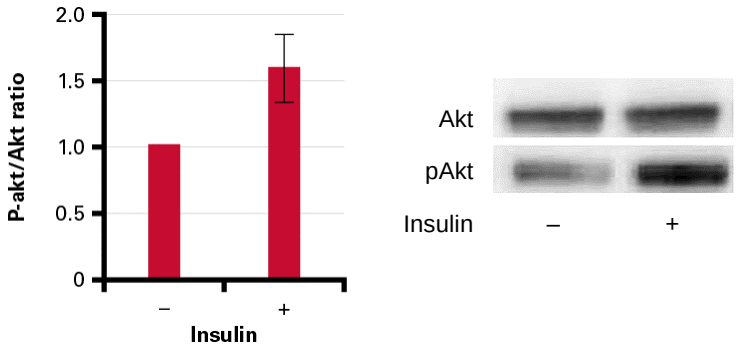


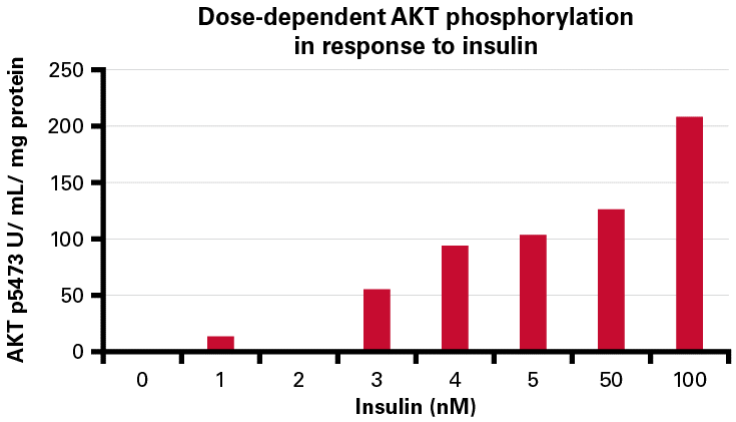


iPSC-derived hepatocytes respond to insulin—energy metabolism

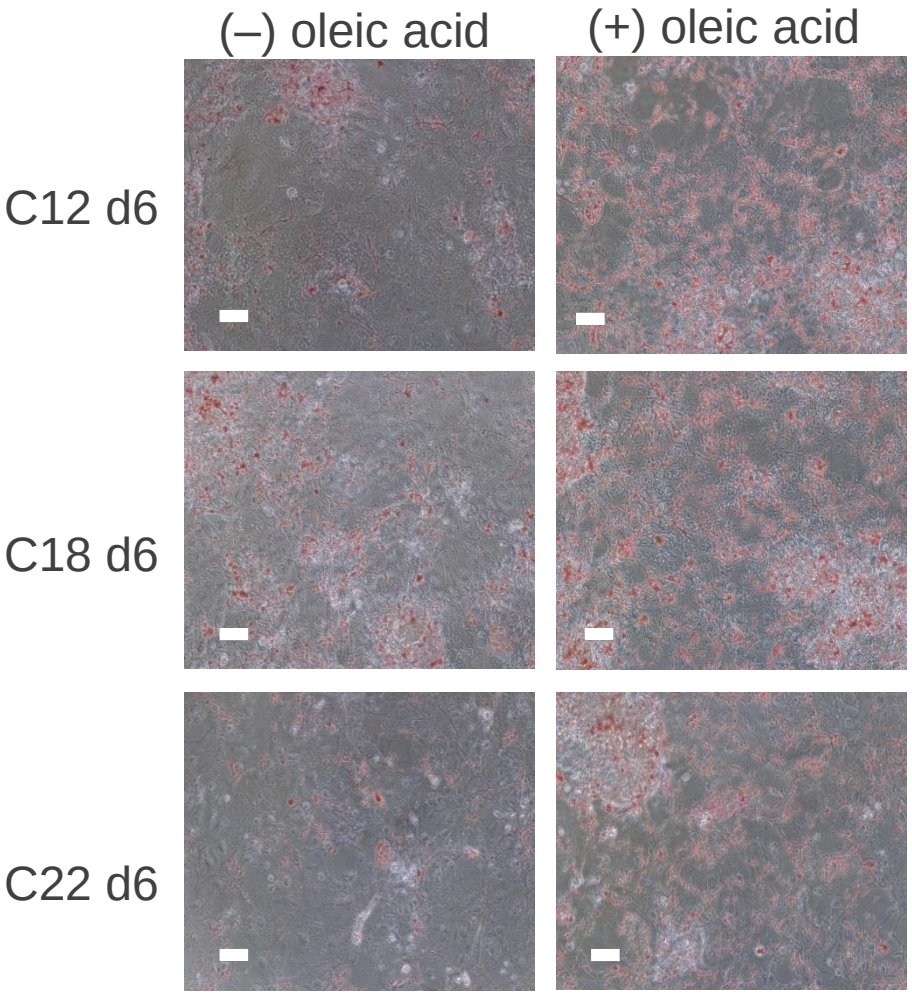
- Genes for insulin signaling expressed in similar levels as in primary hepatocytes
- AKT is phosphorylated in response to insulin

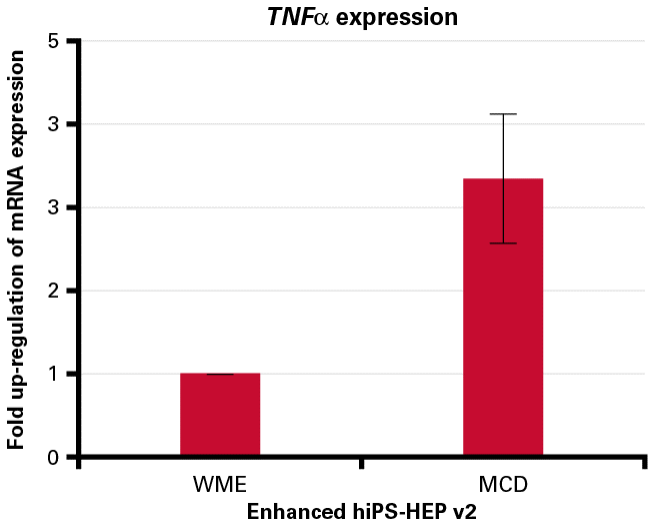






hiPS-HEP v2 cells as a potential NAFLD model

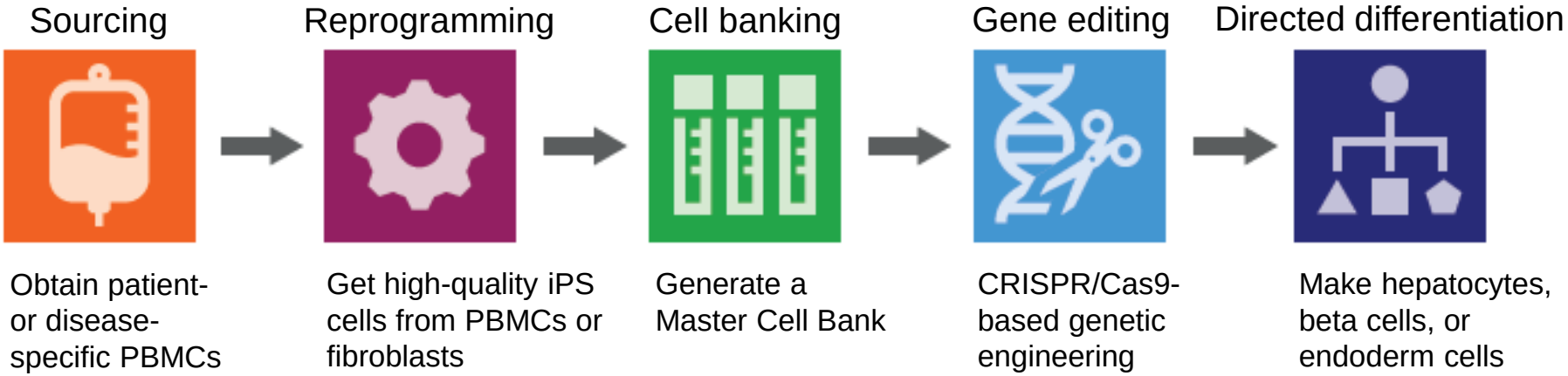




Up-regulation of inflammation marker *TNFα* in response to steatosis-inducing medium

