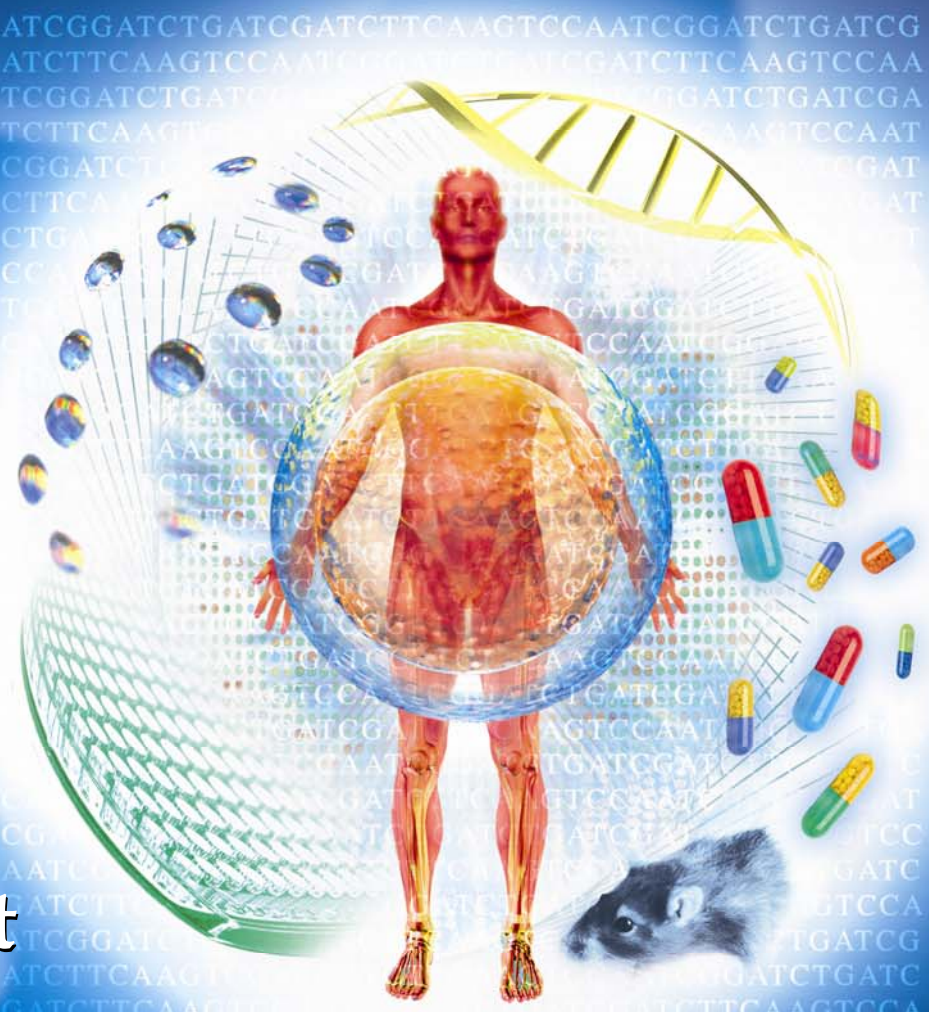
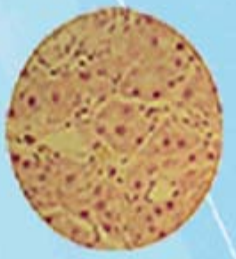
RegeneMed

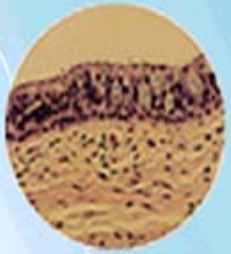


High Throughput Human Tissues for Drug Discovery and Therapies

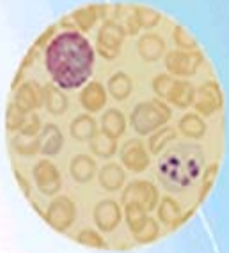
Advanced Tissue Sciences, Inc.
Human Solutions in Tissue Engineering™



LIVER



GASTROINTESTINAL TRACT



BONE MARROW

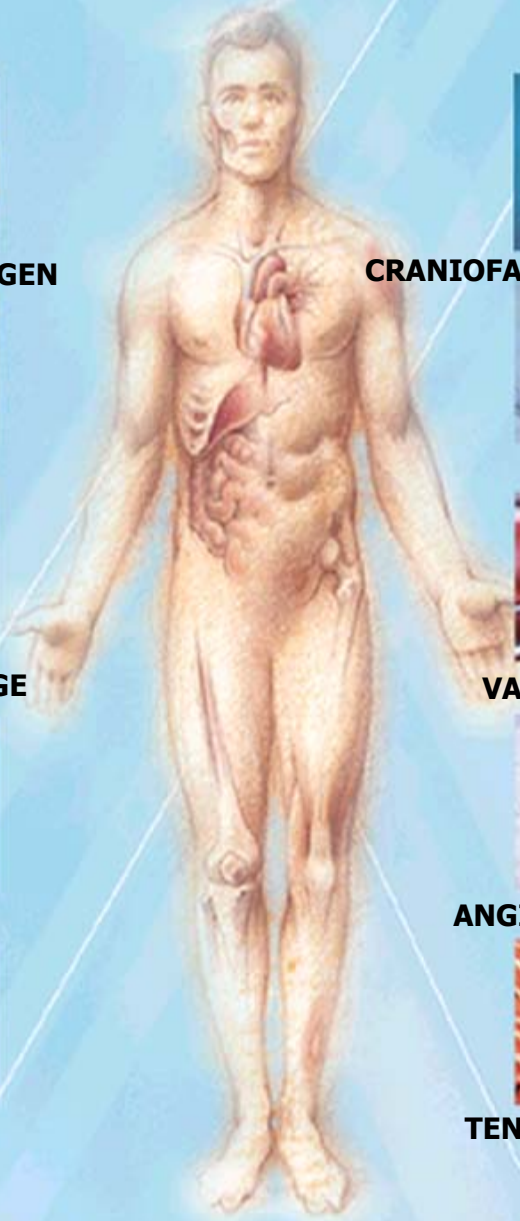
INJECTABLE COLLAGEN

BONE

BONE / CARTILAGE

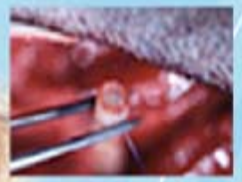
MENISCUS

CARTILAGE

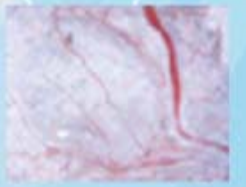


CRANIOFACIAL RECONSTRUCTION

EAR



VASCULAR GRAFTS



ANGIOGENESIS PATCH

TENDON / LIGAMENT

HEART VALVES

TRANSCYTE™ BURNS

DERMAGRAFT®
SKIN ULCERS

Proven Technology

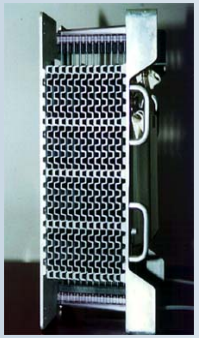
New Business Model

Non-regulated, Research use, Quick to market

- **35 patents: Tissue engineered human and animal organs**
- **Advanced Tissue Sciences, Inc. - Proven technology:**
  3 FDA-approved products, 1 toxicity, 2 consumer

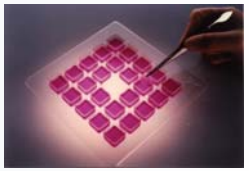
FDA Approved Therapeutics | **Consumer** | **Toxicity Testing**











TRANSCYTE ® *full- and partial-thickness burns*

DERMAGRAFT® *chronic wounds*

COLLAGEN *wrinkles & scars*

COSMETIC *wrinkle cream*

SKIN [2] *skin toxicity model*

RegeneMed

Platform Technology Extensions

Liver → All Tissues

Drug Safety	Drug Efficacy	Personalized Medicine	Medical Devices	Tissue Transplants
• ADMET **($3B)**	• Hepatitis	• SNP's	• ECLAD	• Liver
• DMPK	• HIV	• Genotyping	• Diagnostics	• Pancreas
• Drug Reactions	• Cirrhosis/ Cancer	• Molecular Diagnostics	• Drug Delivery	• Bone Marrow
• Drug-Drug Interactions	• Diabetes/ Cardiovasc.	• Biopsy Chemo Screen	• Tissue Biosensors	• Kidney / lung, etc.
$5 B	**$10 B**	**$5 B**	**$15 B**	**$20 B**

personalized, preventive and regenerative medicine

Increasing donors, tissue mass, regulation, approvals

RegeneMed

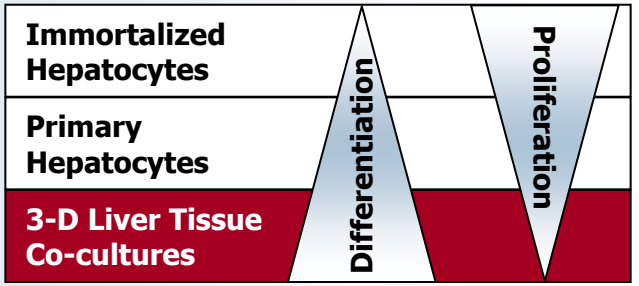
RegeneMed 3-D Tissue Technology

More Physiologically Relevant *In vitro* Models

3-D vs 2-D **All cells of organ** **Mimic *in vivo* environment**

1. **Replace unicellular (hepatocyte), short-lived (2-D) cell function**

2. **With *ALL* cells of the native tissue, in 3-D structure to mimic *in vivo*, to form a full tissue with longevity of tissue-specific function**
   - **Cells responsible for tissue formation: stromal/microenvironmental cells**
   - **Cells responsible for tissue function: parenchymal cells**
   - **ALL cells for**
     - **Cell-cell communication**
       - **Extracellular matrix (ECM) and growth factor (GF)/ cytokine expression**



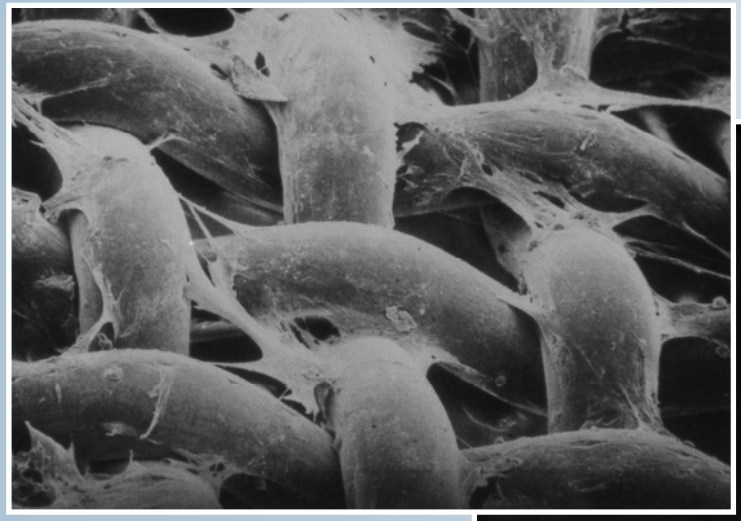
3. **3-D *interconnecting porous, contractible* structure (vs gel) for cell seeding**
   - **Not: gels, sandwich cultures, spheroids, or hanging drops**
   - **Significantly upregulate ECM and GF expression to form a tissue**
     - **Cells in 2D don't express much ECM / GF and grow faster than in 3D**

RegeneMed

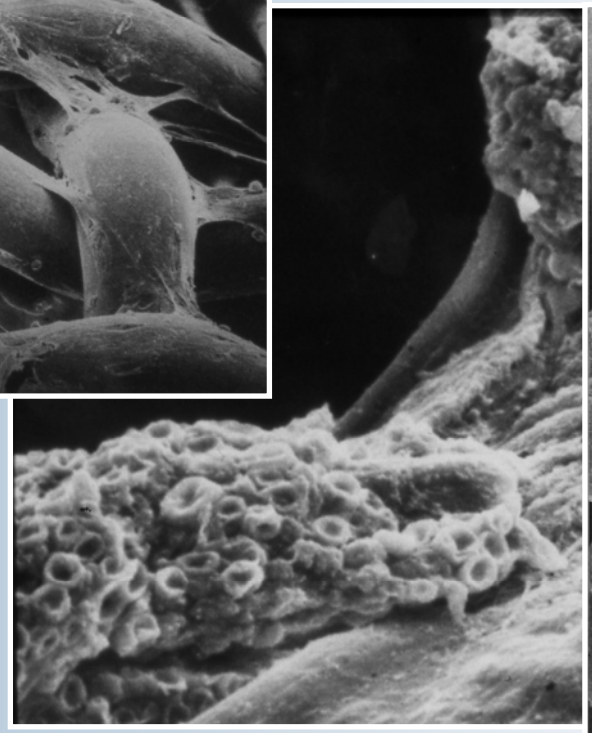
RegeneMed 3-D Tissue Technology

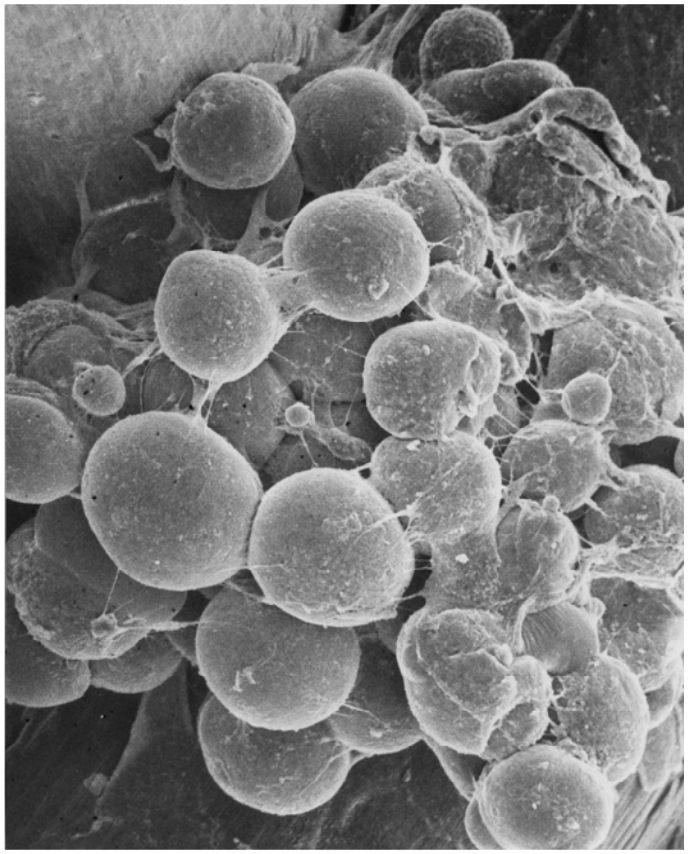
More Physiologically Relevant *In vitro* Models

3-D vs 2-D



ALL cells of the organ





Mimic the *in vivo* environment

RegeneMed

RegeneMed 3-D Tissue Technology

More Physiologically Relevant *In vitro* Models

- **Insufficient to have multiple cell types in 2D culture**
  - **Customer data**
  - **RegeneMed data**
- **Multilayer culturing on 2D ECM or gels often non-physiologic**
- **Goal should be 3D *in vitro* to *in vivo* correlations, not to current 2D**
  - **More complex readouts (omics, SAR-to-pathway, GWAS)**
  - **Translating *in vivo* assays to *in vitro***
    - **clinical chemistry, imaging (MRI, CT, ultrasound), pathology**
  - **Separating and defining single-cell molecular biology in multicellular readout**
  - **Effects of serum on drug ADMET in vivo vs in vitro**
- **Balancing defined *in vitro* conditions versus physiologic relevance**
  - **Single versus multicellular**
  - **Serum versus serum-free**
  - **2D versus 3D with ECM (assay and imaging challenges)**