→ Inflammation is physiologic response to steatosis in the liver

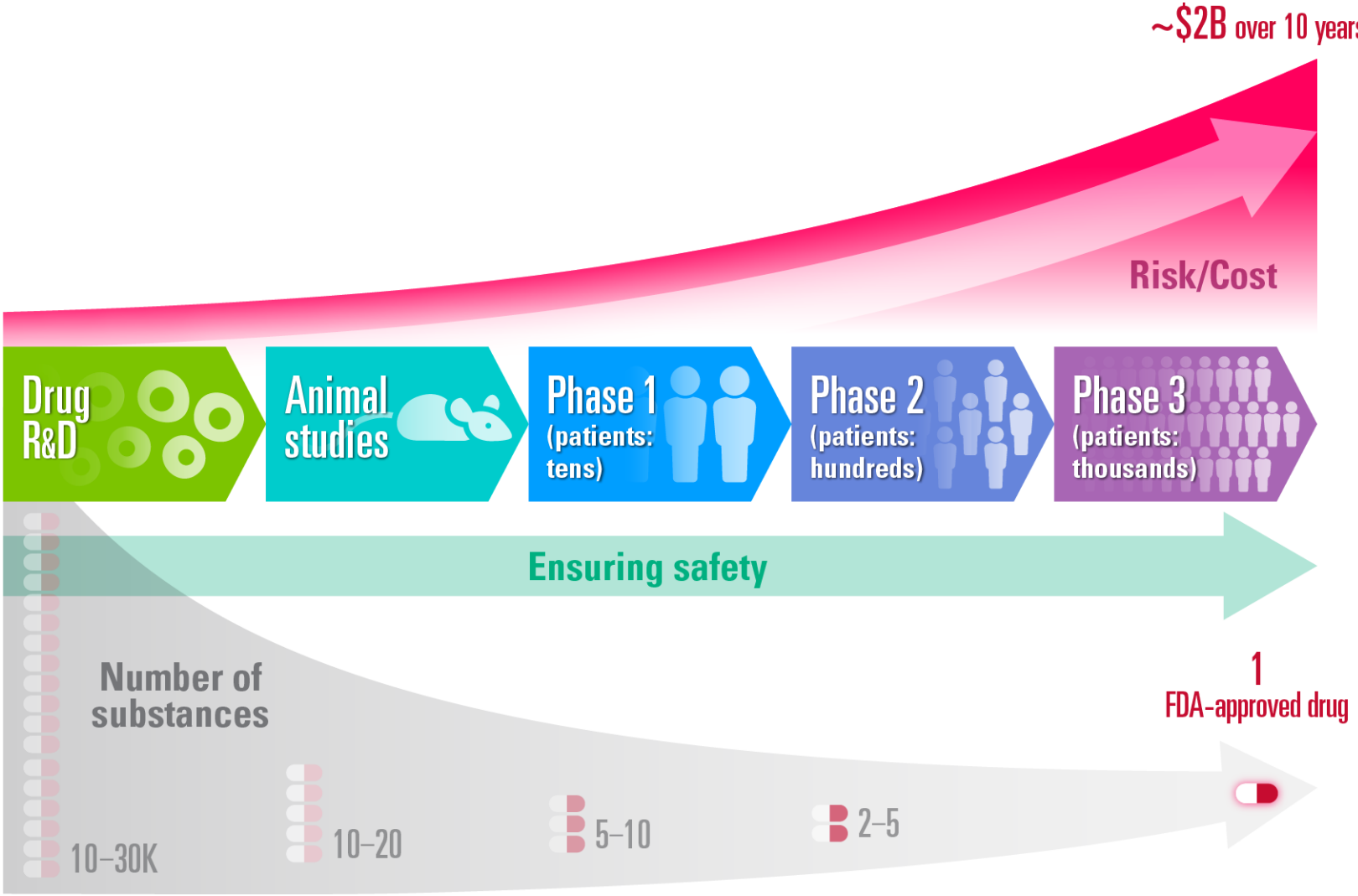
Customized iPSC-derived hepatocytes



- **Flexibility:** customer can pick the level of support they will need
- **Disease modeling:** customer can specify donor requirements or request gene editing for generation of custom disease model

POWER MEDIUM FOR DRUG DEVELOPMENT STUDIES

Biopharmaceutical research and development process



Important terms

- **DMPK**: Drug Metabolism & Pharmacokinetics
  - Originally associated with safety evaluation, but now a core discipline within drug discovery
- **Pharmacokinetics:** study of what the body does to medicine
  - Absorption, distribution, metabolism, and excretion of the medicine (ADME)
  - Bioavailability
- **ADME:** determines concentration of medicine in the body and onset, duration, and intensity of the effect
- **Clinical pharmacokinetics:** application of the above principles for safe and effective therapeutic management of patients
  - Dosage, interval, route, form of drug, and therapeutic drug monitoring

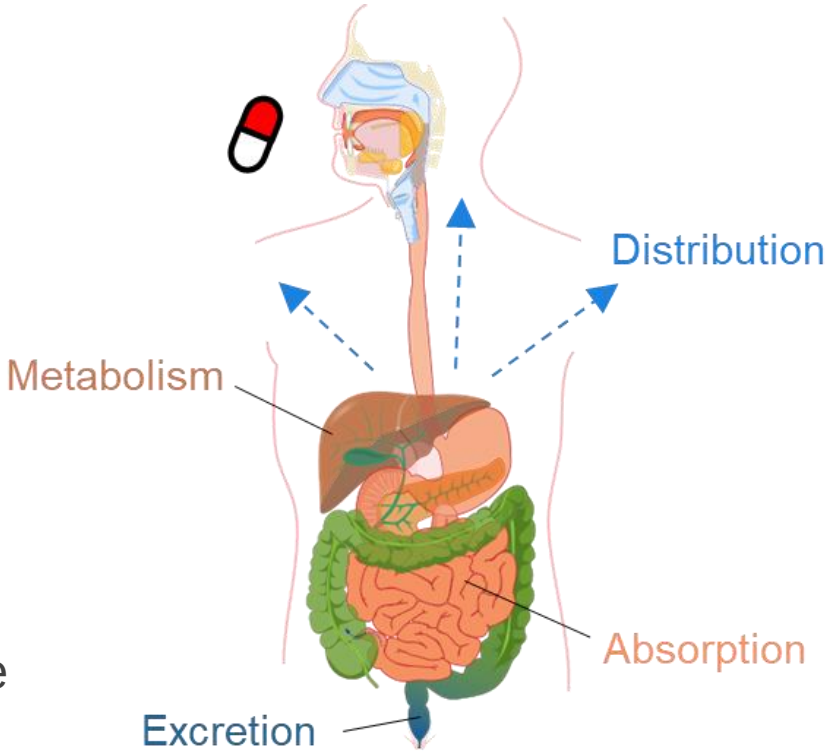
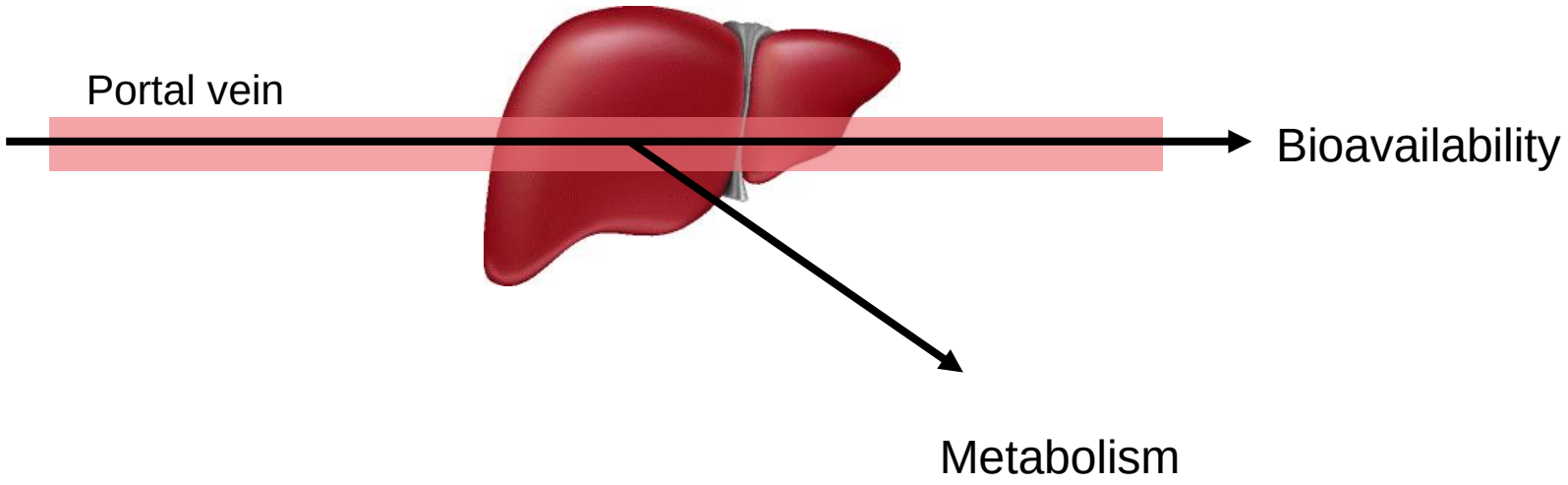


Image adapted from "Digestive system without labels" by user: LadyofHats / Wikimedia Commons / Public Domain

Metabolism of drugs in the liver

- The liver is the primary organ responsible for drug metabolism and the major route of elimination for ~70% of a marketed drug
- **Hepatic clearance** is the loss of a drug as it passes through the liver
  - A function of hepatic blood flow, plasma protein binding or bioavailability, and the intrinsic ability of liver enzymes to metabolize a drug (intrinsic clearance, $\mathbf{CL_{int}}$)



Understanding how a drug is metabolized *in vitro* can provide insight into how the drug will be metabolized *in vivo,* and allows more accurate patient dosing

Ideal *in vitro* hepatocyte cell model for drug metabolism studies

- Mimics the functionalities of the liver
  - Drug clearance and metabolism activities with phase I and phase II metabolizing enzymes, transporters
- Metabolic activity stably maintained over time in culture
- Easily adaptable to high throughput
- User friendly
- Compatible with multiple assay readouts
- Allows for cocultures and advanced culture systems
- Controls for donor variation and batch-to-batch consistency
- Accurately predicts *in vivo* drug metabolism

Human primary hepatocytes (hpheps) are the gold standard for drug metabolism research

Limitations of commercially available primary HEP systems

- Optimized for hpheps from each vendor → no clear winner
- Have short assay window in 2D culture, lose hepatocyte phenotype within 2–7 days
- Require daily feeding schedule
- Media has a short shelf life; can't refreeze/aliquot
- Often requires use of an overlay to keep cells functional
  - Interferes with some assays
  - Labor intensive—additional step in the culturing procedure
- Not able to accurately and reliably measure the disappearance rates (hepatic clearance) of compounds

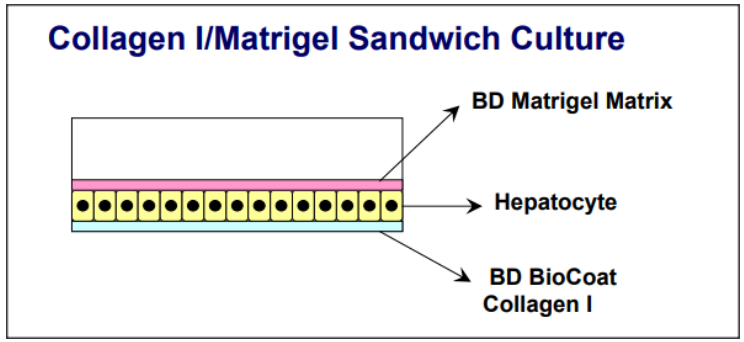
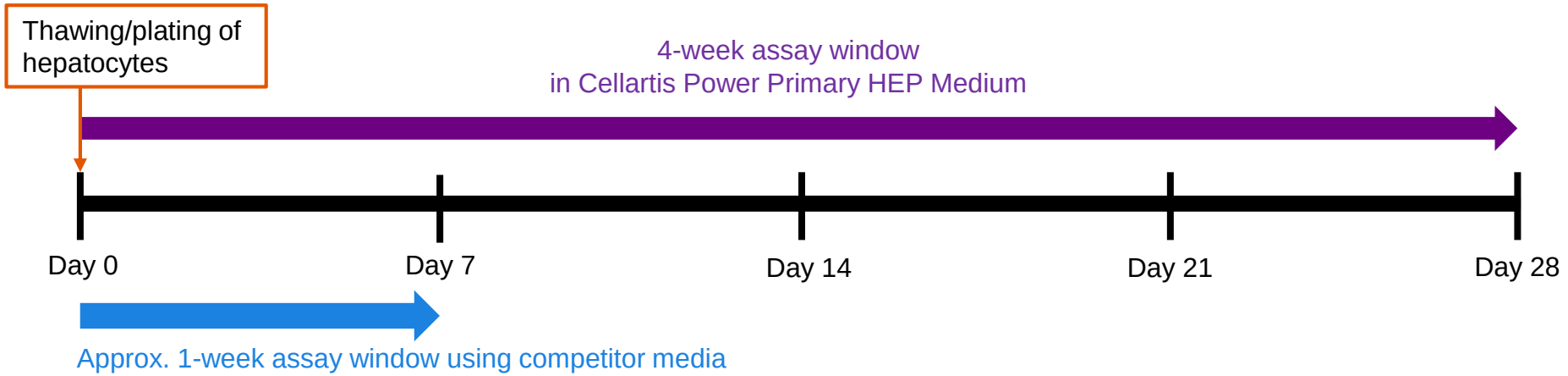


Image courtesy of BD Biosciences.

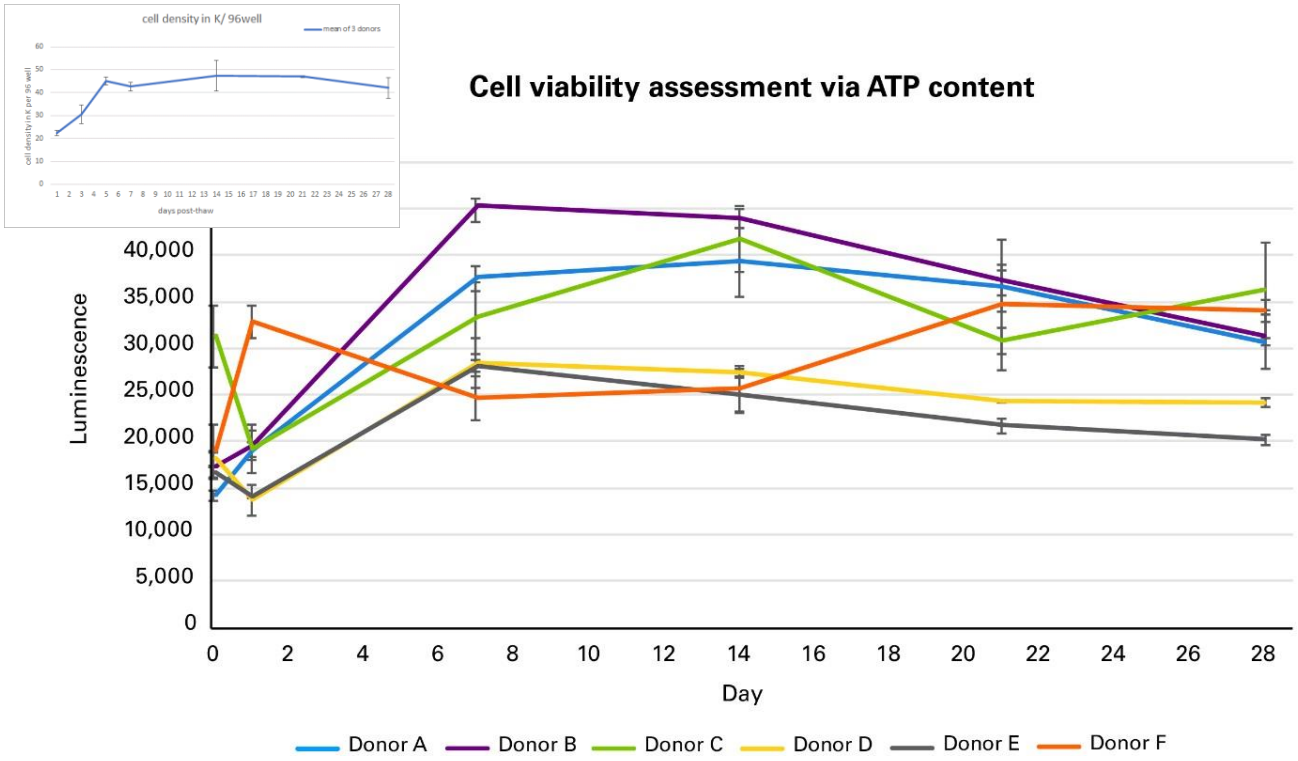
Cellartis Power Primary HEP Medium

Enables long-term drug metabolism studies:

- Extends viability
- Maintains morphology
- Maintains hepatocyte function
- Supports compound clearance assays
- Enables CL_{int} prediction for low clearance compounds



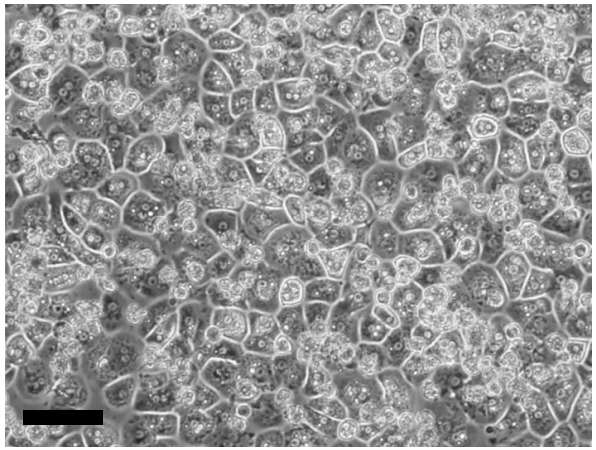
Viability maintained for 4 weeks



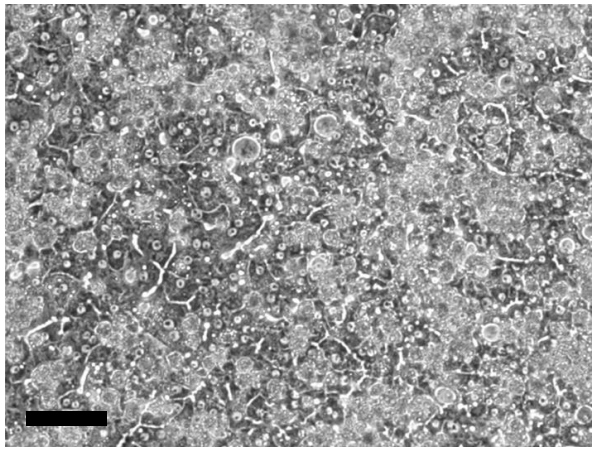
- Hpheps from 6 donors (4 vendors) cultured in Cellartis Power Primary HEP Medium were viable for 4 weeks
- Plated based on manufacturer's conditions—changed to Cellartis Power Primary HEP Medium 4 hr post-thaw
- Donor-dependent recovery period—one population doubling in 5 days (proliferating)