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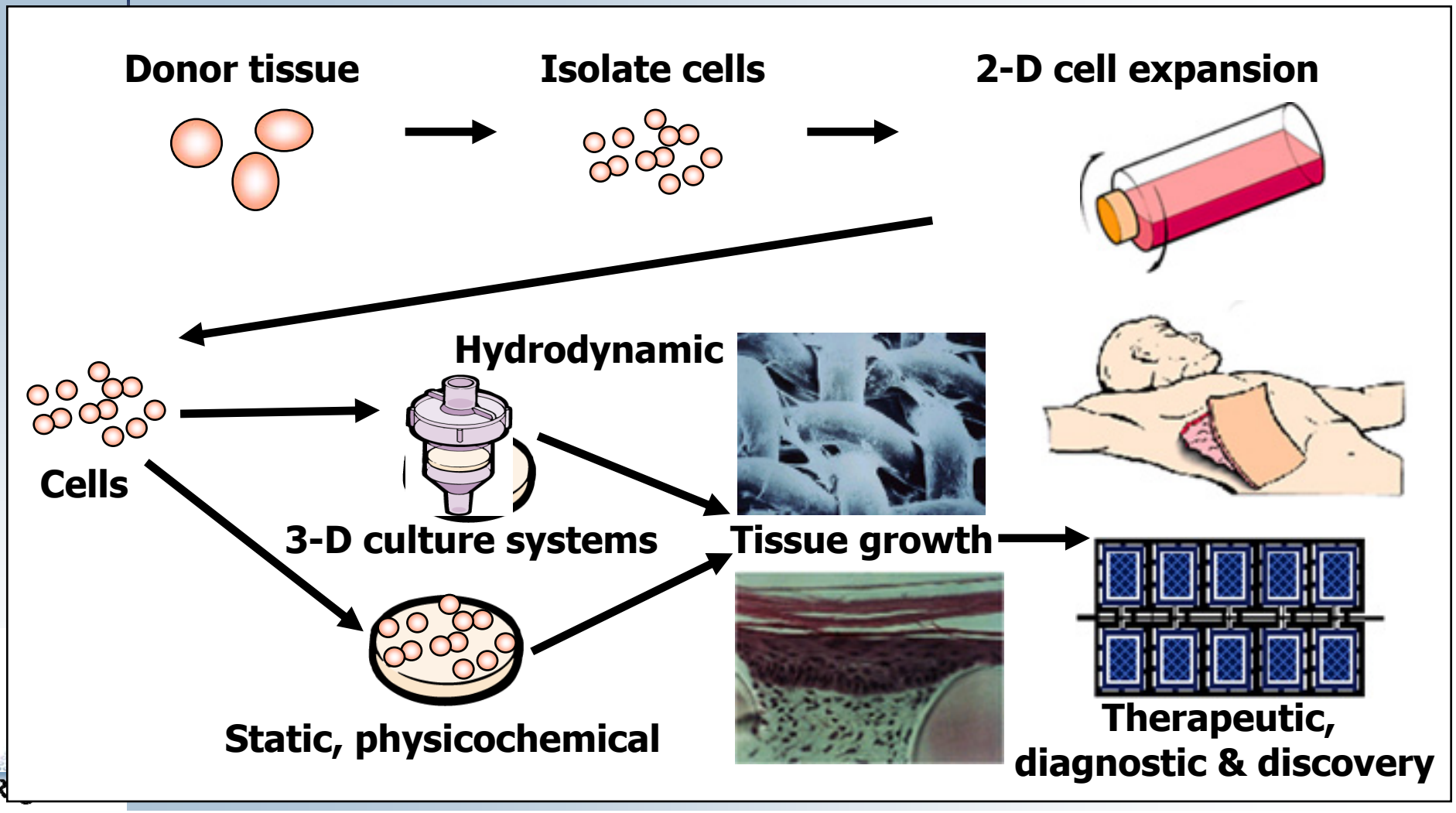
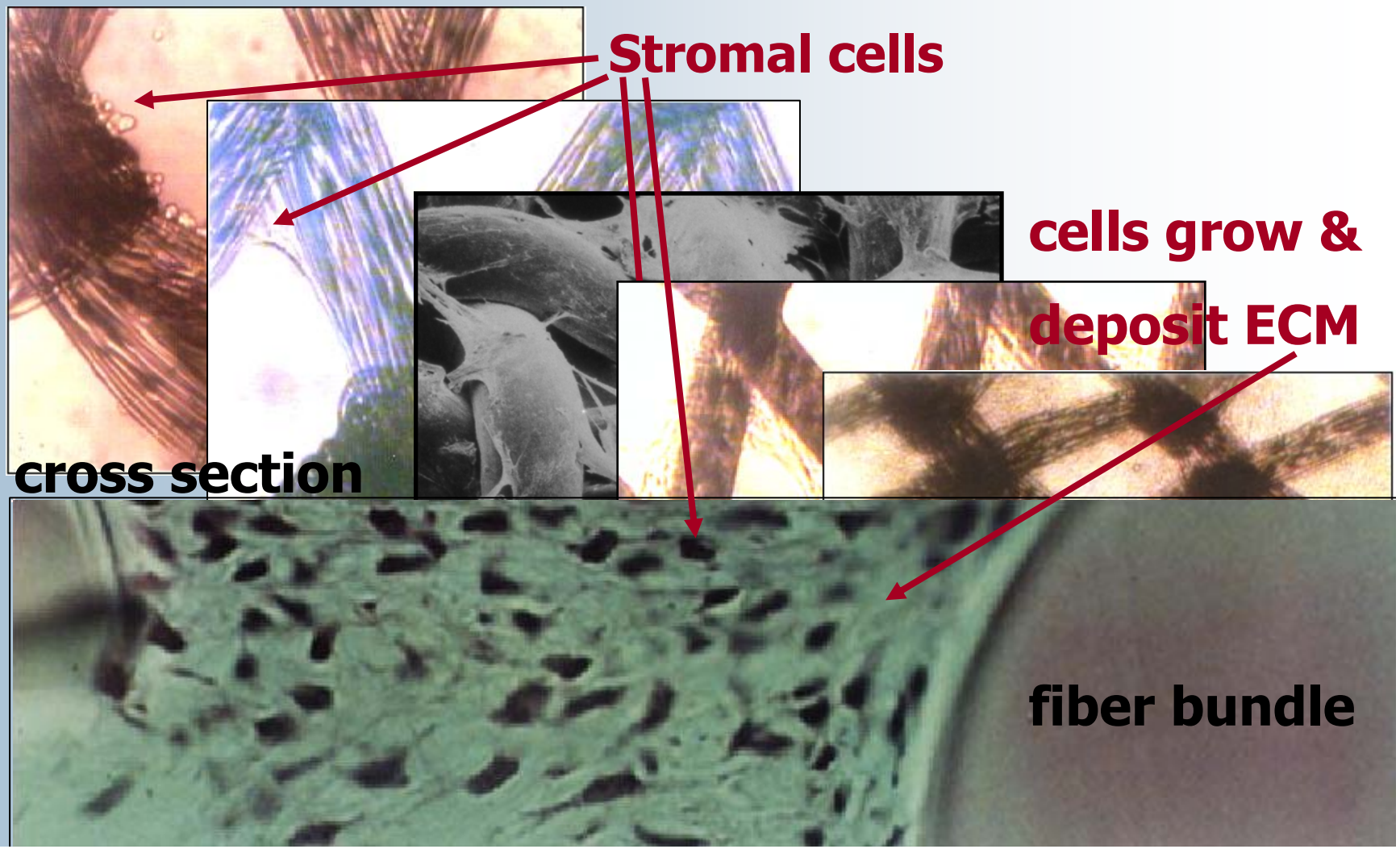
Tissue Growth Process

Common for any tissue type and application



Stromal Cells in 3-D Porous Scaffold

Creation of a Tissue Equivalent



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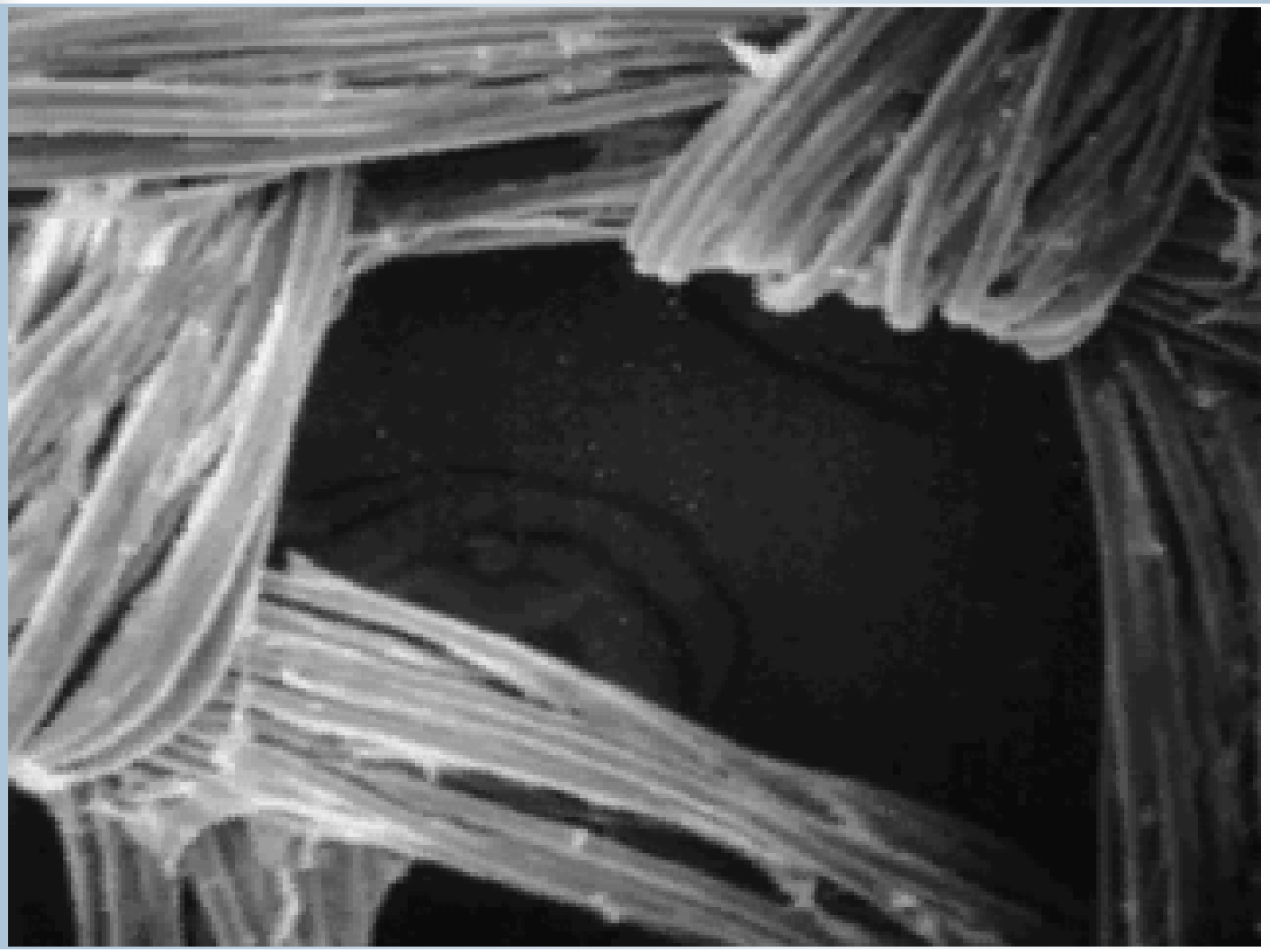
Fibroblasts in 3-D Porous Scaffold

Dermal Tissue Growth Time Lapse



RegeneMed

Skin^{2} ™

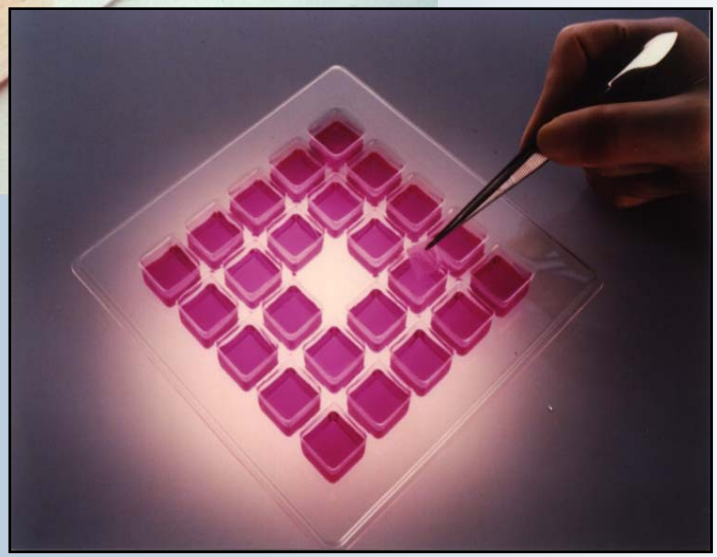
Skin Irritancy, Corrosivity, Penetration, Toxicity

All simple viability-based assays (MTT); mechanistic models needed

- 13 skin toxicology models (full- & partial-thickness)
- Alternative to animal testing
- Completely human skin tissues
- GLP manufacturing systems

Skin^{3}

- immune cells (T, B, Langerhan cells)
- Sensitization, inflammation, immune models



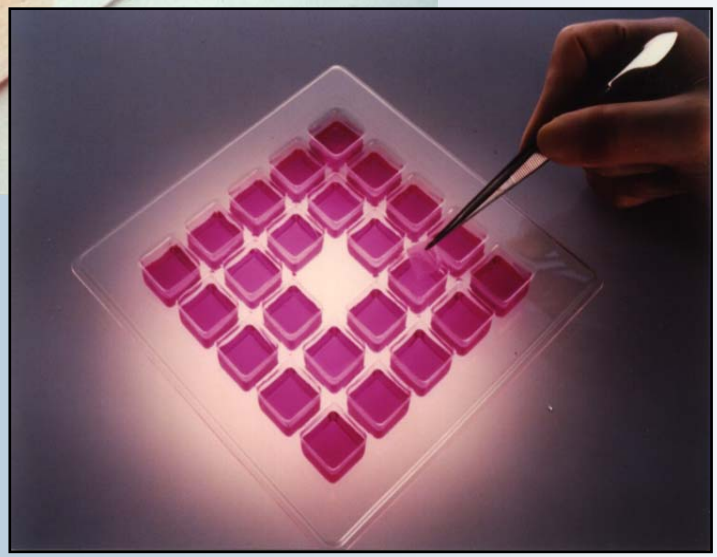
- Fresh preserved
- Validated in U.S., Europe, Asia
- Major customers: Amway, Exxon, Helene Curtis, Procter & Gamble, SC Johnson, Witco

RegeneMed

Skin^{2} TM

Skin Irritancy, Corrosivity, Penetration, Toxicity

- **EU 7th Amendment**
  - **No animal testing beyond**
    - **2009 Cosmetics**
    - **2013 all chemicals**
- **Industry response**
  - **Return to chem/bio assays**
  - **Not in vitro cell/tissue based**



- **Legally defensible chem/bio**
- **Non-validated in vitro models**
- **Cost**
- **Low throughput, 24-well, handled 1 well at a time**
- **Market potential**

Skin^{3}

- **immune cells (T, B, Langerhan cells)**
- **Sensitization, inflammation, immune models**

RegeneMed

Liver Toxicity

Leading Cause of Drug Failures

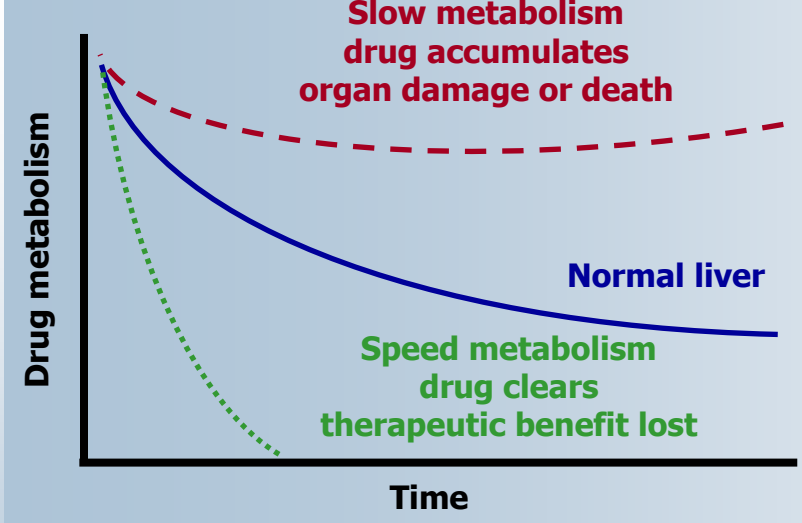
- **Drug development cost $1.1B**
- **Failed drugs prominent cost**
- **Liver toxicity & metabolism drug failures**
  (2/3 clinical, 1/3 withdrawals, 1/2 warnings, 40% liver failures)
- **Lab models not predictive of humans**
- **RegeneMed 3-D human liver tissue in the lab**

RegeneMed

Liver Toxicity

Leading Cause of Drug Failures

Parent Substance toxic to any organ



Liver metabolism breakdown products toxic to liver or other organs

- **Toxicity via cell death or tissue damage**
- **Drug-induced liver metabolism changes**
- **Drug-drug interactions**
- **CYP450-mediated**

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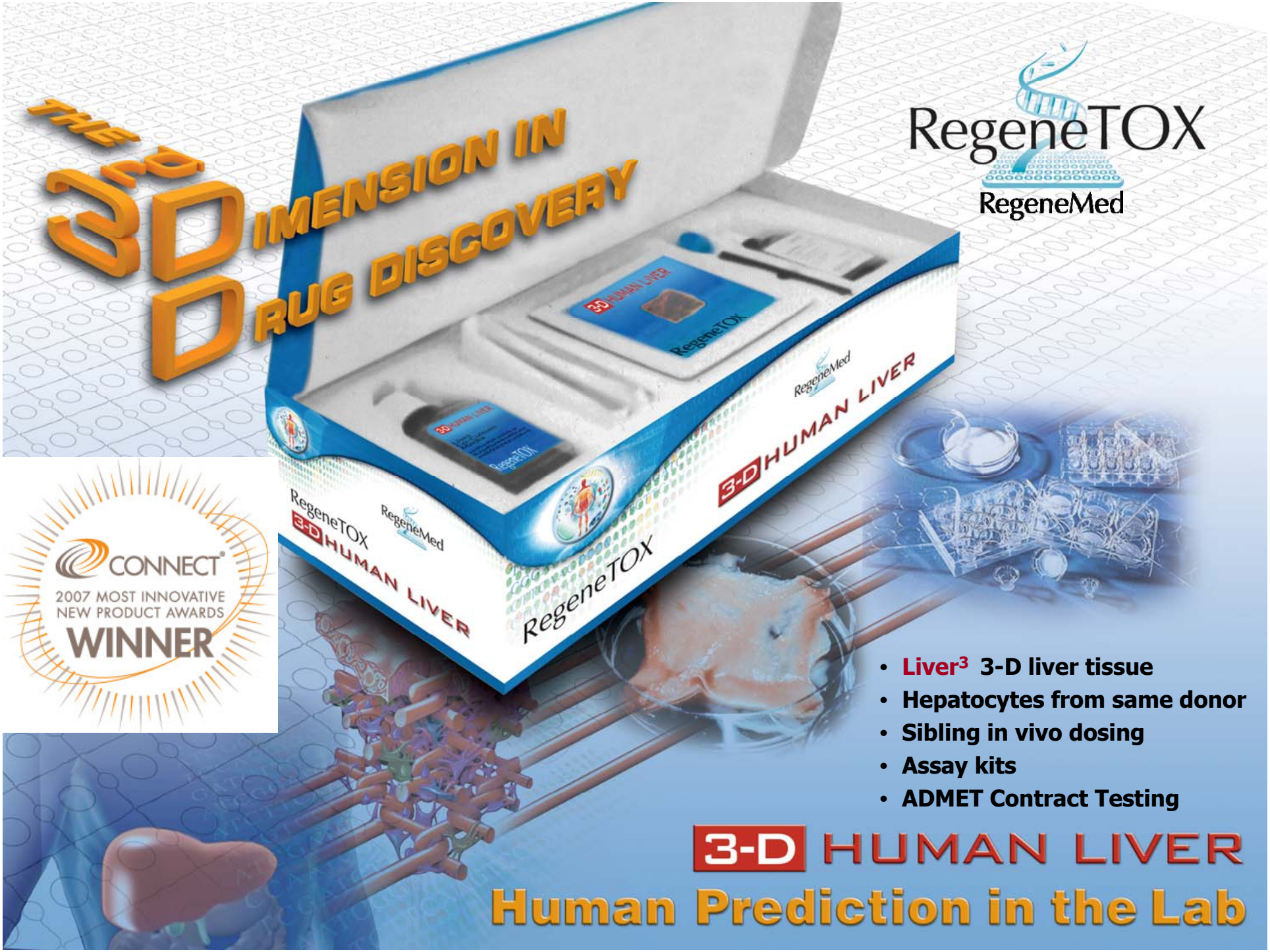
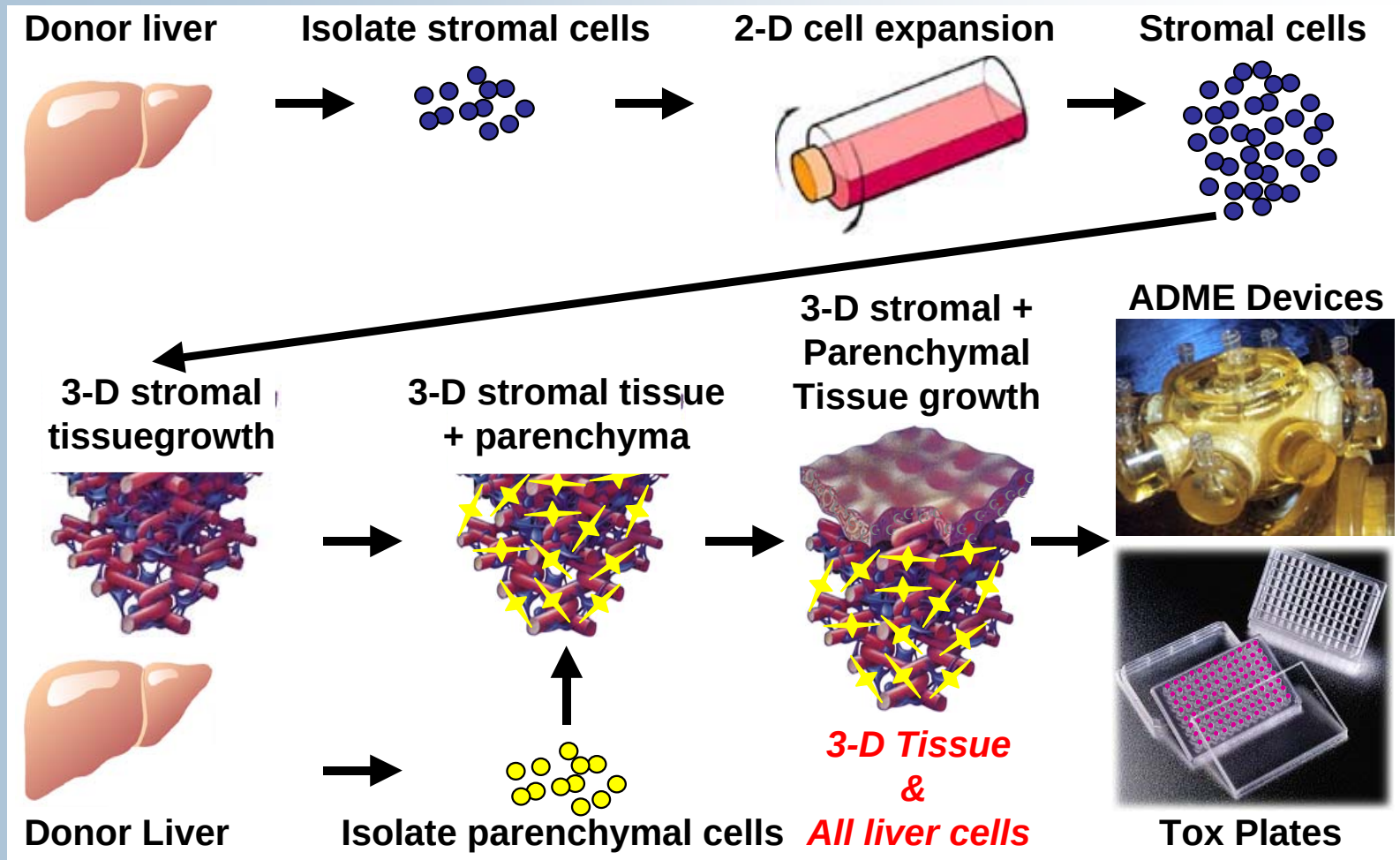

THE 3rd DIMENSION IN DRUG DISCOVERY
RegeneTOX
RegeneMed
CONNECT
2007 MOST INNOVATIVE NEW PRODUCT AWARDS
WINNER
RegeneTOX
3-D HUMAN LIVER
RegeneMed
• Liver3 3-D liver tissue
• Hepatocytes from same donor
• Sibling in vivo dosing
• Assay kits
• ADMET Contract Testing
3-D HUMAN LIVER
Human Prediction in the Lab

RegeneMed 3-D Liver Tissue

Liver Tissue Co-Culture Growth Process

1-step vs. 2-step



30 pharma equivs * 100 systems/day * 260 days/year = 780,000 systems/yr

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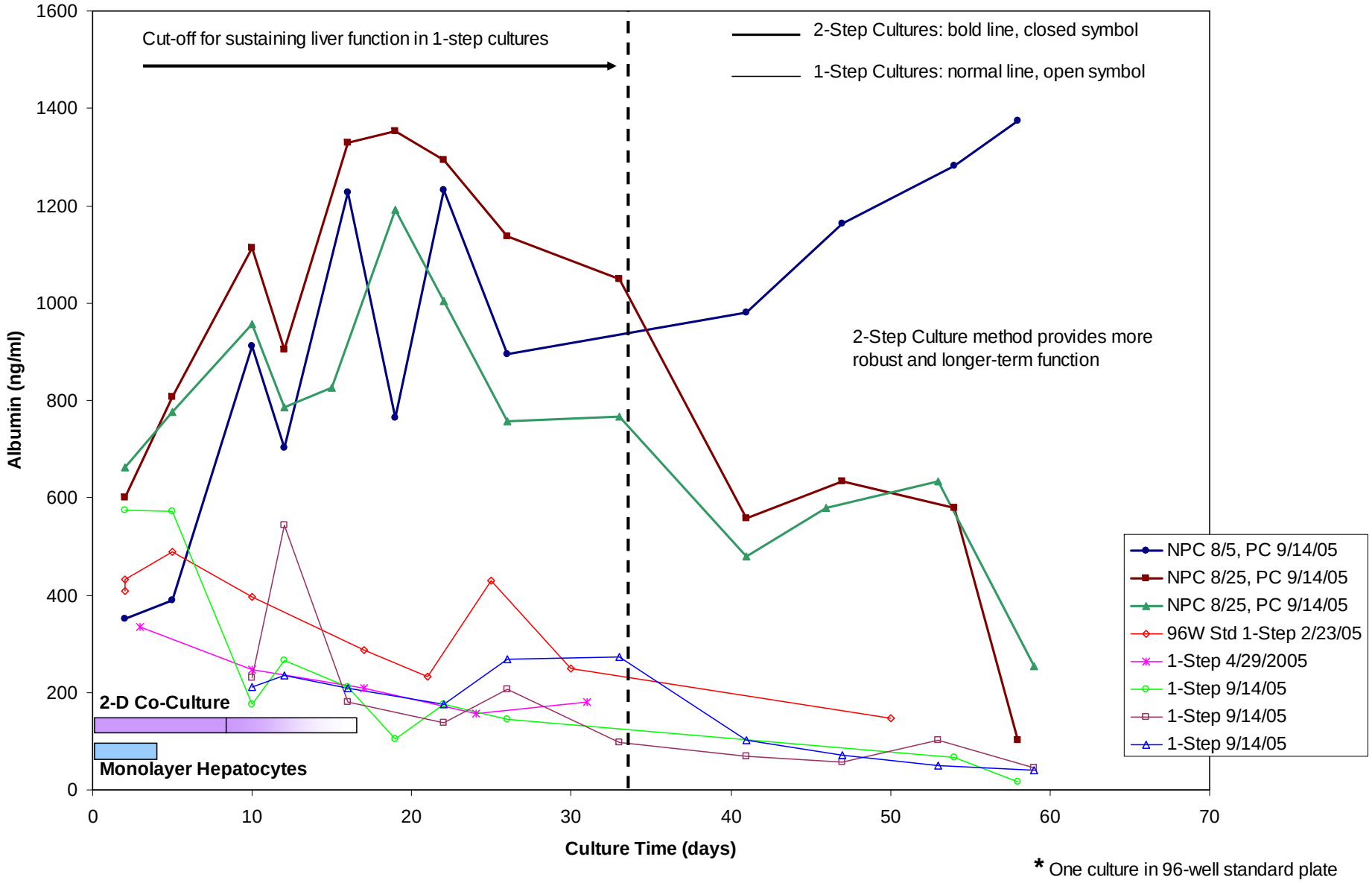
Rat Albumin Expression in 3-D Liver Co-Cultures Grown in 12-well Transwells*

2-Step versus 1-Step Growth Process

Cultures Initiated 2/05 to 9/05



* One culture in 96-well standard plate

Comparison of Liver-Specific Protein Expression

From 1980's to 2007

T-flasks and Bioreactors to Multiwell Plates

Protein	Plate ($ug/10^6$ cells)	Bioreactor ($ug/10^6$ cells)
Albumin	0.57-3.47	0.80-4.00
Transferrin	0.08-0.76	0.70-4.22
Fibrinogen	0.01-1.31	0.12-1.40
α-Fetoprotein	Not meas'd	0.50-5.00
HAAT	0.06-1.38	0.05-2.00
GST	268-376 (nmole/mg/min)	290-720 (nmole/mg/min)

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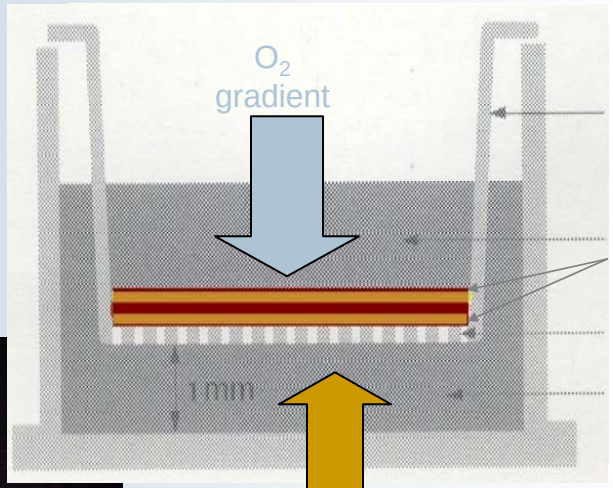
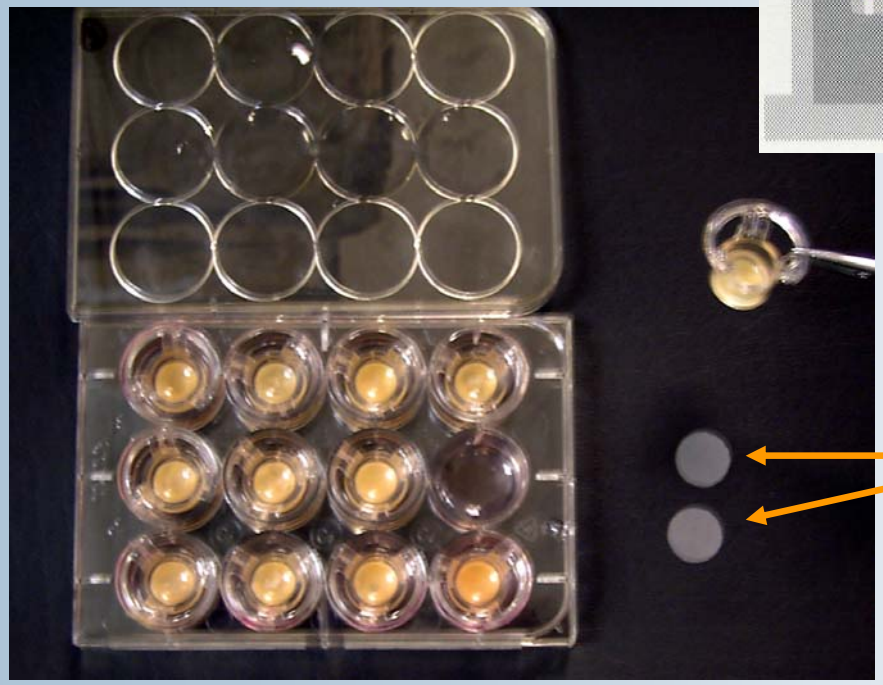
Liver^3: 3-D Liver Tissue Co-cultures

Grown in Static Transwell Plates for seamless integration pharma workflow

Method modified for metabolism studies:

- Use highly porous TW to maximize diffusion
- Nylon screens provide 3-dimensionality
- Cell seeding efficiencies are 100%
- Functional longevity to study chronic toxicity
- Amenable to multi-well plate formats





nylon screens

Transwell size: 6, 12, 24, 96, 384
√ √ √ √ in devl
Tissue size: 12, 24, 48, 384, 1536

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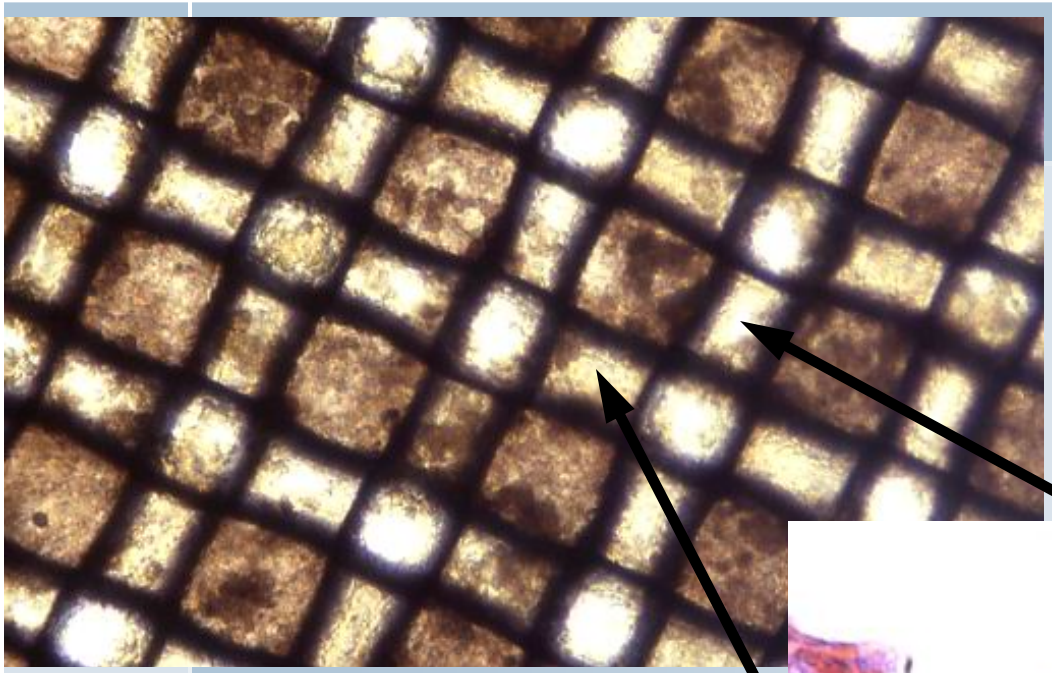
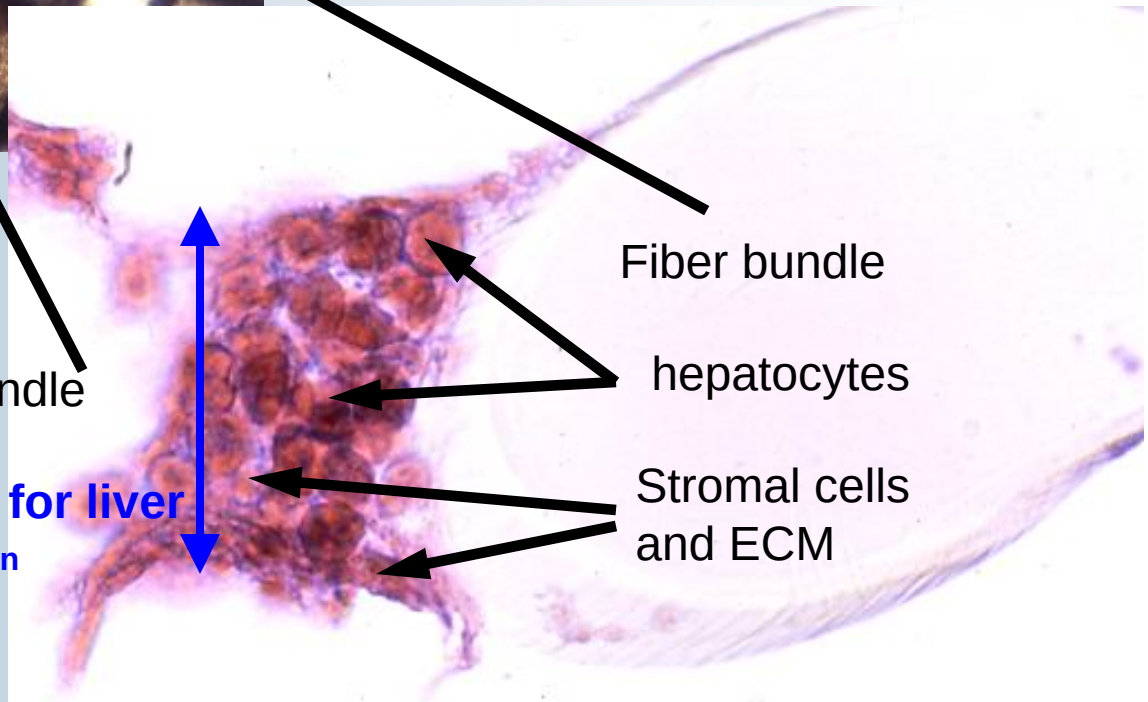
Rat Liver Co-culture