Maintained hepatocyte morphology during entire culture period

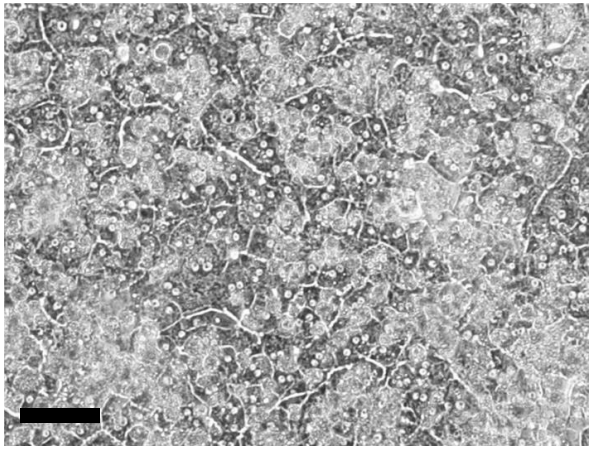
Day 1



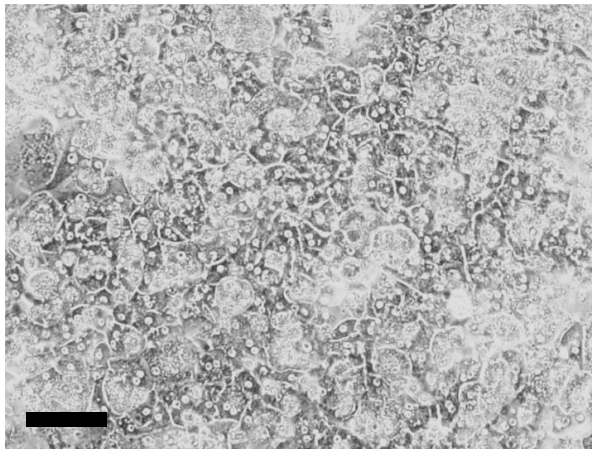
Day 7



Day 14



Day 21



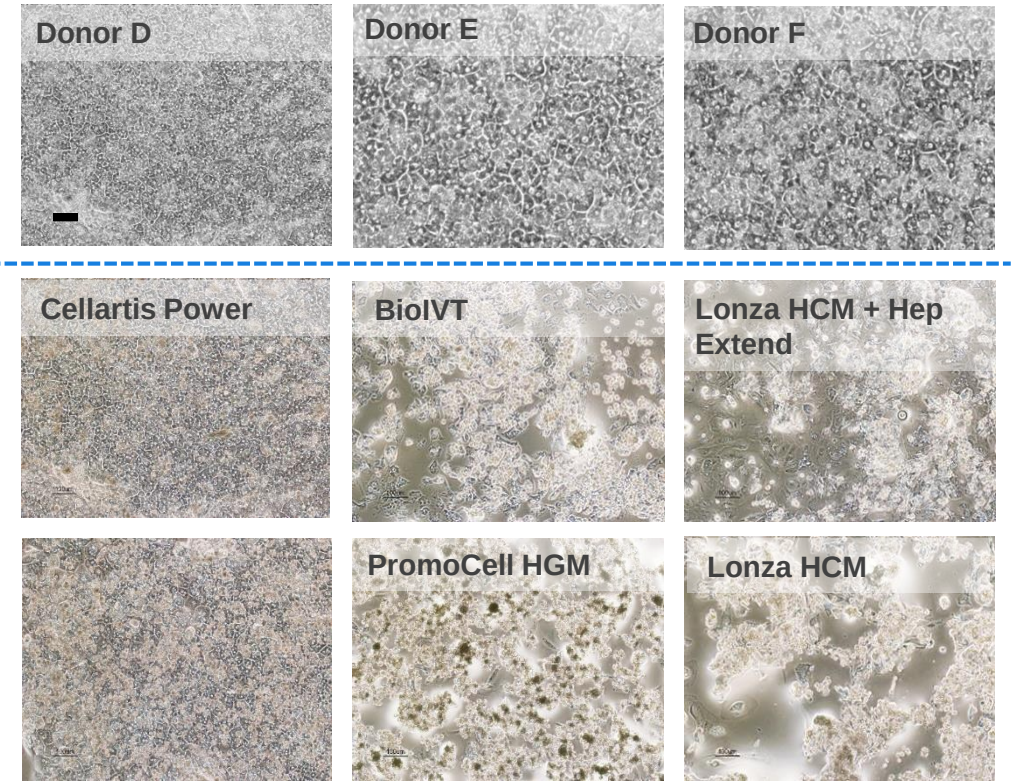
A healthy hepatocyte morphology is observed during the entire culture period in Cellartis Power Primary HEP Medium.

Scale bar = 100 µm

Healthy morphology—Day 28

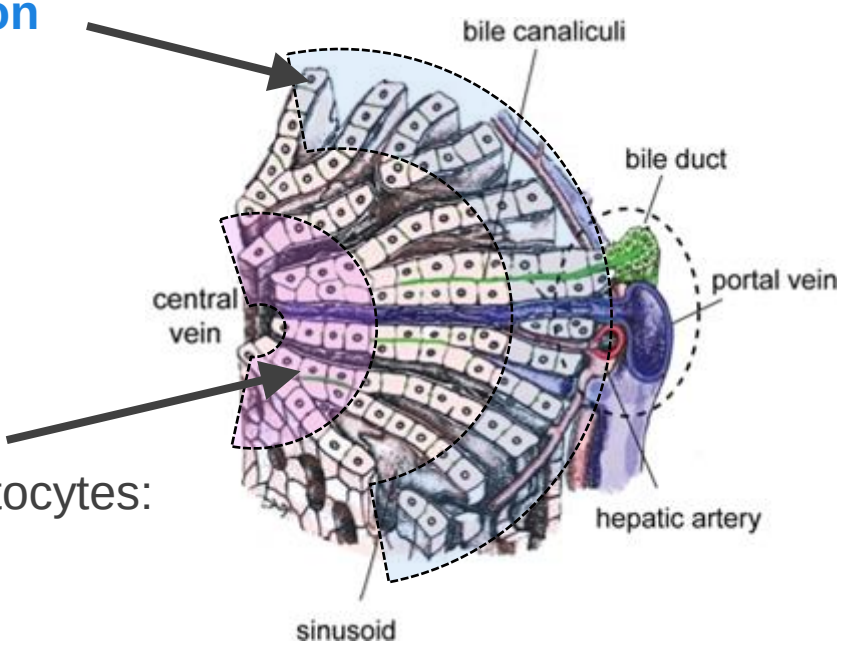
- Primary hepatocytes cultured in Cellartis Power Primary HEP Medium display a healthy hepatocyte morphology for 28 days

- Morphology on Day 28 using media from different vendors—no dedifferentiation or cell death in Cellartis Power Primary HEP Medium



Hepatocyte function—metabolic zonation

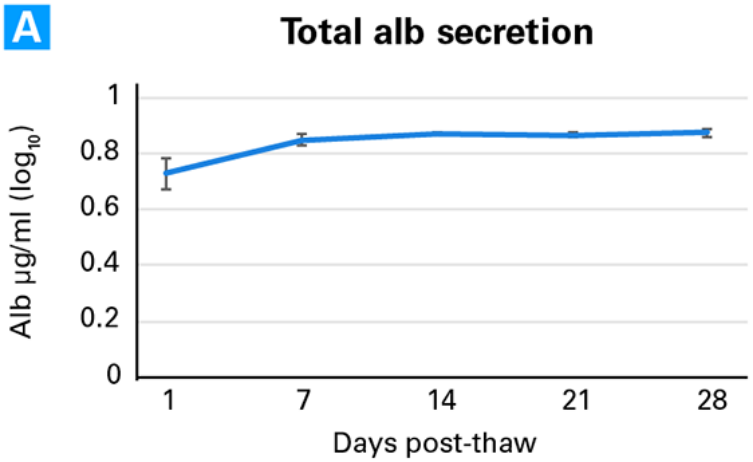
Periportal hepatocytes:
albumin secretion

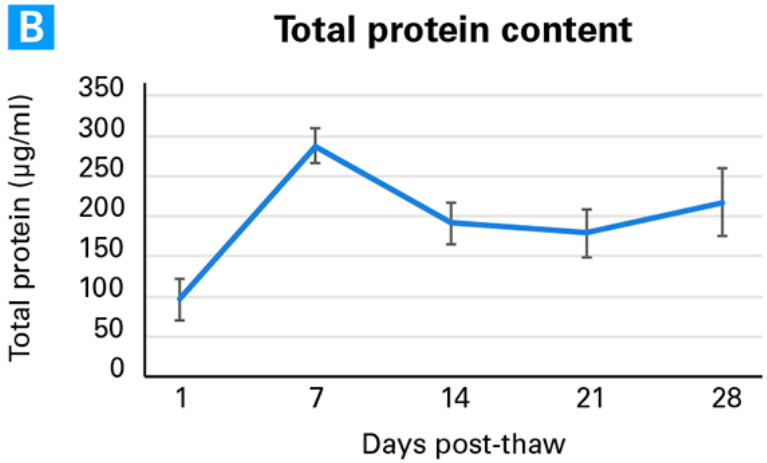


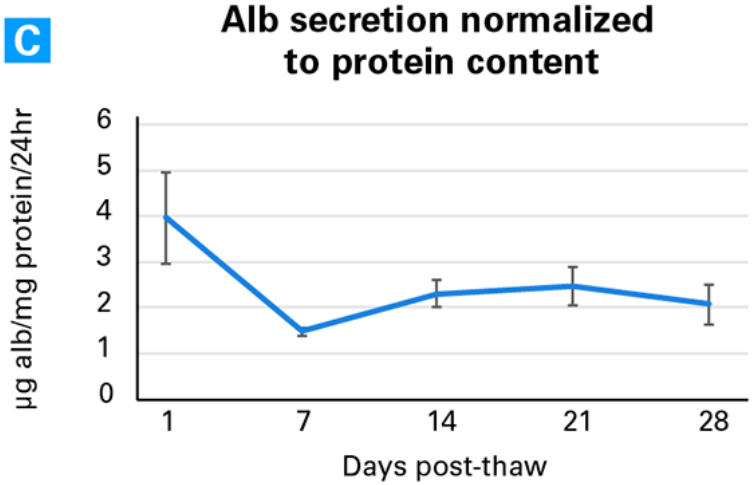
Perivenous hepatocytes:
CYP activity

Adapted from "Cellular architecture of the liver." © 2013 Massachusetts General Hospital / http://www.stembook.org / CC BY 3.0

Albumin secretion



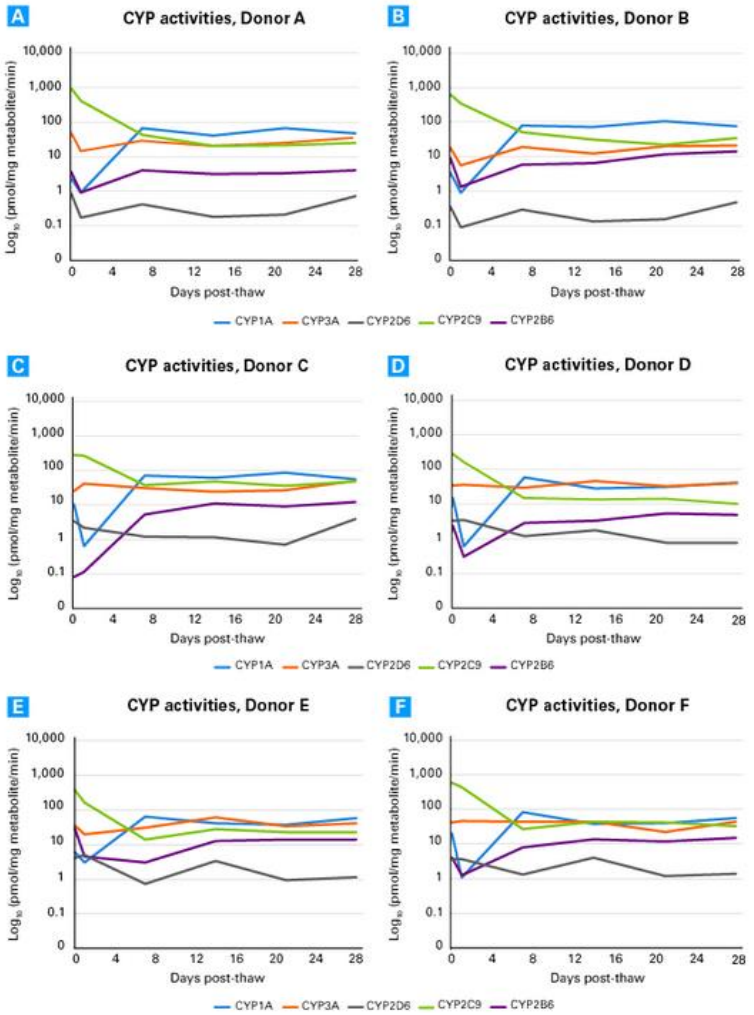




A. Albumin slightly increasing, then stable
B. Protein content initially increasing
C. Normalized albumin secretion appears to decrease initially due to increasing protein content
   - Correlates with recovery period post-thawing/plating

Maintained CYP activities

- 6 donors from 4 different vendors
  - Cultured in Cellartis Power Primary HEP Medium for 28 days
  - Donor-dependent recovery period, interindividual variation is preserved



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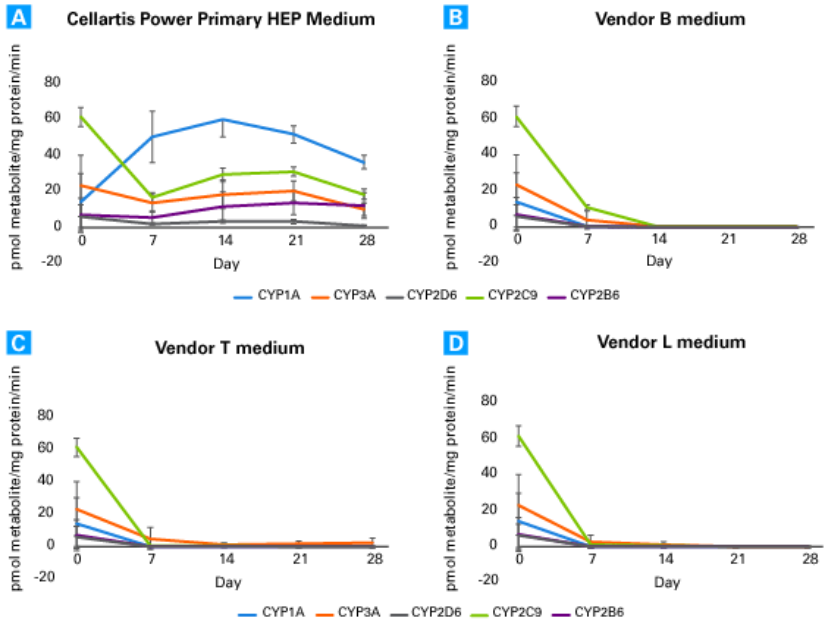
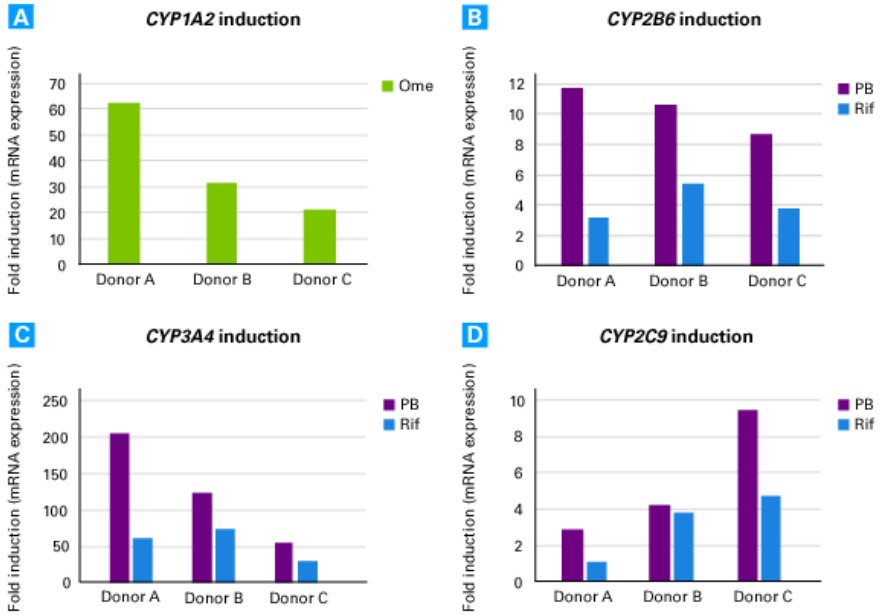
- 3 different vendor media (benchmark)
  - Cultured in Cellartis Power Primary HEP Medium or competitor media for 28 days
  - Rapid loss of CYP activity in competitor media