Inverted Phase: 140X, no stain



Cross-Section Through a Rat Liver Co-culture 52 days After PC Inoculation



60 uM – 500 uM for liver

Note: 500 uM is the limit of thickness in static culture due to oxygen diffusion limitations. Thicker tissues are possible using fluid flow bioreactors

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Maintenance of Tissue Cell Types

With Culture Age

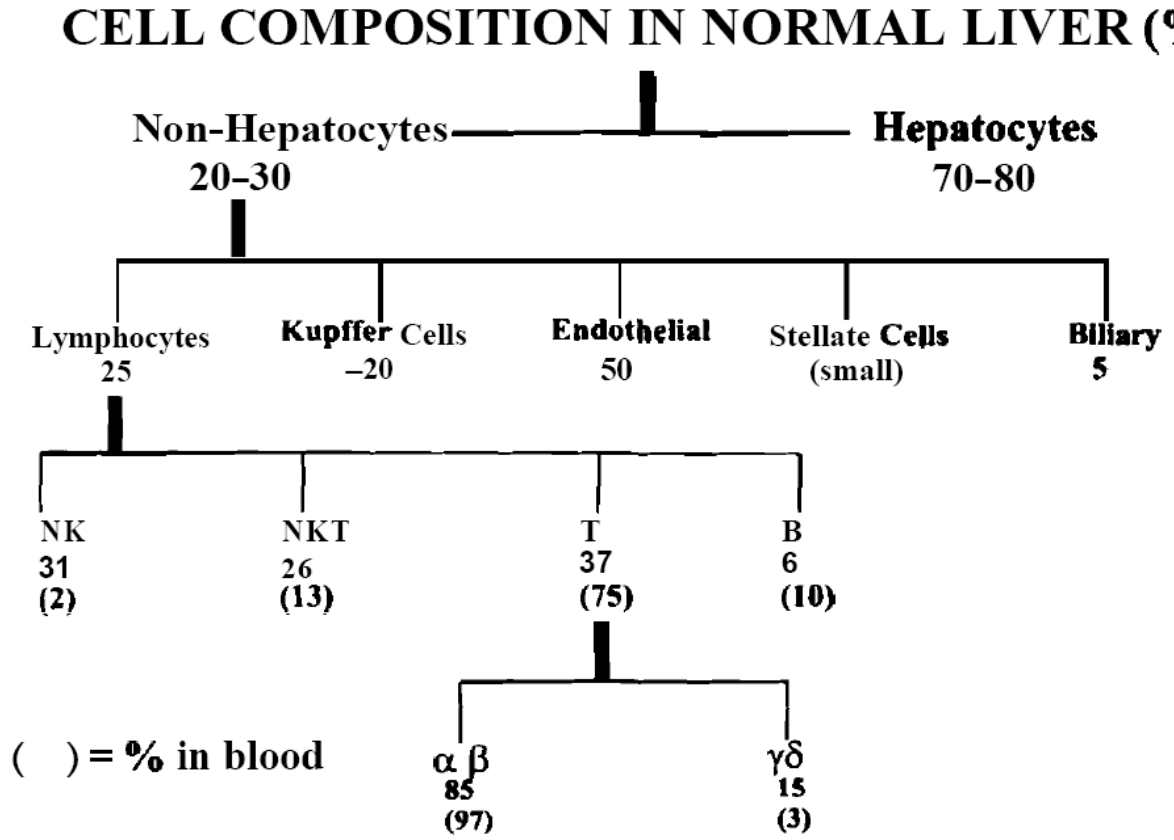

Figure 1. Percentage proportions of different cell types in the normal liver, showing subproportions for non-hepatocytes, total lymphocytes, and T lymphocytes, and comparisons with proportions in blood. (Reproduced from reference 2, with permission from the publishers of *Immunology and Cell Biology,* Blackwell Science, Asia.)

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Maintenance of Tissue Cell Types

With Culture Age

Table I. Mean absolute numbers (±1 SEM) of type I and type II parenchymal cells (P), stromal cells (S), and type III parenchymal (acidophilic) cells (A) and mean ratio of parenchymal cell numbers (all types) to stromal cell numbers in cocultures[a] of various ages.

Age of co-culture (days)	P	S	A	P:S
0	$6.82 \times 10^5 \pm 1.8 \times 10^4$	$3.69 \times 10^5 \pm 9.8 \times 10^4$	$4.95 \times 10^4 \pm 1.3 \times 10^3$	1.98
1	$9.30 \times 10^5 \pm 1.5 \times 10^5$	$5.03 \times 10^5 \pm 8.0 \times 10^4$	$6.75 \times 10^4 \pm 1.1 \times 10^3$	1.98
7	$1.20 \times 10^6 \pm 2.1 \times 10^5$	$6.65 \times 10^5 \pm 1.2 \times 10^4$	$3.84 \times 10^4 \pm 6.6 \times 10^2$	1.86
14	$1.63 \times 10^6 \pm 2.1 \times 10^5$	$1.00 \times 10^6 \pm 1.3 \times 10^5$	$6.48 \times 10^4 \pm 8.4 \times 10^2$	1.69
21	$2.56 \times 10^6 \pm 1.7 \times 10^5$	$1.68 \times 10^6 \pm 1.1 \times 10^5$	$6.45 \times 10^4 \pm 4.2 \times 10^3$	1.56
28	$3.86 \times 10^6 \pm 2.1 \times 10^5$	$2.88 \times 10^6 \pm 1.6 \times 10^5$	$1.52 \times 10^5 \pm 8.1 \times 10^3$	1.39
35	$5.57 \times 10^6 \pm 3.5 \times 10^5$	$4.15 \times 10^6 \pm 2.6 \times 10^5$	$6.83 \times 10^5 \pm 4.2 \times 10^4$	1.51
42	$5.83 \times 10^6 \pm 2.8 \times 10^5$	$4.83 \times 10^6 \pm 2.3 \times 10^5$	$5.31 \times 10^5 \pm 2.5 \times 10^4$	1.32
48	$6.34 \times 10^6 \pm 4.5 \times 10^5$	$4.27 \times 10^6 \pm 3.0 \times 10^5$	$4.88 \times 10^5 \pm 3.4 \times 10^4$	1.60

[a]Data derived from the evaluation of 3 to 5 cultures for each time period (SEM = standard error of the mean).

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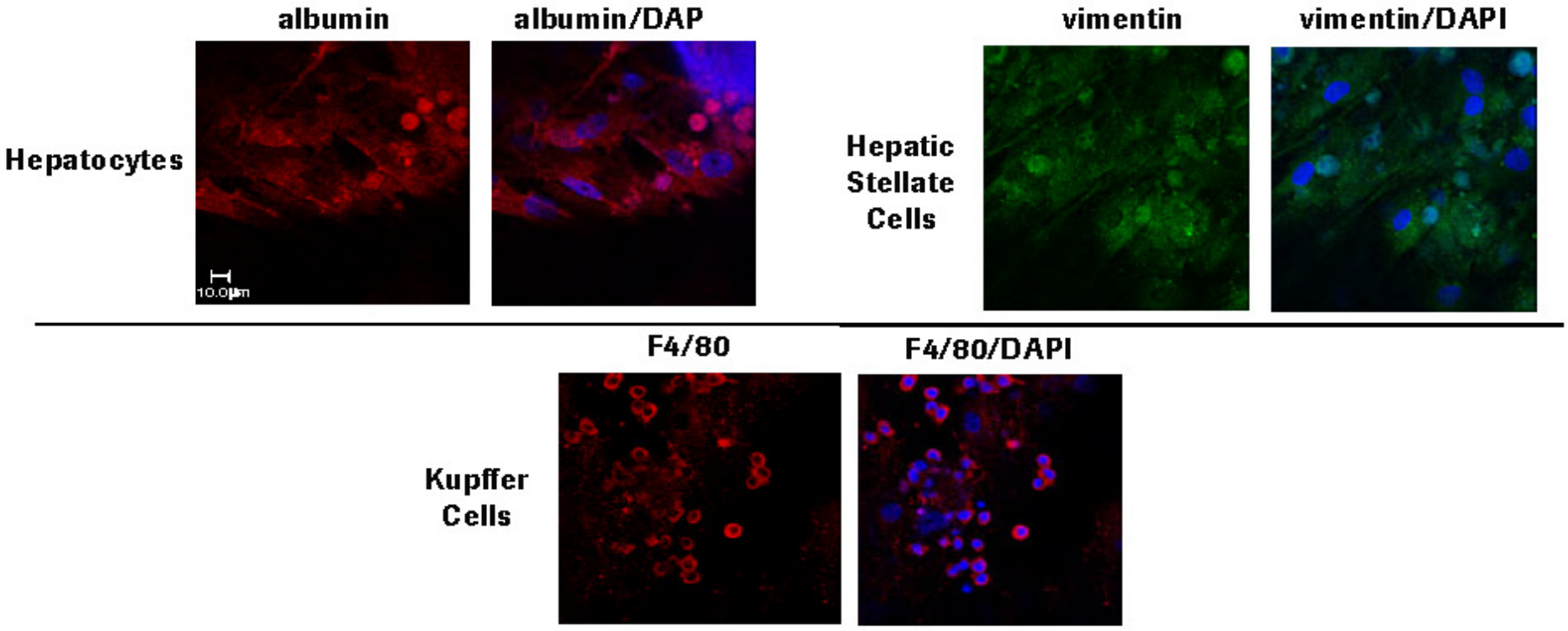
Human3D liver co-cultures contain main liver cell types found *in vivo*

Hoffmann-La Roche AG, Basel



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3D Bone Marrow Cultures

Maintenance of all cell types to 121 days

Table II. Phenotypic Analysis of Cells Dissociated from the Adherent Zones of Nylon Screen Cultures of Various Ages[a]

Culture age (days)	NK	OX-8	OX-54	$NK^+/IL\text{-}2R^{+b}$	$NK^+/IL\text{-}2R^-$
0	22.2 ± 1.32	4.0 ± 1.20	13.7 ± 2.05	8.1 ± 2.56	86.4 ± 5.69
15	57.5 ± 3.90	—	—	—	—
24	52.0 ± 4.24	15.4 ± 1.52	25.8 ± 2.07	47.5 ± 2.52	53.7 ± 3.08
49	44.5 ± 3.21	17.7 ± 1.29	22.9 ± 2.41	50.3 ± 2.03	48.6 ± 1.71
75	69.0 ± 6.20	—	—	—	—
105	53.5 ± 3.88	11.6 ± 1.17	15.3 ± 0.95	43.8 ± 4.53	49.9 ± 3.27
121	35.4 ± 2.79	5.6 ± 0.91	17.8 ± 1.24	51.0 ± 3.00	41.8 ± 2.92

TABLE 3. Mean Percent Reactivity[a] (± 1 SEM) of Uncultured Bone Marrow and Cells from LTBMC with Various FITC-Labelled Monoclonal Antibodies

	Monoclonal Antibodies				
Sample[b]	B-1	T-3	Plt-1	Mo-1	MY-9
Human					
2 wk LTBMC[c]	10.20 ± 1.43	18.64 ± 1.88	4.40 ± 1.33	20.10 ± 1.04	3.98 ± 0.26
7 wk LTBMC	6.76 ± 0.98	11.18 ± 1.86	8.08 ± 0.92	17.26 ± 2.29	3.70 ± 0.68
10.5 wk LTBMC	22.73 ± 1.37	13.01 ± 1.84	17.05 ± 4.10	20.98 ± 1.41	3.46 ± 0.25
uncultured marrow	11.96 ± 1.13	9.90 ± 0.64	8.72 ± 1.83	15.60 ± 0.84	1.46 ± 0.54
Macaque					
7 wk LTBMC[d]	31.01	18.13	46.50	40.87	21.64
uncultured marrow	8.37 ± 0.99	11.56 ± 2.10	8.53 ± 1.09	24.64 ± 2.25	5.49 ± 0.83

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3D Bone Marrow Cultures

Maintenance of all cell types to 208 days and in response to model toxins

Table I. The Effects of Various Doses of CP, Ara-C, MTX, and 5FU on the Phenotypic Distribution of Hematopoietic Cells in the Adherent Zones of Rat Nylon Screen Bone Marrow Cultures[a]

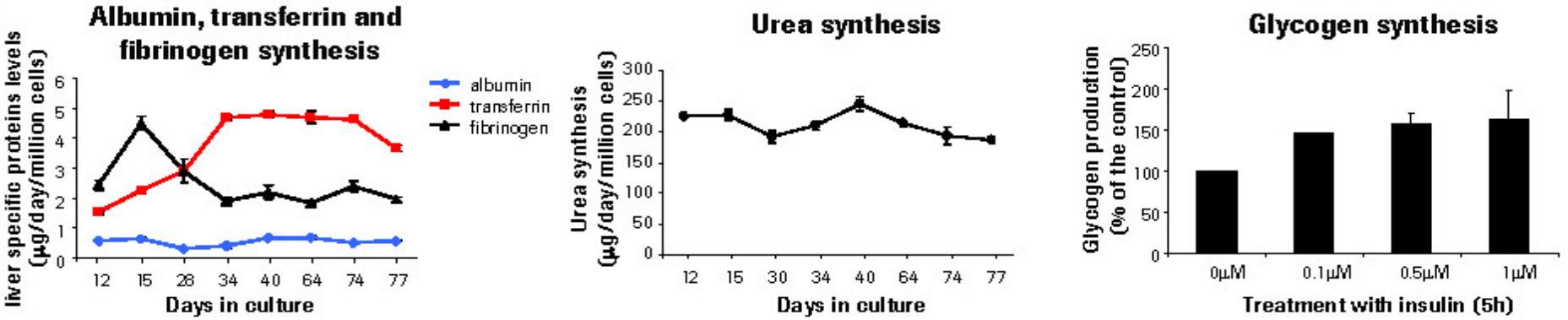
Drug—dose	OX-33 (B)	W3/25 (T_4)	OX-8 (T_8)	MOM/3F12/F2 (myeloid)
Untreated	7.84 ± 0.99 [100]	5.62 ± 0.83 [100]	6.01 ± 0.96 [100]	12.88 ± 0.72 [100]
CP				
0.025%	8.09 ± 0.11 [103]	5.71 ± 0.71 [102]	5.94 ± 0.62 [99]	15.41 ± 4.72 [120]
0.05%	4.80 ± 0.96 [59][b]	3.74 ± 0.95 [67][b]	2.97 ± 0.39 [49][b]	8.26 ± 2.03 [64][b]
0.10%	5.09 ± 2.02 [65]	2.94 ± 0.91 [38][b]	2.73 ± 0.70 [45][b]	7.63 ± 1.25 [59][b]
Ara-C				
0.02 mg/ml	8.37 ± 1.10 [107]	5.96 ± 0.89 [106]	7.54 ± 0.84 [125]	16.35 ± 0.56 [127]
0.20 mg/ml	8.52 ± 1.93 [108]	4.41 ± 1.31 [78]	7.58 ± 0.90 [126]	8.46 ± 0.82 [66][b]
2.0 mg/ml	4.49 ± 0.77 [57][b]	4.08 ± 0.54 [73][b]	4.60 ± 0.73 [77][b]	5.77 ± 0.96 [45][b]
MTX				
10^{-6} *M*	7.02 ± 0.48 [90]	7.52 ± 1.70 [134]	6.03 ± 1.48 [100]	10.36 ± 1.10 [80]
10^{-5} *M*	8.19 ± 1.72 [104]	5.32 ± 0.11 [95]	9.51 ± 1.83 [158]	7.61 ± 0.54 [59][b]
10^{-4} *M*	5.53 ± 0.74 [71][b]	3.78 ± 0.72 [67][b]	3.93 ± 0.53 [65][b]	7.47 ± 0.83 [57][b]
5FU				
0.1 mg/ml	4.47 ± 0.92 [57][b]	5.41 ± 0.75 [96]	3.51 ± 0.89 [58][b]	9.52 ± 0.88 [74][b]
0.2 mg/ml	3.26 ± 0.61 [42][b]	4.39 ± 0.38 [78][b]	2.32 ± 0.14 [39][b]	8.75 ± 0.70 [68][b]
2.0 mg/ml	1.23 ± 0.24 [16][b]	0.82 ± 0.08 [15][b]	0.69 ± 0.09 [11][b]	2.42 ± 0.36 [19][b]

[a] Represents the mean positive fluorescent events for each antibody and each drug and dose level as pooled from cultures of various ages (10, 33, 100, 150, 170, and 208 days). n = 3–11. The bracketed numbers indicate percentage of control.
[b] Significantly different from controls.