Maintained CYP activities

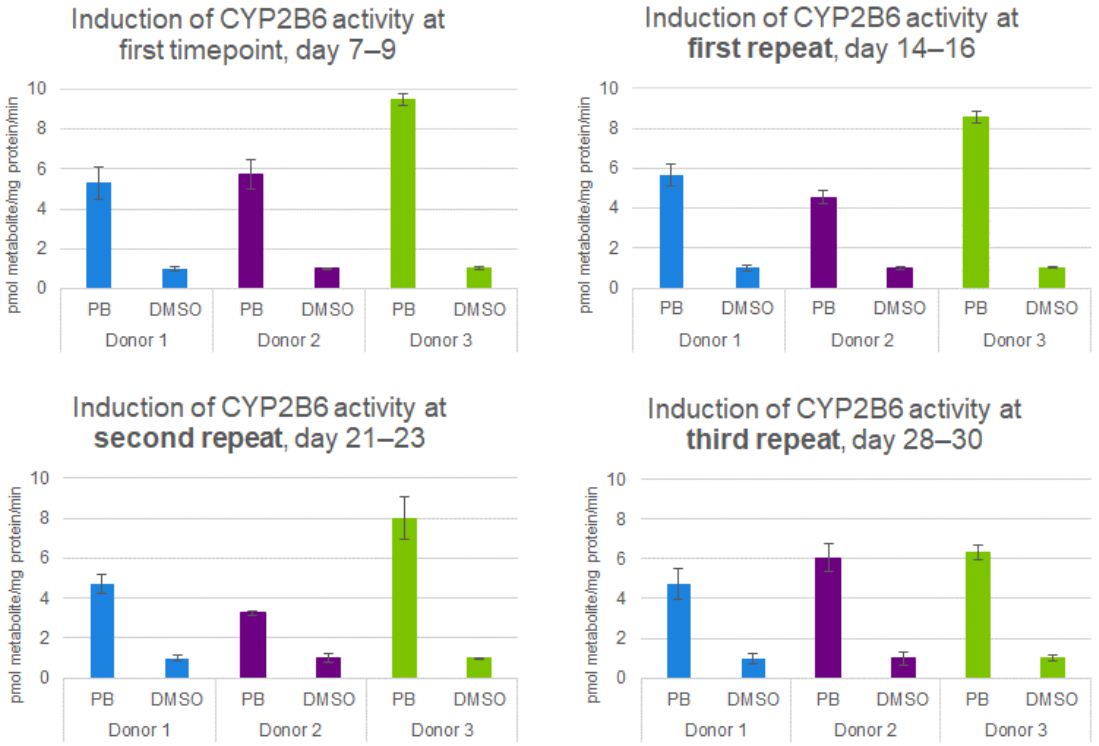
- 6 donors from 4 different vendors
  - Cultured in Cellartis Power Primary HEP Medium for 28 days
  - Donor dependent recovery period, interindividual variation preserved
- 3 different vendor media (benchmark)
  - Cultured in Cellartis Power Primary HEP Medium or competitor media for 28 days
  - Rapid loss of CYP activity in competitor media
- 3 different donors, 4 different CYPs
  - All 4 CYPs are induced to high levels
  - Induction of *CYP2B6* by Rif and *CYP3A4* by PB due to overlap in substrate specificity
  - *CYP2C9* is not inducible with Rif in one donor, consistent with literature

Drugs can increase CYP enzyme levels by inducing their mRNA expression, which can cause a change in the effects of co-administered drugs, leading to serious problems for patients taking multiple medications.



Repeated CYP2B6 induction

- 3 different donors, multiple inductions
  - Induction of CYP2B6 with PB for 48 hours
  - Normalized to CYP activity post-DMSO treatment
  - Mean values of triplicates per donor and timepoint, +/- SD



Same hpheps used for repeat induction studies—lowers cost of the assays and allows more sophisticated studies to be performed with repeat dosing, etc.

Compound clearance assays

Question 1: Can hpheps cultured in Cellartis Power Primary HEP Medium survive 10 days **without medium change**?

Experimental setup

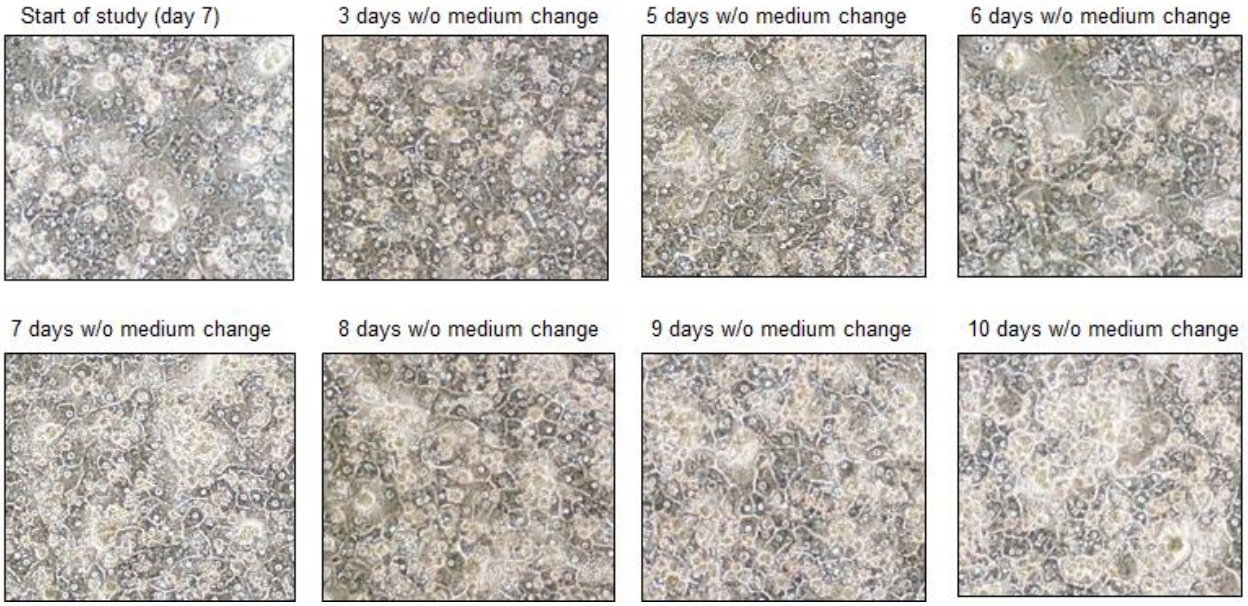
- Plated hpheps from BioIVT
- 3 donors
- Seeding density: 1.5 x 10^5 cells/cm^2
- Study initiated on Day 7 post-thaw by adding 230 µl of Cellartis Power HEP Medium per well of a 96-well plate
- Cells cultured for 10 days without medium change, Days 7–17 post-thaw

Readouts

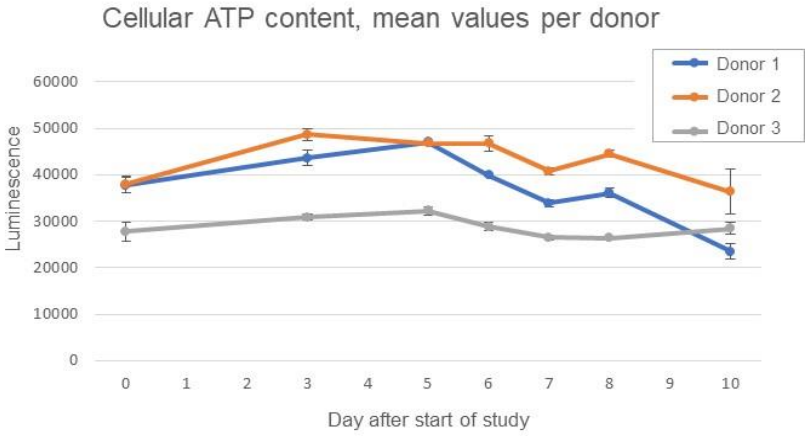
- Morphology
- Cellular ATP content
  - CellTiter-Glo Luminescent Cell Viability Assay (Promega)
- CYP activity assay
  - Incubation with CYP substrates
  - Metabolite formation measured by LC/MS normalized to protein content per well (determined by Pierce BCA assay)

Compound clearance assays—results

Morphology



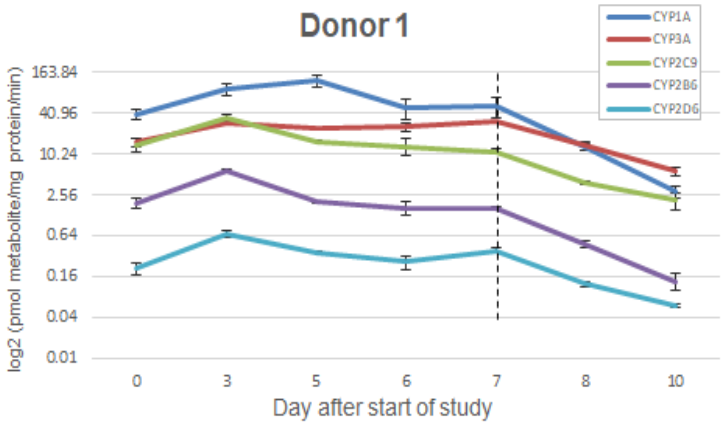
ATP content (viability)