RegeneMed

Preserved liver-specific functions in human 3D liver co-culture over months

Hoffmann-La Roche AG, Basel



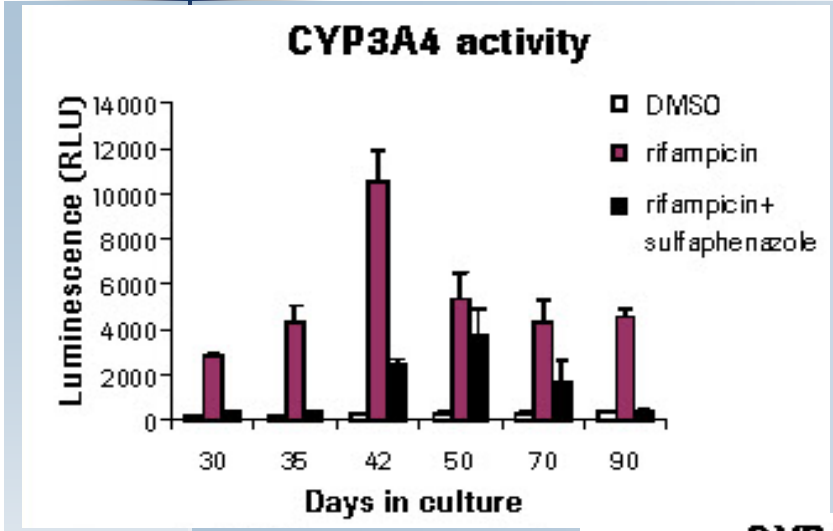
Levels of albumin, transferrin and fibrinogen were measured with ELISA kit (GenWay) and urea synthesis using urea nitrogen kit (Sanbio). The levels of respective proteins in human 2D hepatocytes are: albumin: 0.1-0.4 µg/day/million cells; fibrinogen: 0.2-1 µg/day/million cells and urea: 50-150µg/day/million cells.

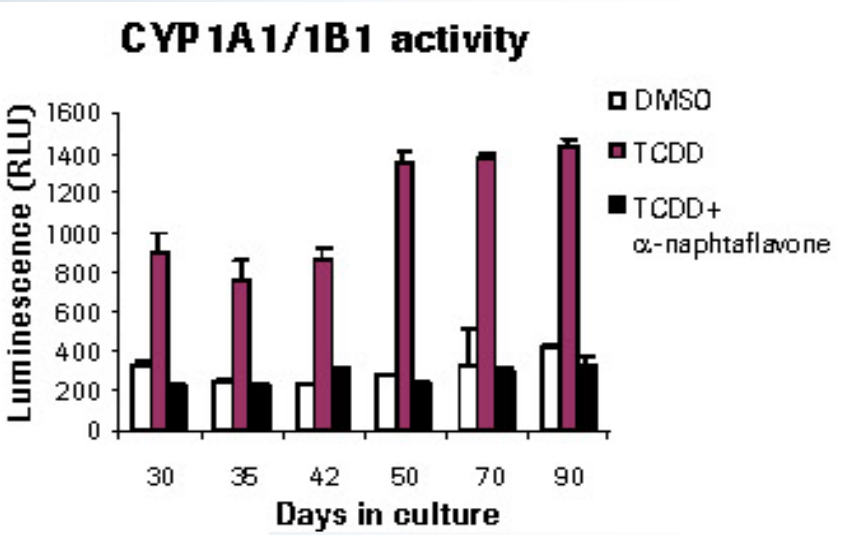
- **All current assay guidelines for higher dose acute response due to short-lived function**
- **Chronic toxicity – no current guidance**
- **Micronucleus assay in multinucleated cells (chronic)**

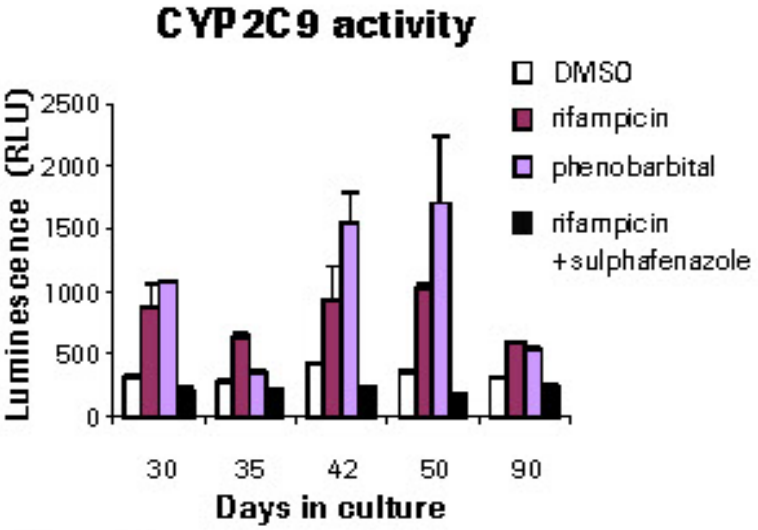
RegeneMed

Preserved basal, inducible and inhibited CYP450 activities in human 3D liver co-culture over months

Hoffmann-La Roche AG, Basel







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Hepatic Transporter Expression (Rat)

Basolateral

Uptake

Transporter	Transcription Factors	Expression	
		Basal Relative to In Vivo	Fold Change *
Oatp1a1 (Slco1a1)	CAR/PXR/ AhR ↓	Lower	-2
Oatp1a4 (Slco1a4)	CAR/PXR/ AhR ↓	Lower	-20
Ntcp (Slc10a1)	RARα ↑ PXR/CAR/ FXR ↓	Lower	22

Efflux

Transporter	Transcription Factors	Expression	
		Basal Relative to In Vivo	Fold Change *
Mrp3 (Abcc3)	CAR/PXR/ FXR ↑	Higher	10
Mrp4 (Abcc4)	CAR/PXR/ FXR ↑	Similar	NA

Canalicular

- **Transporter guidance doc under DILI & DDI**
- **Current assays cannot discern individual transporters**
- **Primary cell-based in development**

Transporter	Transcription Factors	Expression	
		Basal Relative to In Vivo	Fold Change *
Mdr1(P-gp; Abcb1)	AhR/PPARα/ CAR/PXR ↑	Similar	6-8
Bsep (Abcb11)	CAR/PXR/ FXR ↑ AhR ↓	Lower	8
Mrp2 (Abcc2)	CAR/PXR/ FXR ↑	Similar	3
Bcrp (Abcg2)	PPARα/CAR/ PXR ↑	Similar	NA

* Fold change induced by Phenobarbital at 100 uM for 72 hr in 3D liver cultures

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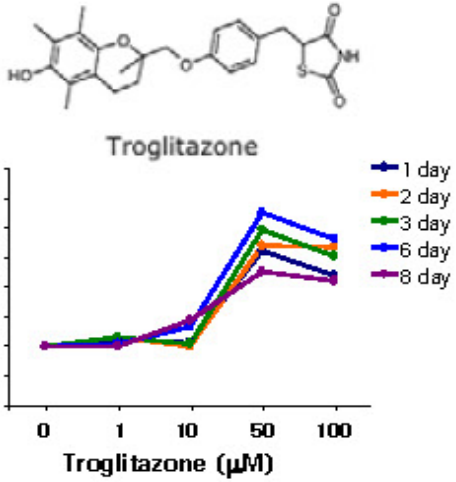
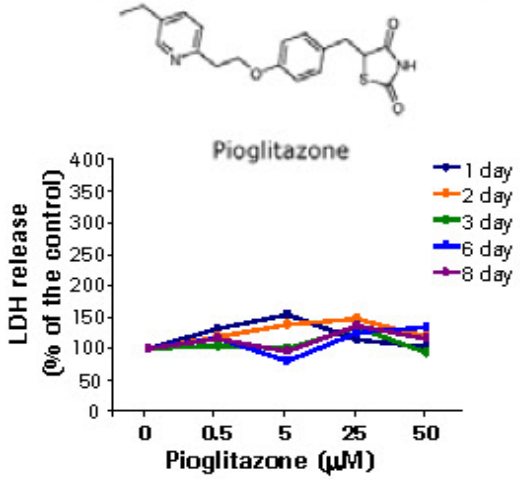
Comparison of structurally similar PPARγ agonists with different *in vivo* toxicity in human 3D liver co-cultures

Hoffmann-La Roche AG, Basel

Troglitazone & Pioglitazone
(anti-diabetic drugs)

- **Troglitazone:** withdrawn from market due to hepatotoxicity in clinic; Human Cmax=7µM
- **Pioglitazone:** marketed. No hepatotoxicity in clinic; Human Cmax=2.7µM





Dose and time-dependent toxicity profiles of hepatotoxic drugs upon treatment for 1-15 days. The media was collected everyday and the amount of released lactate dehydrogenase (LDH) was measured using CytoTox-One assay (Promega). Cmax =maximum therapeutic concentration of the drug found in the human plasma.

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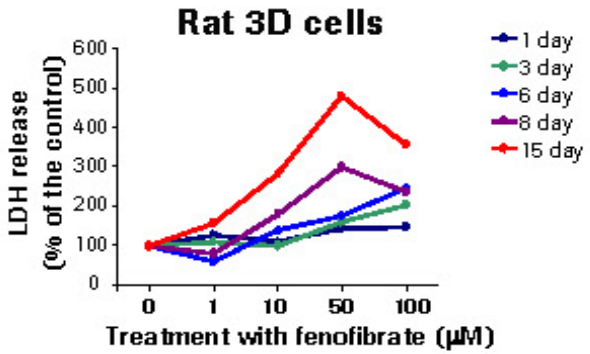
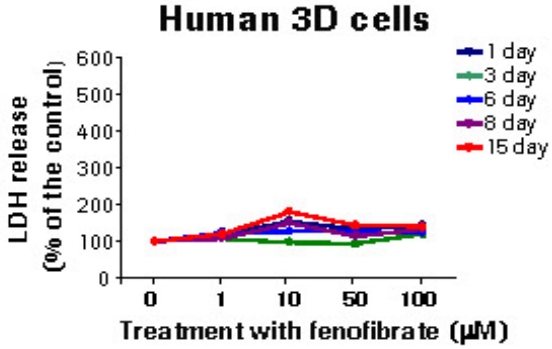
3D liver co-culture reflect species-specific responses to drug treatments

Hoffmann-La Roche AG, Basel

Fenofibrate
(anti-lipidemic drug)

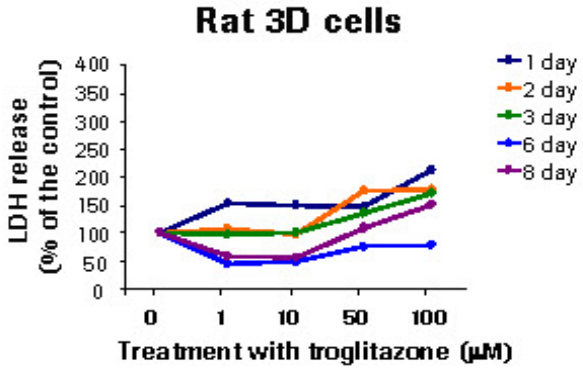
- marketed
- no hepatotoxicity observed in rat/human hepatocytes
- hepatotoxicity in rat pre-clinical model
- no hepatotoxocity in clinic, Human Cmax=12,14µm

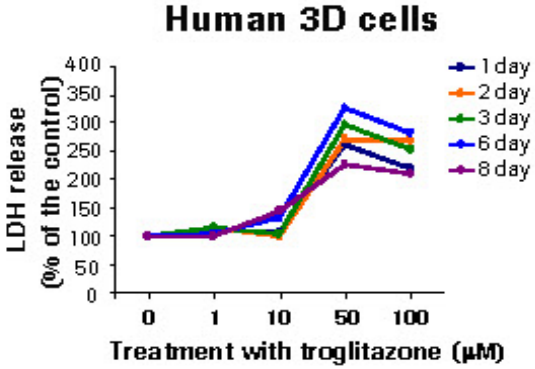




Troglitazone
(anti-diabetic drug)

- withdrawn from the market
- no hepatotoxicity observed in rat/human hepatocytes
- no hepatotoxicity in rat pre-clinical model
- hepatotoxicity in clinic, Human Cmax=7µm





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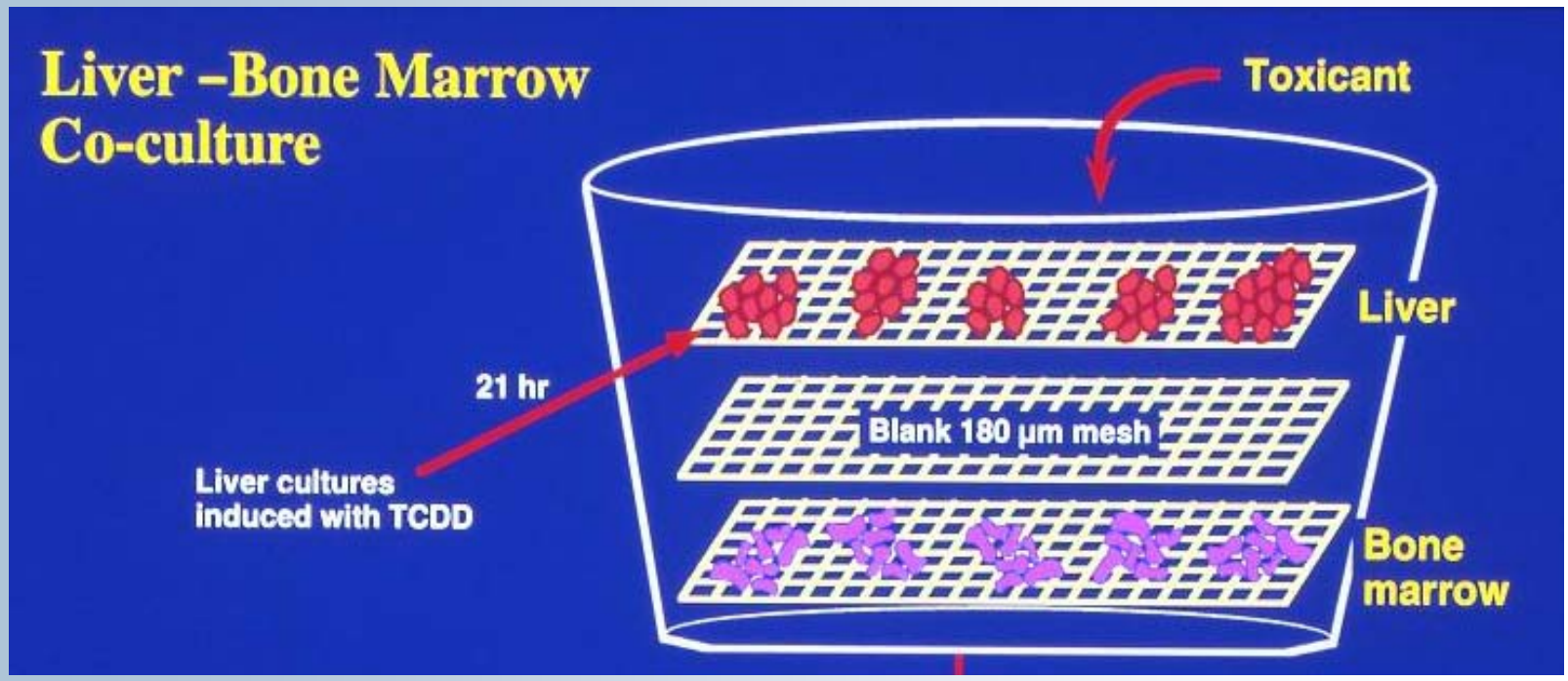
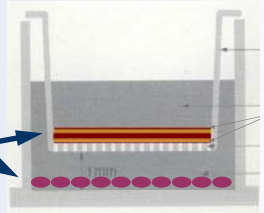
- **Pharm study alongside clinical trial: Animal-to-human prediction pre-animal study provides confidence to proceed with animal study**
- **Replacement of animal testing long term vision**

Combination Systems
Metabolic Activation
3D liver co-culture with cell reporter assay



- **Benzene not toxic to bone marrow until metabolized by liver**
- **Transwell culture system**
  - Reporter cells in 2D on bottom of plate
  - 3D liver co-culture on insert



- **Hit stage: toxic metabolites**
- **Lead optimiz: mechanism**

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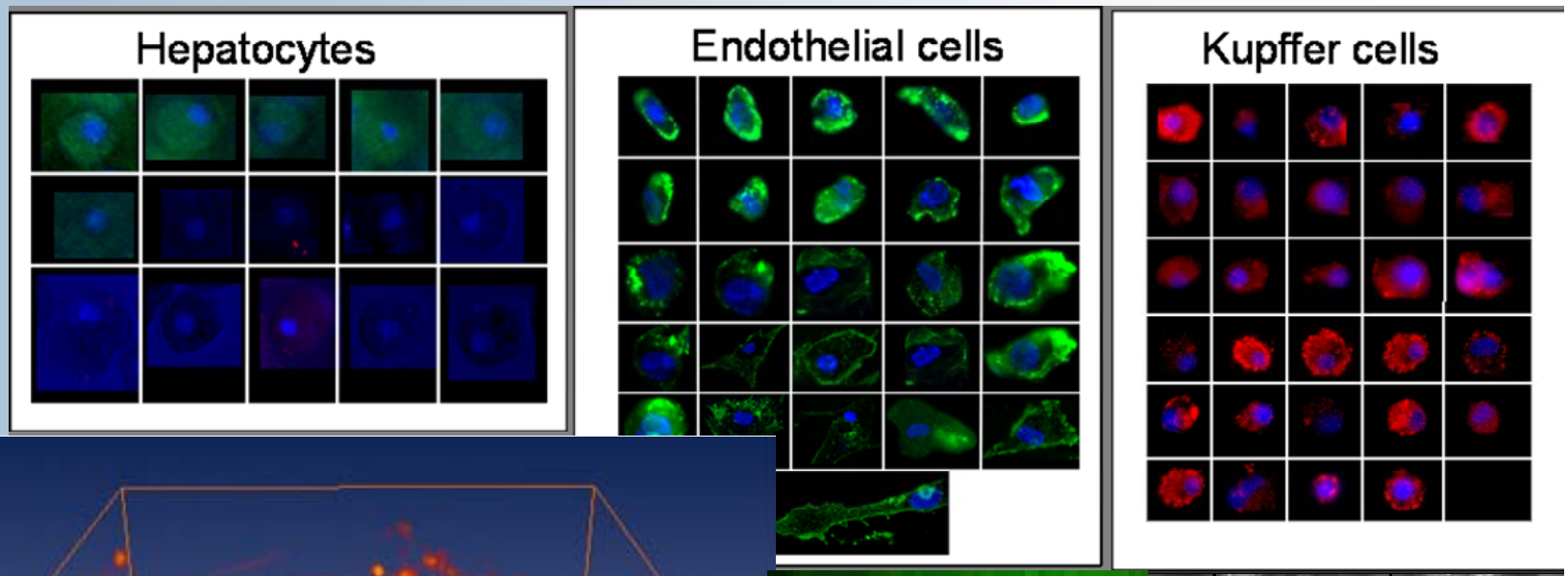
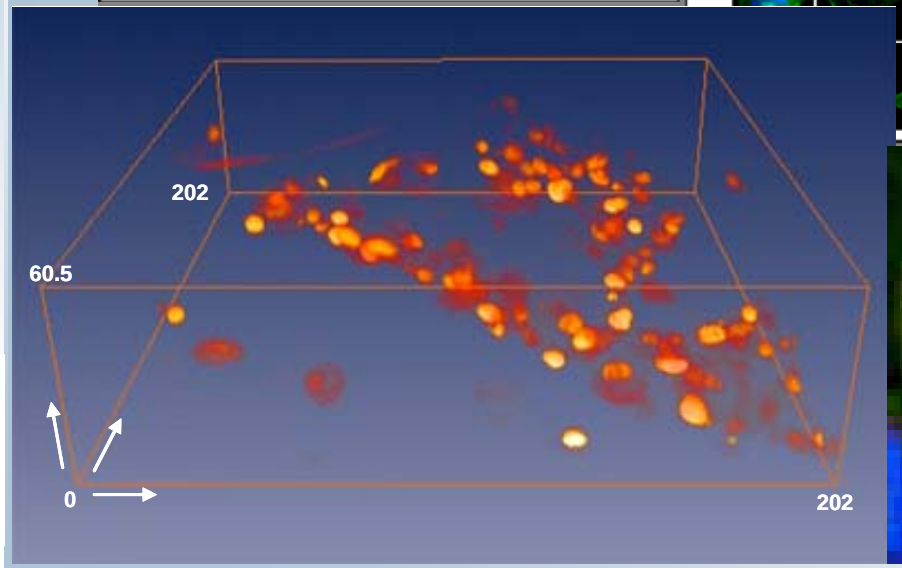
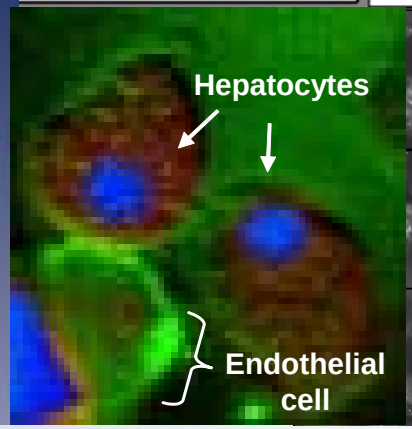
3D *In vitro* Imaging

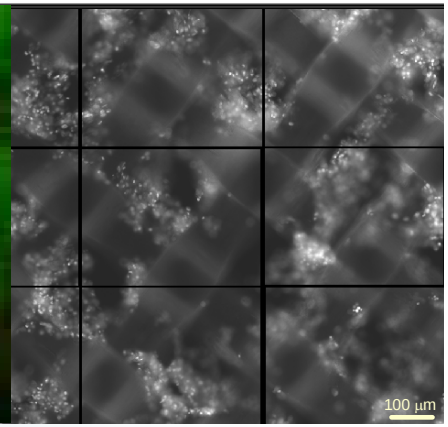
RegeneMed and Vala (Q3DM)

Real-time, min-invasive, non-destructive, *in vivo* to *in vitro* platforms









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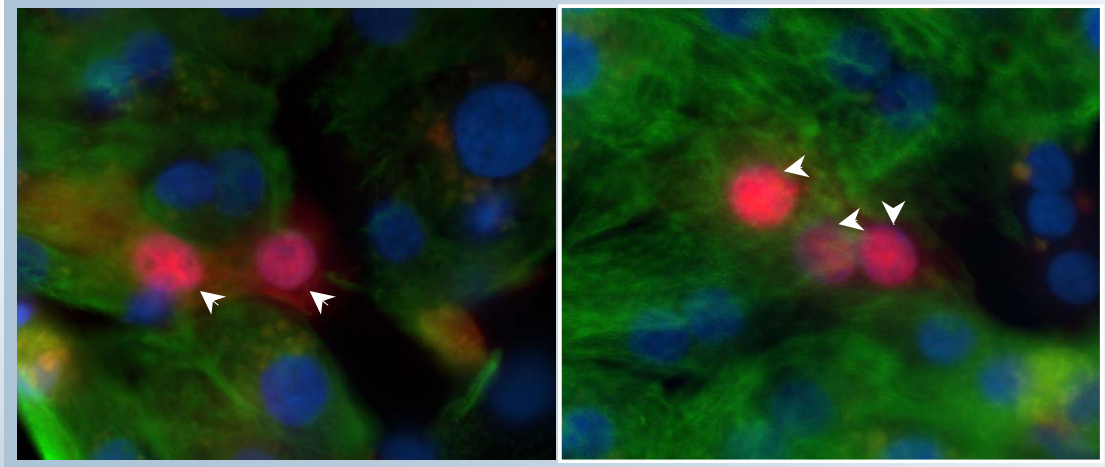
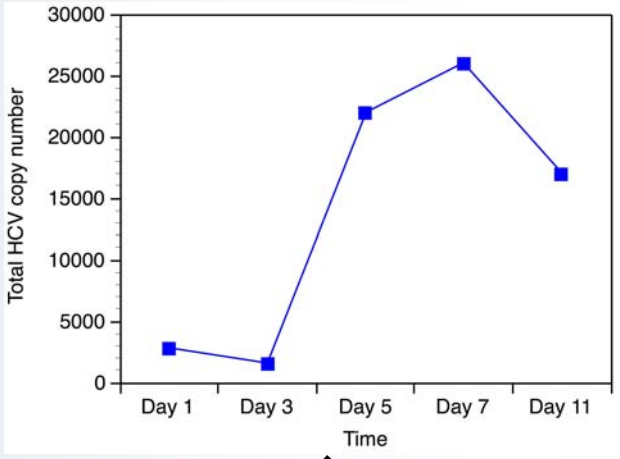
Disease Model

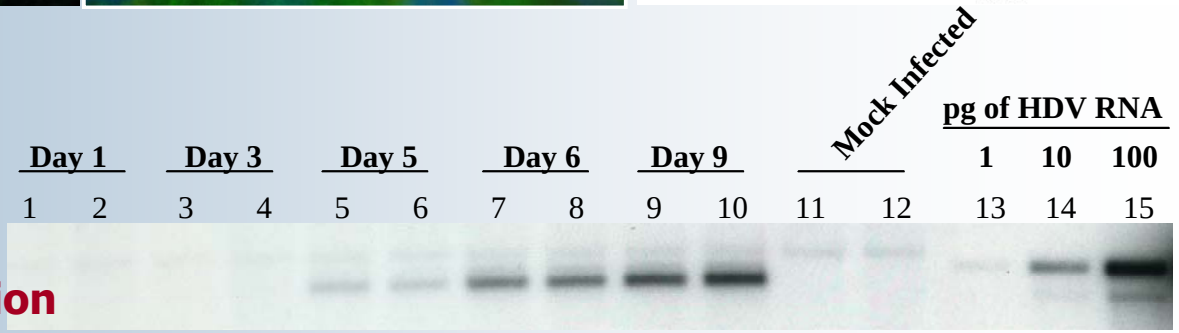
Hepatitis C & D infection/replication

- Humans only species that infects with Hepatitis
- No animal models for development of antivirals
- Chiron and Stanford (Gilead, ICN, others)





- **Multiple cell types**
- **Culture longevity**
- **Patient/infectious sera variation challenges validation**



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Disease Progression

Phenobarbital-induced cancer

Non-genotoxic carcinogen – DNA replication

Tritiated thymidine incorporation with culture age

Animal	Condition	Age of Culures at TT assay	# days culture exposed to PB	DNA ng/ul	RNA ng/ul	TT Counts/ ug DNA	% Increase Over Control
male mouse							
DAY 3 (5)	Control	9 d	3 d	56.7	366	48,768	
	0.1mM Phenobarbital	9 d	3 d	61.2	290	44,956	-7.8%
	1.0 mM Phenobarbital	9 d	3 d	68	443	65,638	34.6%
	2.0 mM Phenobarbital	9 d	3 d	57	382	49,829	2.2%
Day 7 (9)	Control	13 d	7 d	48	269.1	2,555	
	0.1mM Phenobarbital	13 d	7 d	41	357	5,158	101.9%
	1.0 mM Phenobarbital	13 d	7 d	25	292.8	4,485	75.6%
	2.0 mM Phenobarbital	13 d	7 d	16	156.4	5,282	106.8%
Day 16 (18)	Control	22 d	16 d	51	209	3,653	
	0.1mM Phenobarbital	22 d	16 d	70	196	16,566	353.5%
	1.0 mM Phenobarbital	22 d	16 d	69	258	21,256	481.9%
	2.0 mM Phenobarbital	22 d	16 d	24	226	25,653	602.3%

- Rat, Mouse, Human; Male, Female (different mechanisms)
- Comparing to in vivo data (time course different)
- In vitro progression to preneoplastic lesions and cancer?

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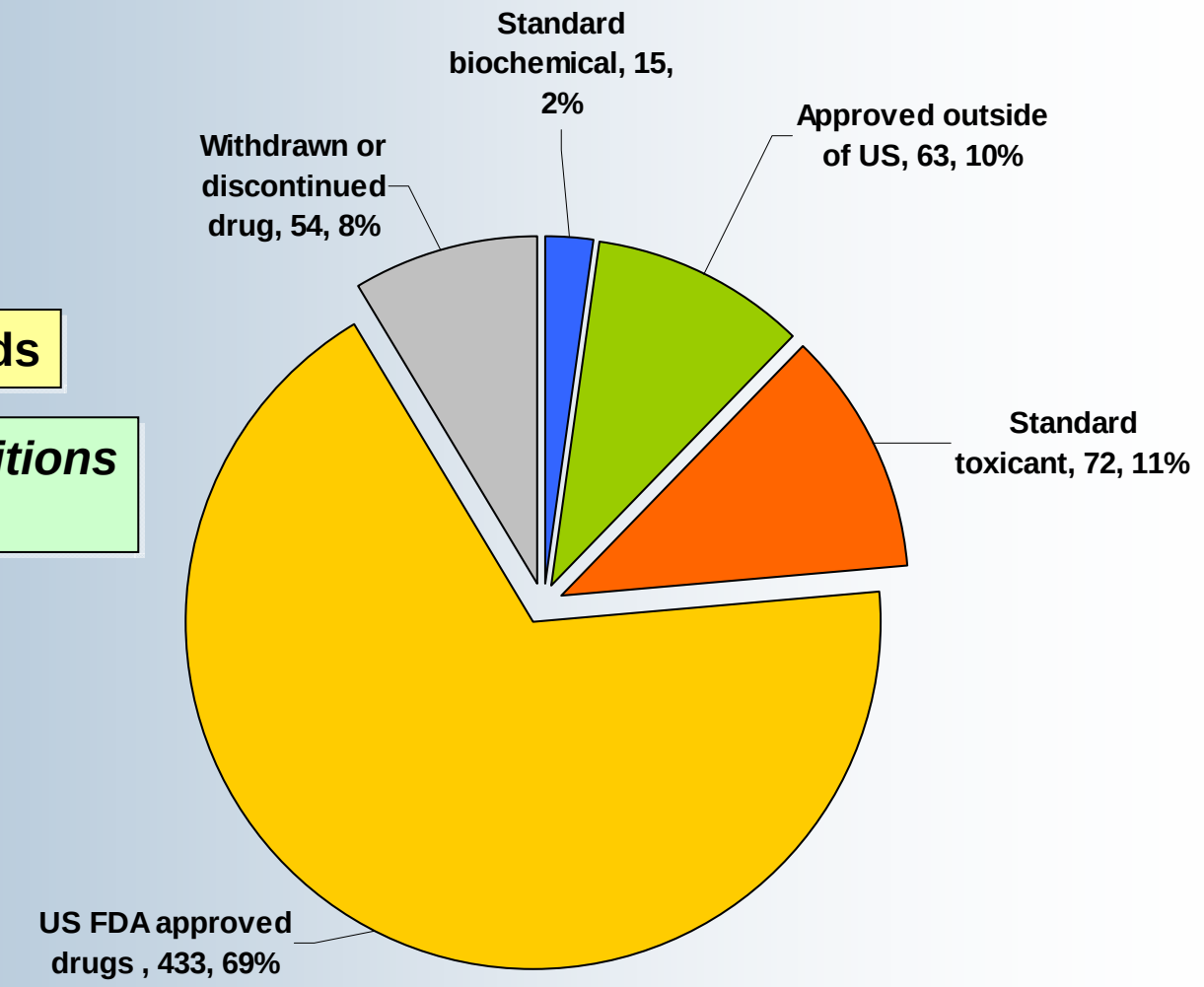
DrugMatrix Compounds

Male Sprague Dawley Rats and 2D hepatocytes

NTP Owns Iconix/Entelos DrugMatrix Database

~640 Compounds

Compound additions ongoing



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DrugMatrix Content

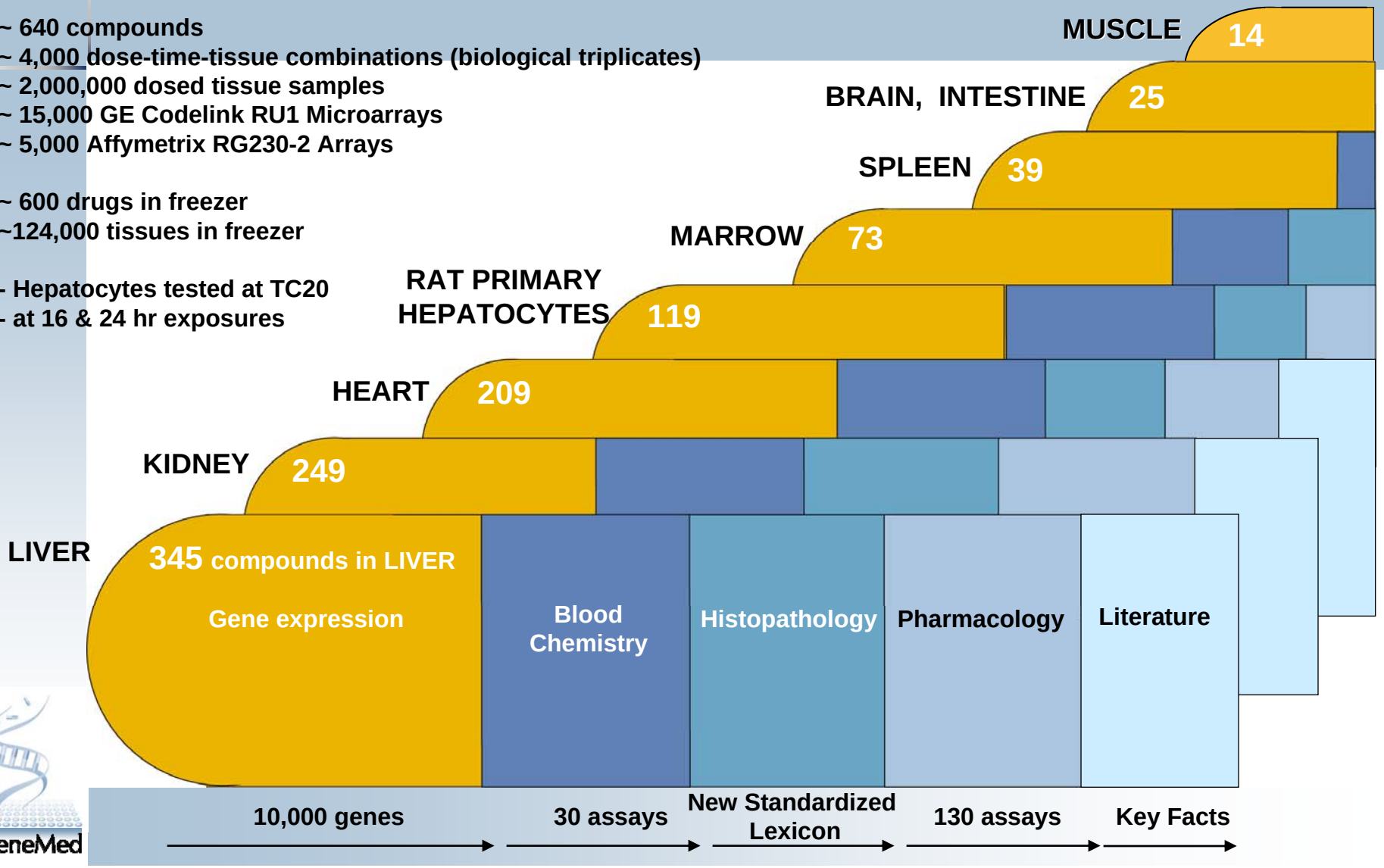
Male Sprague Dawley Rats and 2D hepatocytes