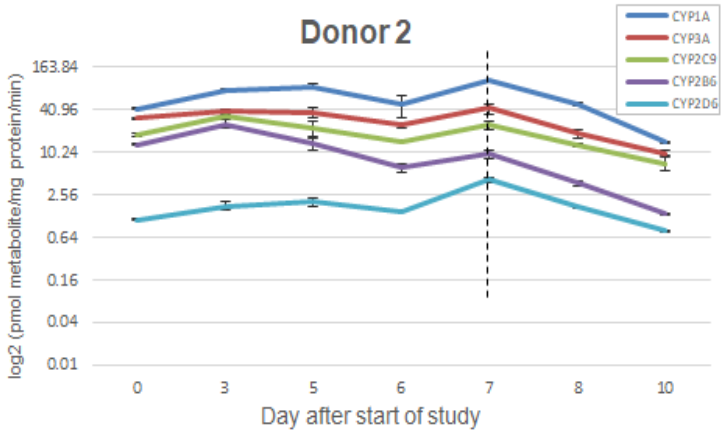


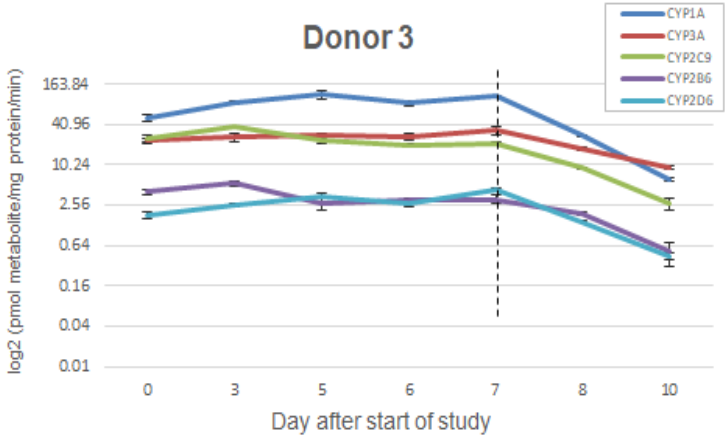
No dedifferentiation or major cell loss observed. Relatively stable ATP content for up to 10 days.

Compound clearance assays—results

CYP activity







CYP enzyme	Metabolite
CYP1A	Paracetamol
CYP3A	OH-Midazolam
CYP2C9	OH-Diclofenac
CYP2B6	OH-Bupropion
CYP2D6	OH-Bufuralol

Hpheps cultured in Cellartis Power Primary HEP Medium show good morphology and viability, stable CYP activities for 7 days without media changes

COLLABORATOR DATA

Kindly provided by AstraZeneca

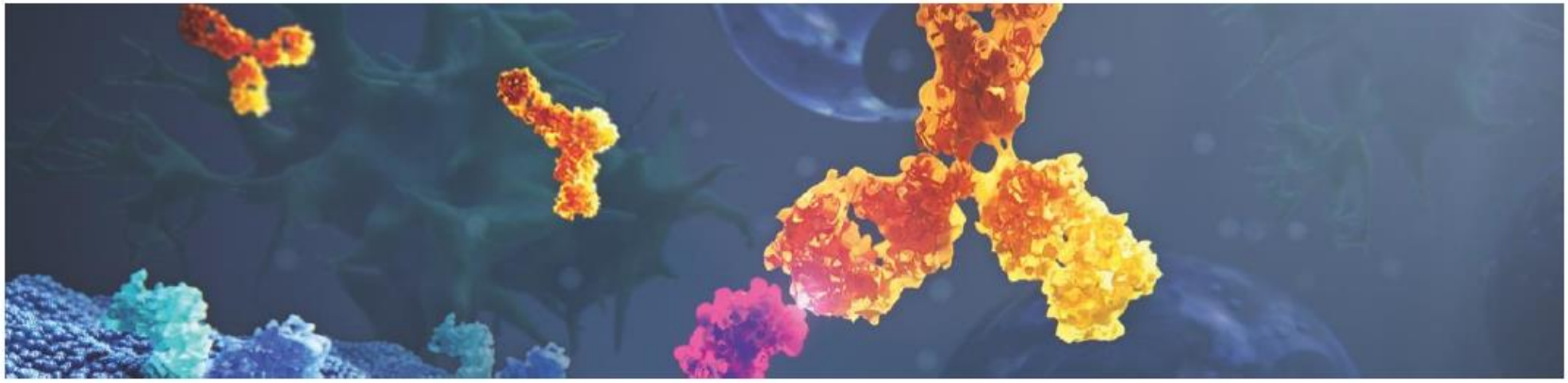
Compound clearance—CL_{int} prediction

AstraZeneca

CLint prediction of Quinidine using Takara Power HEP Medium

Sara Amberntsson
Drug Safety and Metabolism, IMED Biotech Unit, AstraZeneca, Gothenburg

15 Aug 2018



Compound clearance—CL_{int} prediction

Question 2: Can hpheps cultured in Cellartis Power Primary HEP Medium be used to predict intrinsic compound clearance (CL_{int})?

Experimental setup

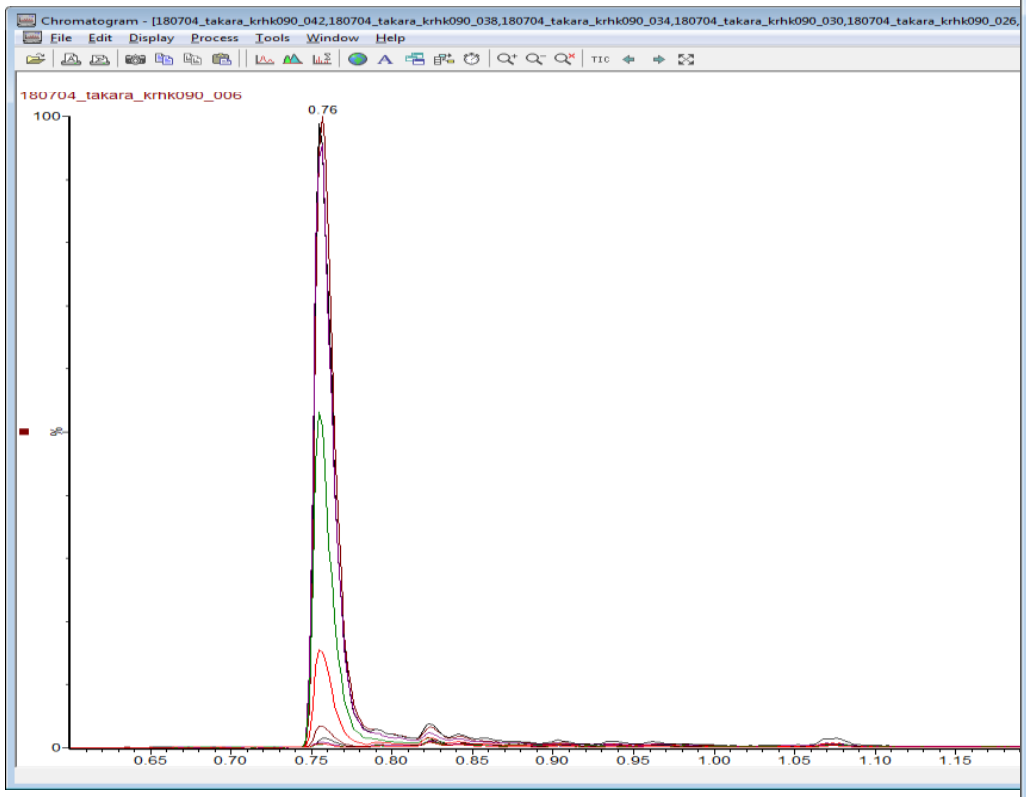
- Plated hpheps from BioIVT
- 1 donor (lot KFF)
- Seeding density 1.5 x 10^5 cells/cm^2
- Study initiated on Day 7 post-thaw by adding 1 µM quinidine in 230 µl Cellartis Power Primary HEP Medium per well of a 96-well plate
- Before dosing, number of cells calculated to 48.3 x 10^3 cells/well
- Cells cultured for 10 days without medium change, Days 7–17 post-thaw

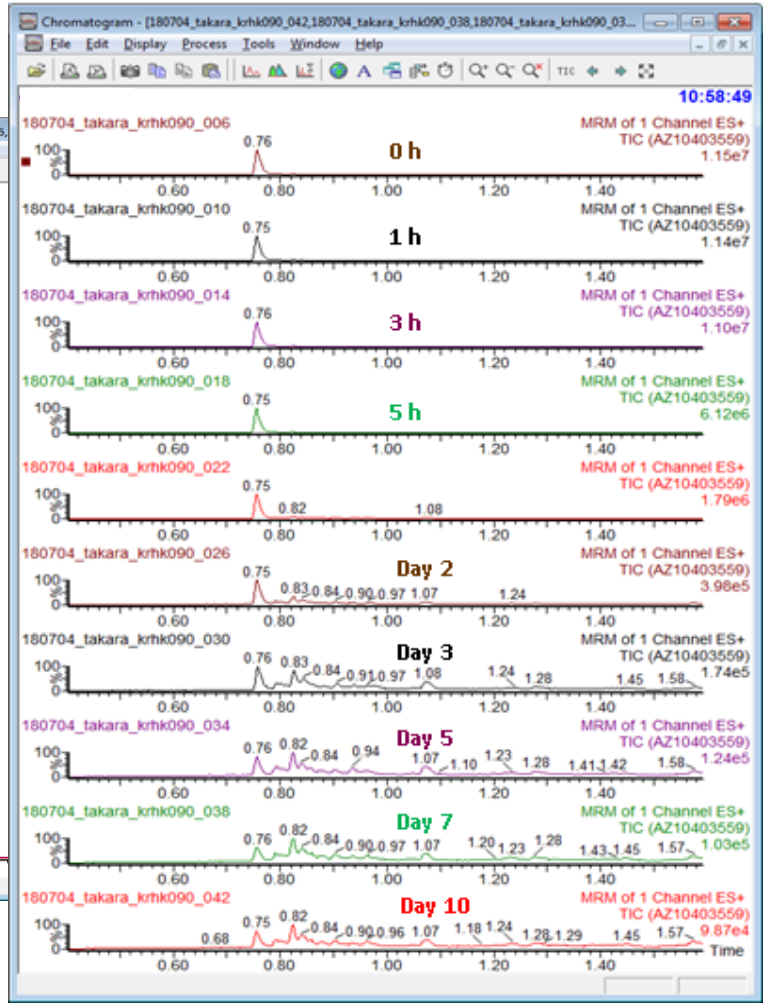
Readouts

- Timepoints
  - 0 hr, 1 hr, 3 hr, 5 hr, Day 1, Day 2, Day 3, Day 5, Day 7, Day 9, Day 10
- LC-MS (Xevo TQ-S, Waters)
- Disappearance of quinidine
  - Monitored by MRM and integration of the chromatographic peak

CL_{int} prediction—quinidine

LC-MS chromatograms



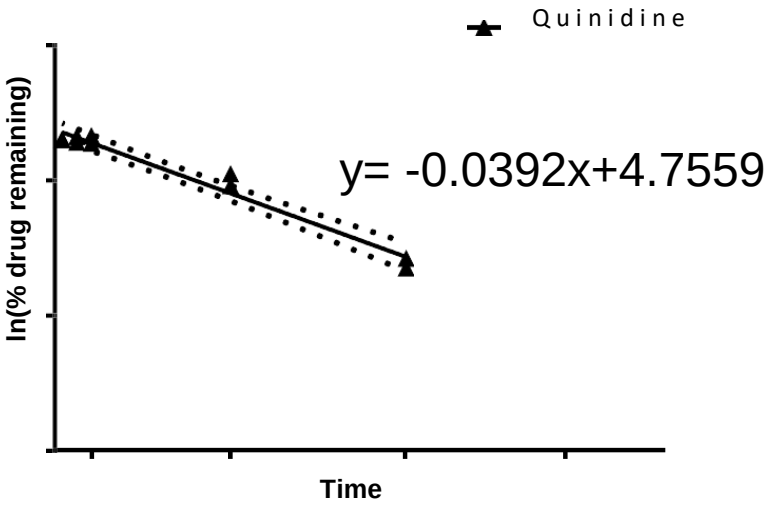


After 3 days, >90% quinidine was metabolized

(Kindly provided by AstraZeneca)

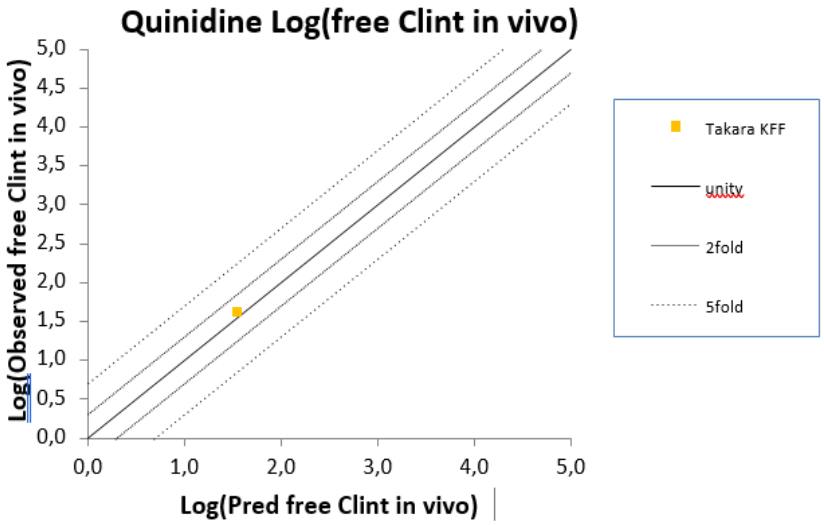
CL_{int} prediction—quinidine

Depletion of quinidine



	Quinidine
$T_{1/2}$	1059 minutes
CL_{int}	3.12 µl/min/million cells
P value	<0.001

Correlation with *in vivo* model



Calculated *in vitro* CL_{int} value correlates with *in vivo* CL_{int} value

(Kindly provided by AstraZeneca)

CL_{int} prediction—quinidine

- No dedifferentiation or major cell loss observed
- Relatively stable ATP content for up to 10 days
- Stable CYP activities for approximately 7–8 days without medium change
- CL_{int} value for quinidine was calculated to 3.12 µl/min/million cells based on 5 time points
- Predicted *in vivo* CL_{int} is in agreement with the observed free CL_{int} *in vivo*

Flexible solutions for liver disease & drug development studies

iPSC-derived hepatocytes

DO IT YOURSELF

Cellartis iPS Cell to Hepatocyte Differentiation System

- Cellartis DEF-CS Culture System
- Cellartis Definitive Endoderm Differentiation Kit
- Cellartis Hepatocyte Differentiation Kit

Cellartis Hepatocyte Maintenance Medium

READY MADE

- Cellartis Human iPS Cell Lines
- Cellartis Definitive Endoderm Cells
- Cellartis Enhanced hiPS-HEP v2 Cells

CUSTOM MADE

Cellartis Human Pluripotent Stem Cell Services

Sourcing | Reprogramming | Cell Banking | Gene Editing | Differentiation

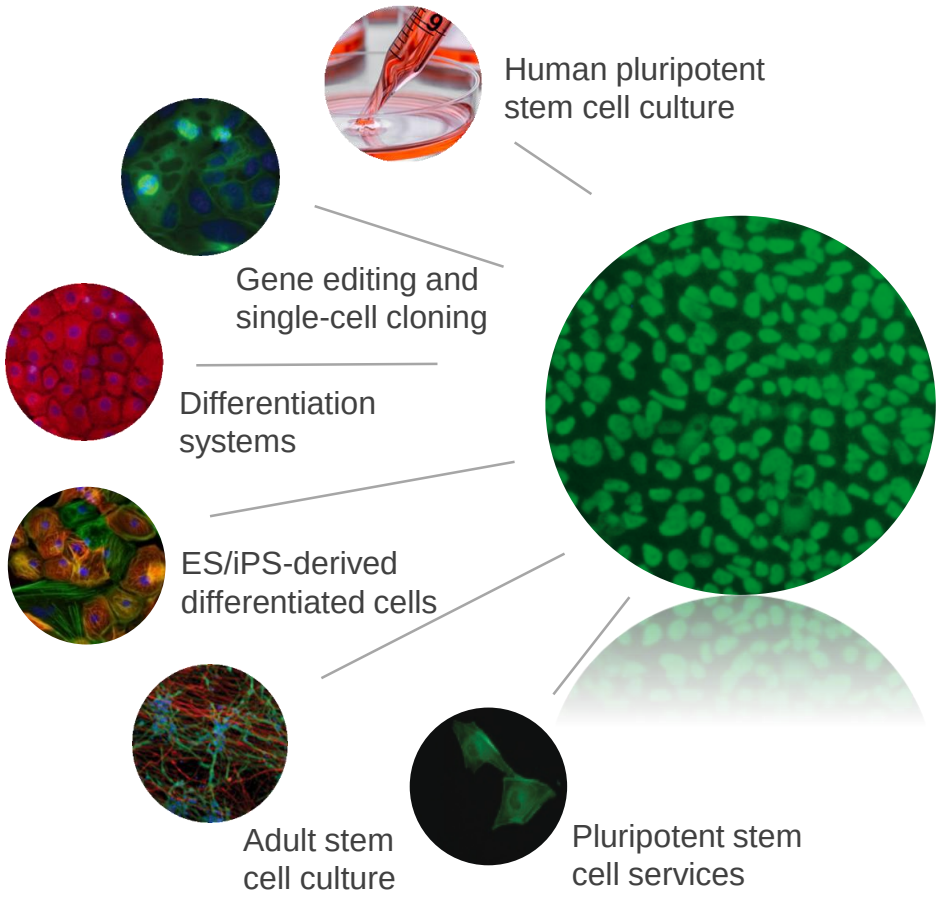
Primary hepatocytes

Cellartis Power Primary HEP Medium

- Metabolically active phenotype for up to 4 weeks
- Minimizes variability
- No daily feedings or sandwich overlay
- Convenient, complete medium
- Enables accurate intrinsic clearance (CL_{int}) studies

Stem cell products and services

Stem cell innovations for today and the future



Featured service:

Cellartis clinical-grade hES cell derivation

- Blastocysts are sourced from FDA-compliant sources
- Animal- & human-component-free method
- Seed Banks and Master Cell Banks of hES cell lines
- Manufacturing license since 2018 (Swedish MPA)
- Tissue establishment license since 2017 (Swedish MPA)

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