NTP Owns Iconix/Entelos DrugMatrix Database

~ 640 compounds
~ 4,000 dose-time-tissue combinations (biological triplicates)
~ 2,000,000 dosed tissue samples
~ 15,000 GE Codelink RU1 Microarrays
~ 5,000 Affymetrix RG230-2 Arrays

~ 600 drugs in freezer
~124,000 tissues in freezer

- Hepatocytes tested at TC20
- at 16 & 24 hr exposures



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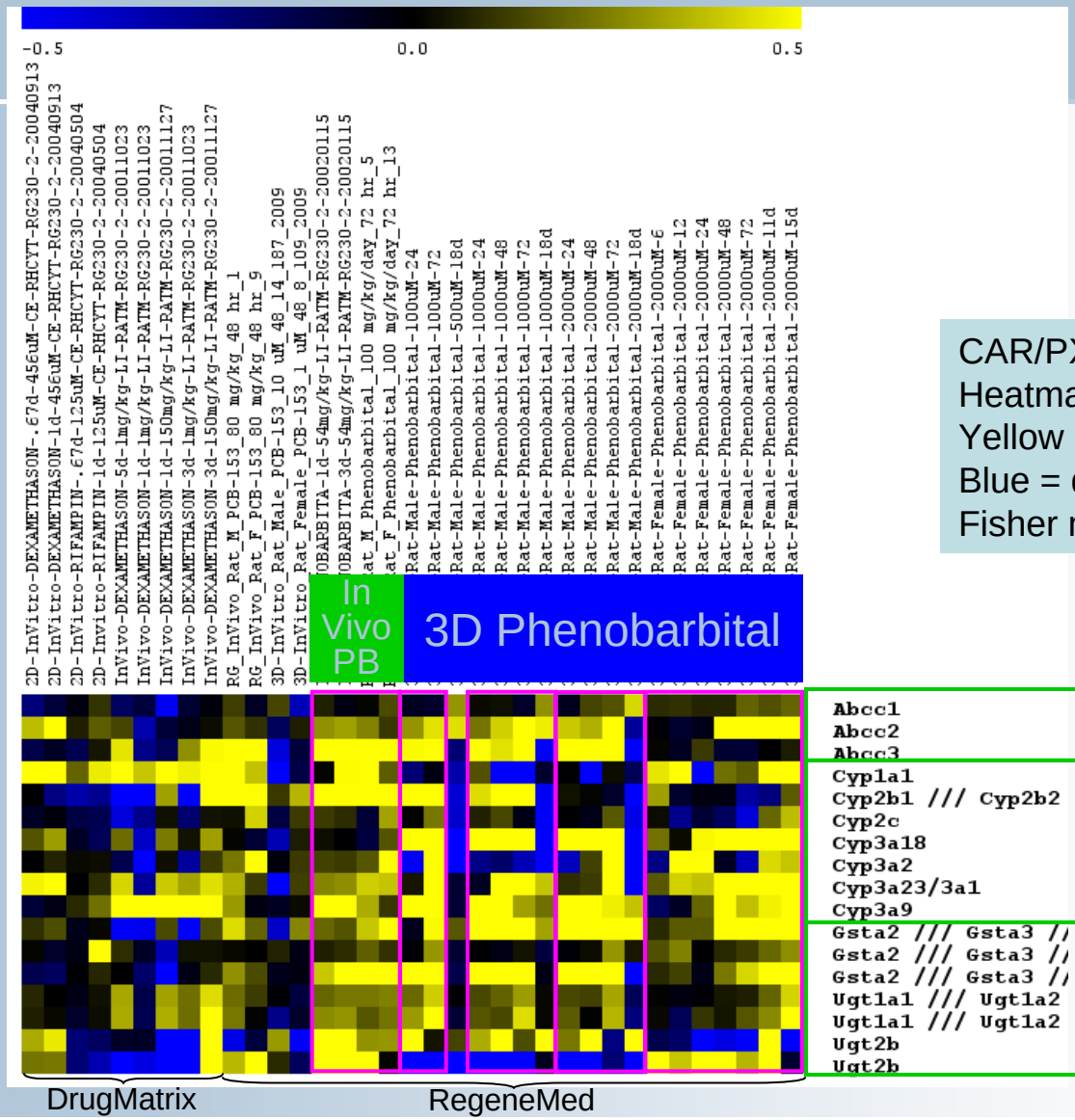
Phenobarbital, CAR/PXR agonists induce gene expression of Phase I, II, and III genes



Phase III

Phase I

Phase II

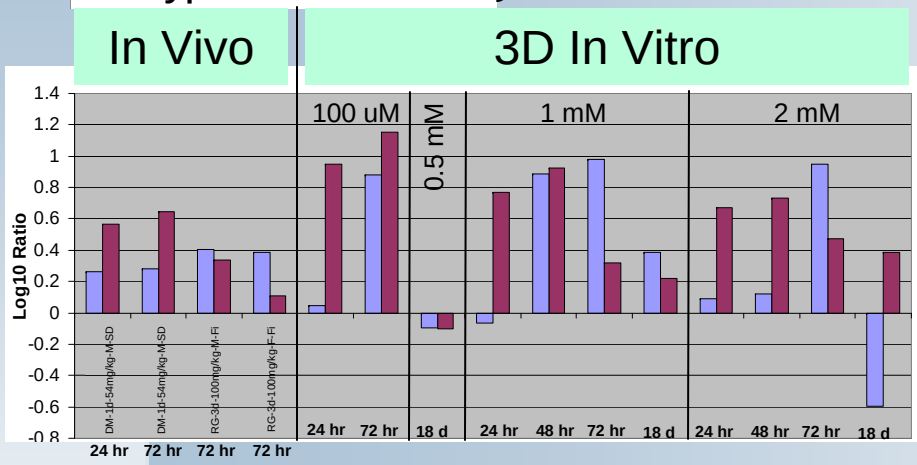
CAR/PXR-inducible genes
Heatmap of log10 ratios
Yellow = upregulation
Blue = downregulation
Fisher male rats

Rat 230A and 230 2.0 GeneChip
Mas5 Processed data
Log10 ratios shown

Phenobarbital (CAR/PXR agonist) induces Cyp3A and Cyp2B gene expression in 3D cultures

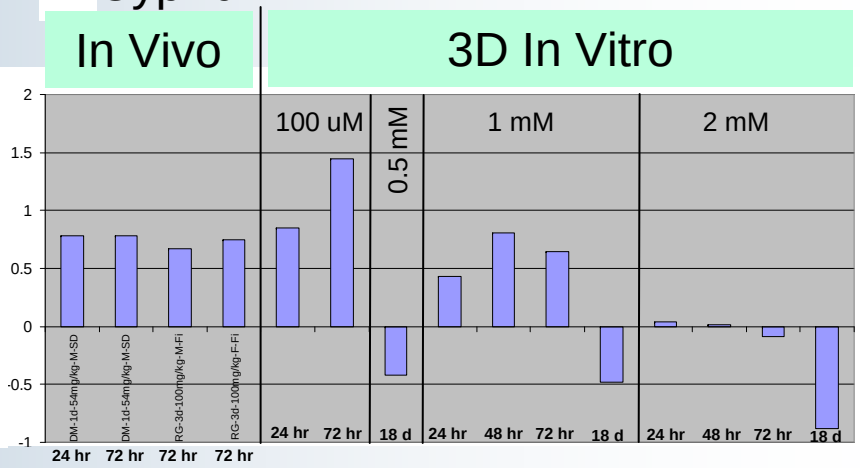
Cyp3a23/Cyp3a1
Cyp3a9

Similar baseline expression between 3D and in vivo



Cyp2b1

Lower baseline expression in 3D than in vivo

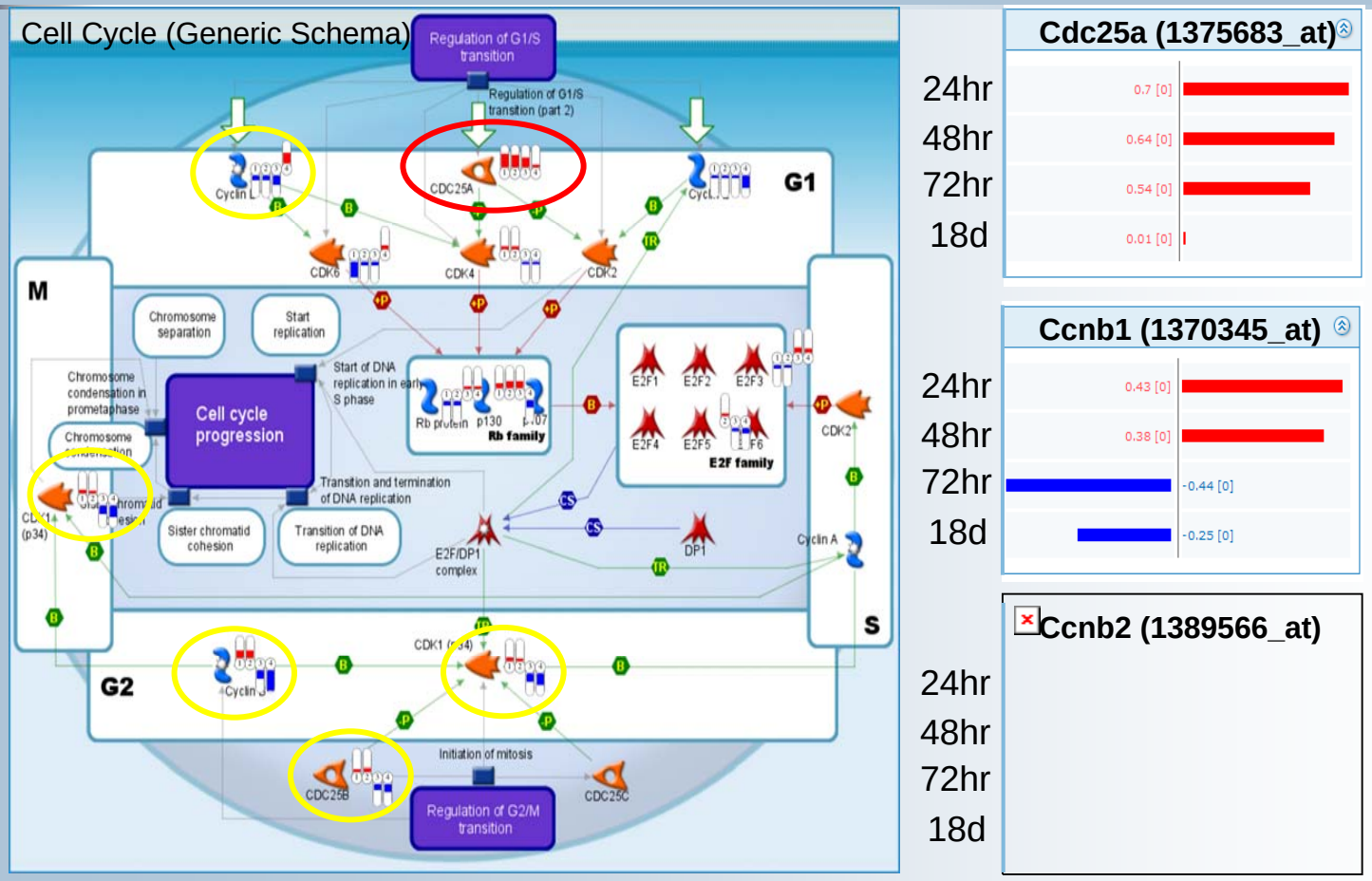


Both Cyp3A and Cyp2B are upregulated by Phenobarbital in vivo and in 3D cultures
Most doses tested upregulate both genes at least 2-fold or more
18 day 3D cultures show downregulation similar to that reported in vivo

RegeneMed

Fisher 344 Male Rat
RegeneMed 3D InVitro and InVivo
DrugMatrix InVivo (Sprague Dawley)
Rat 230 2.0 data
Mas5 Processed data
Log10 ratios shown

Phenobarbital upregulates genes in the cell cycle at early time points but downregulates them at later time points in 3D liver cultures



3D rat male liver cultures upregulate Cdc25a and Ccnb at 24 and 48 hours, but are unchanged or downregulated at 18 days, as shown in the cell cycle pathway and bar graphs from GeneGo. Red = upregulation; Blue = downregulation

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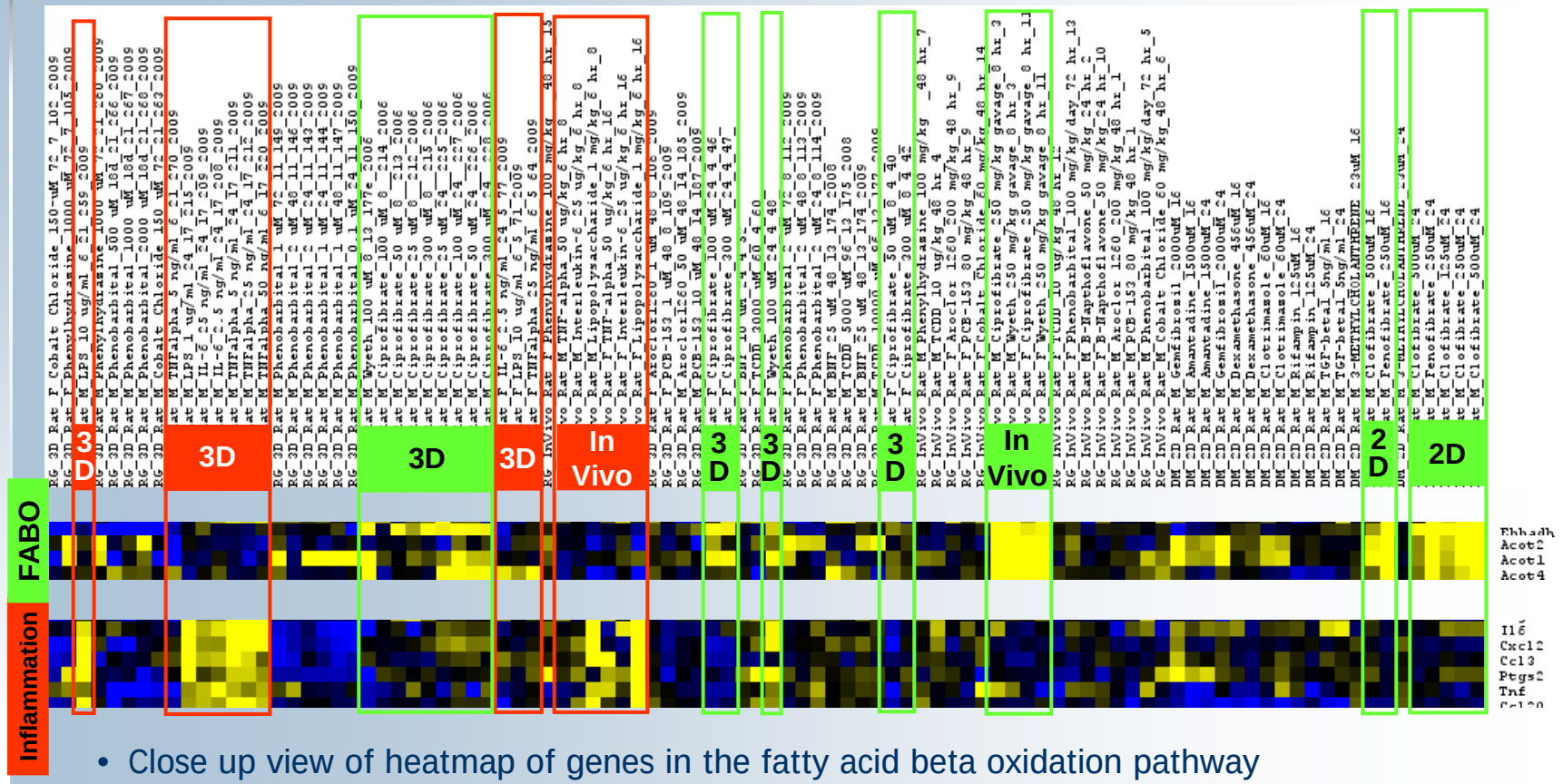
PPAR Activation vs. Inflammation in 3D in vitro cultures and in vivo liver



- Close up view of heatmap of genes in the fatty acid beta oxidation pathway (PPARalpha treatments outlined in green).
- Close up view of heatmap of genes upregulated during inflammation (Inflammatory treatments outlined in red).

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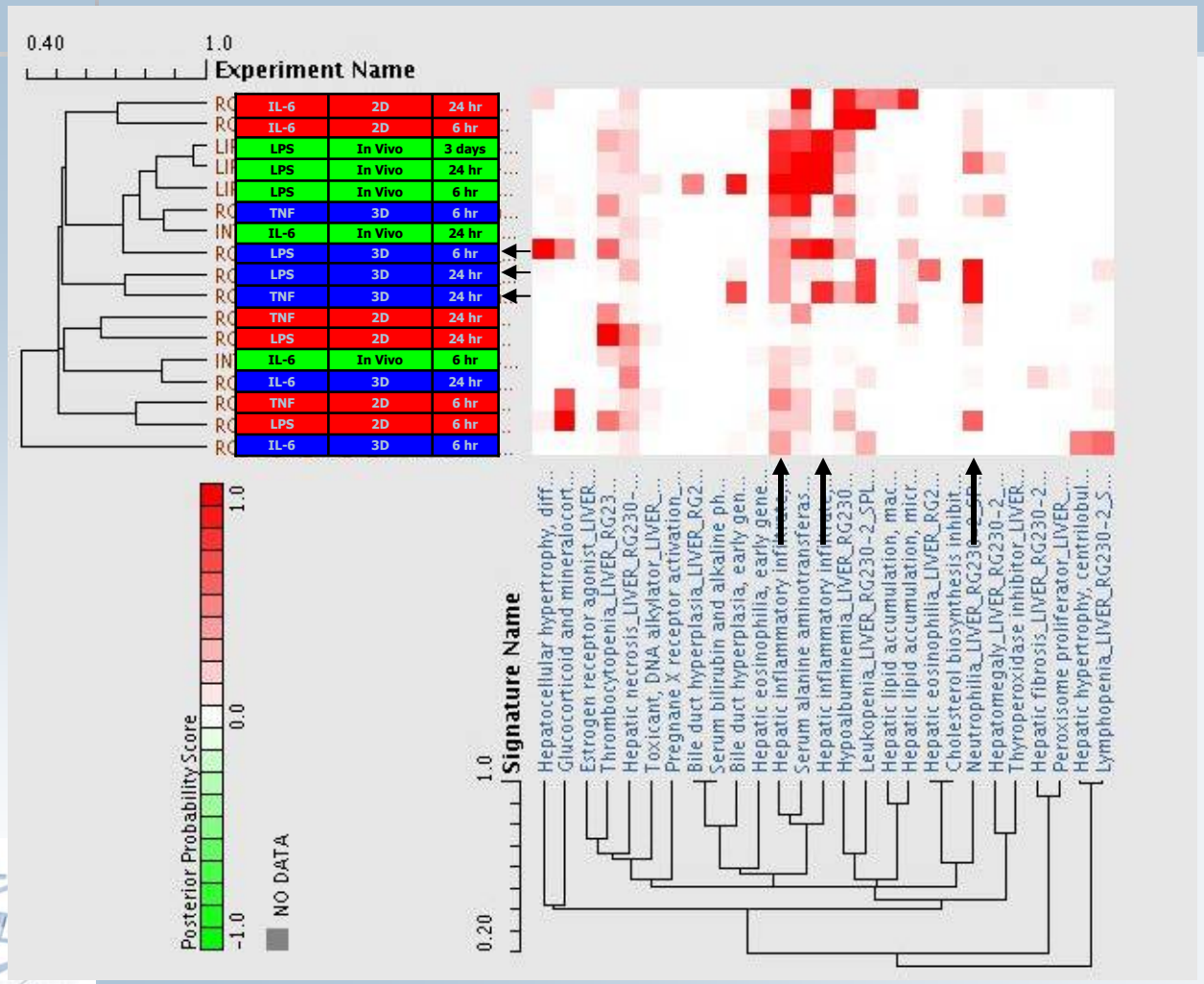
3D cultures treated with LPS and TNFα match inflammatory signatures from DrugMatrix



- 2D cultures treated with same compounds have no or weak matches
- 6 hr treatments tend to have stronger matches than 24 hr treatments
- LPS and TNFalpha elicit stronger responses than IL-6 both in vivo and in 3D cultures

- DrugMatrix signatures derived from in vivo gene expression data
- Red color indicates magnitude of match; posterior probability score
- Arrows indicate inflammatory signatures and 3D treatments that match these signatures

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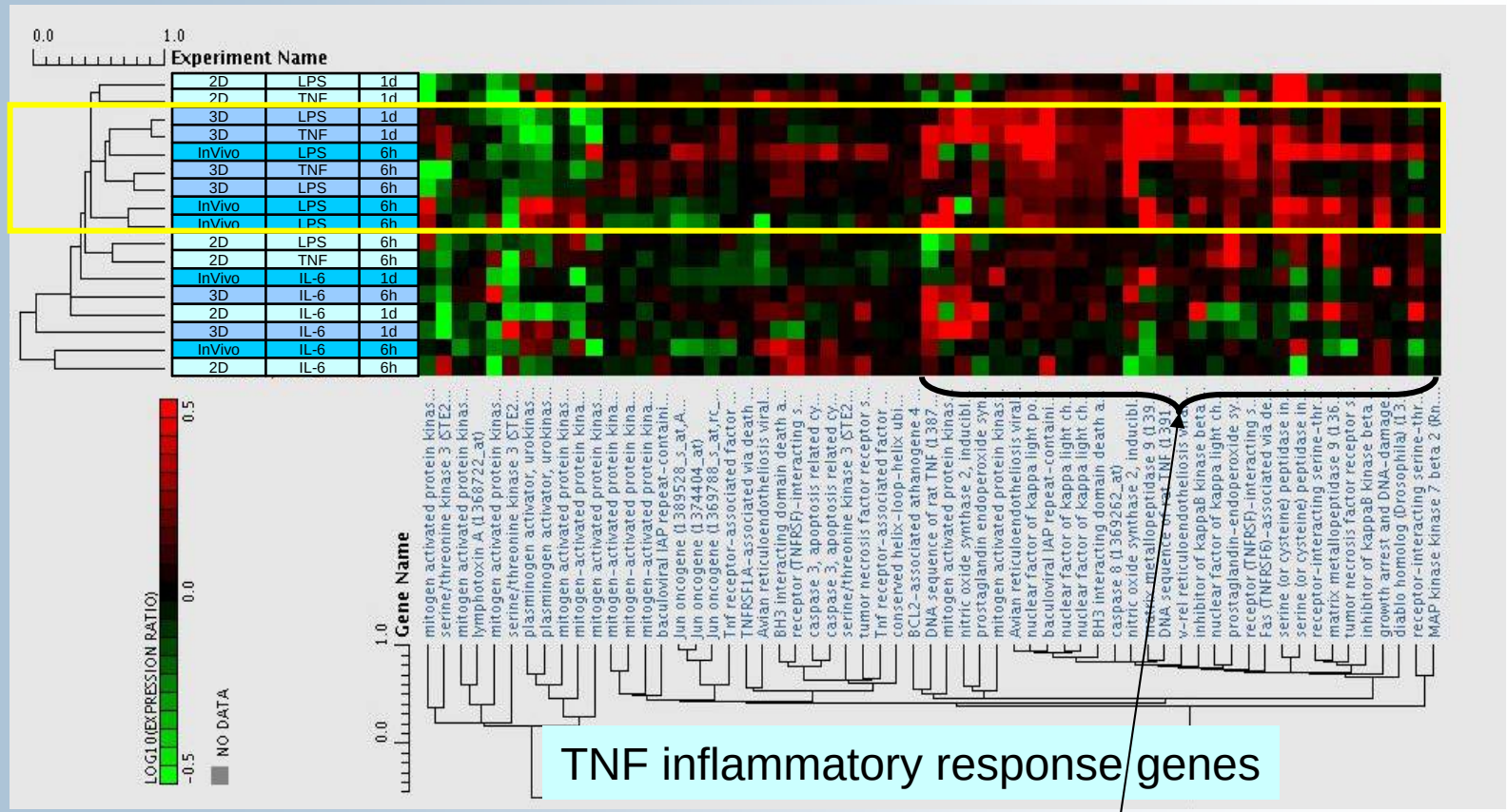
3D in vitro inflammatory treatments are most similar to 6 hr in vivo treatments across the TNF inflammatory response pathway genes

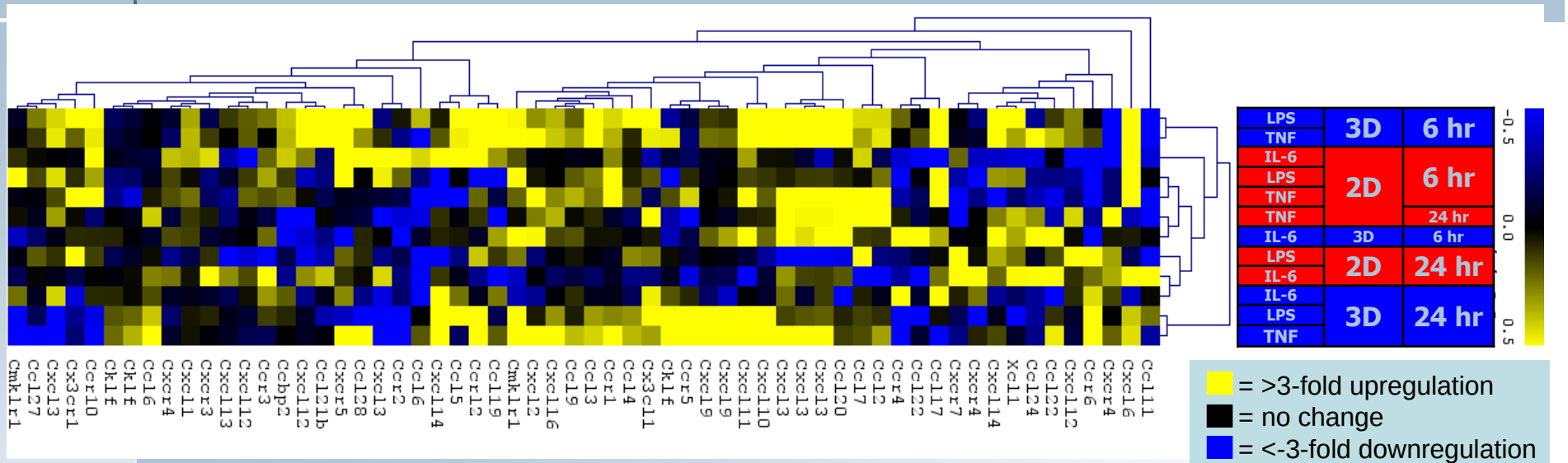


- Similar upregulation between 3D and in vivo LPS and TNF treatments
- 2D LPS and TNF treatments less intense

Log10 ratios shown for chemokine genes on Affy Rat 230 2.0 GeneChip
Two dimensional hierarchical clustering
Sprague Dawley male rat
Rat 230 2.0 data processed with Mas5

RegeneMed

Chemokine genes are upregulated more consistently in 3D cultures than in 2D



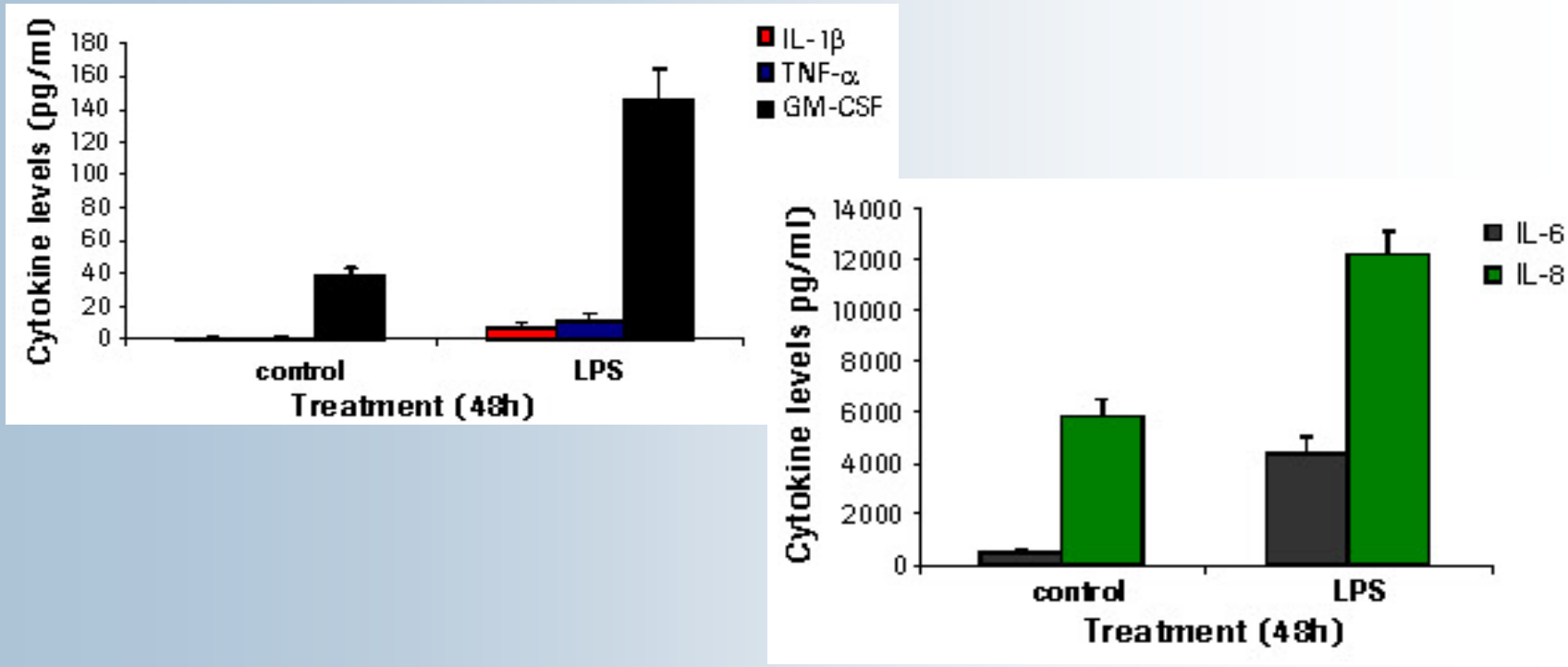
- 2D cultures upregulate a subset of the chemokines that 3D cultures upregulate
- 6 hr time point has most robust response in 3D LPS and TNFalpha cultures
- IL-6 treatment is least robust in 3D cultures

RegeneMed

Log10 ratios shown for chemokine genes
on Affy Rat 230 2.0 GeneChip
Two dimensional hierarchical clustering
Sprague Dawley male rat
Rat 230 2.0 data processed with Mas5

Human 3D liver co-culture response to inflammatory stimuli by release of cytokines

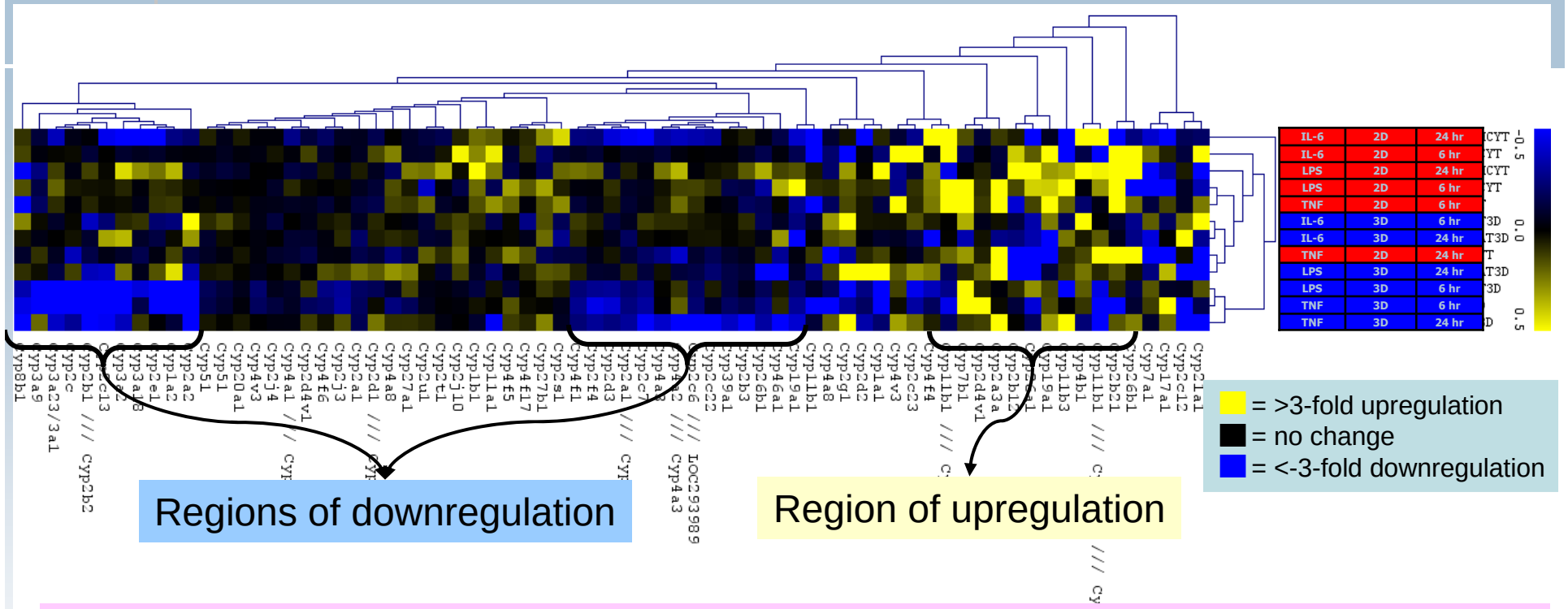
Hoffmann-La Roche AG, Basel



Release of inflammatory cytokines in the medium upon treatment with 10ug/mL LPS in 1-month-old human 3D liver co-cultures

RegeneMed

3D cultures tend to downregulate P450 genes in response to inflammatory agents

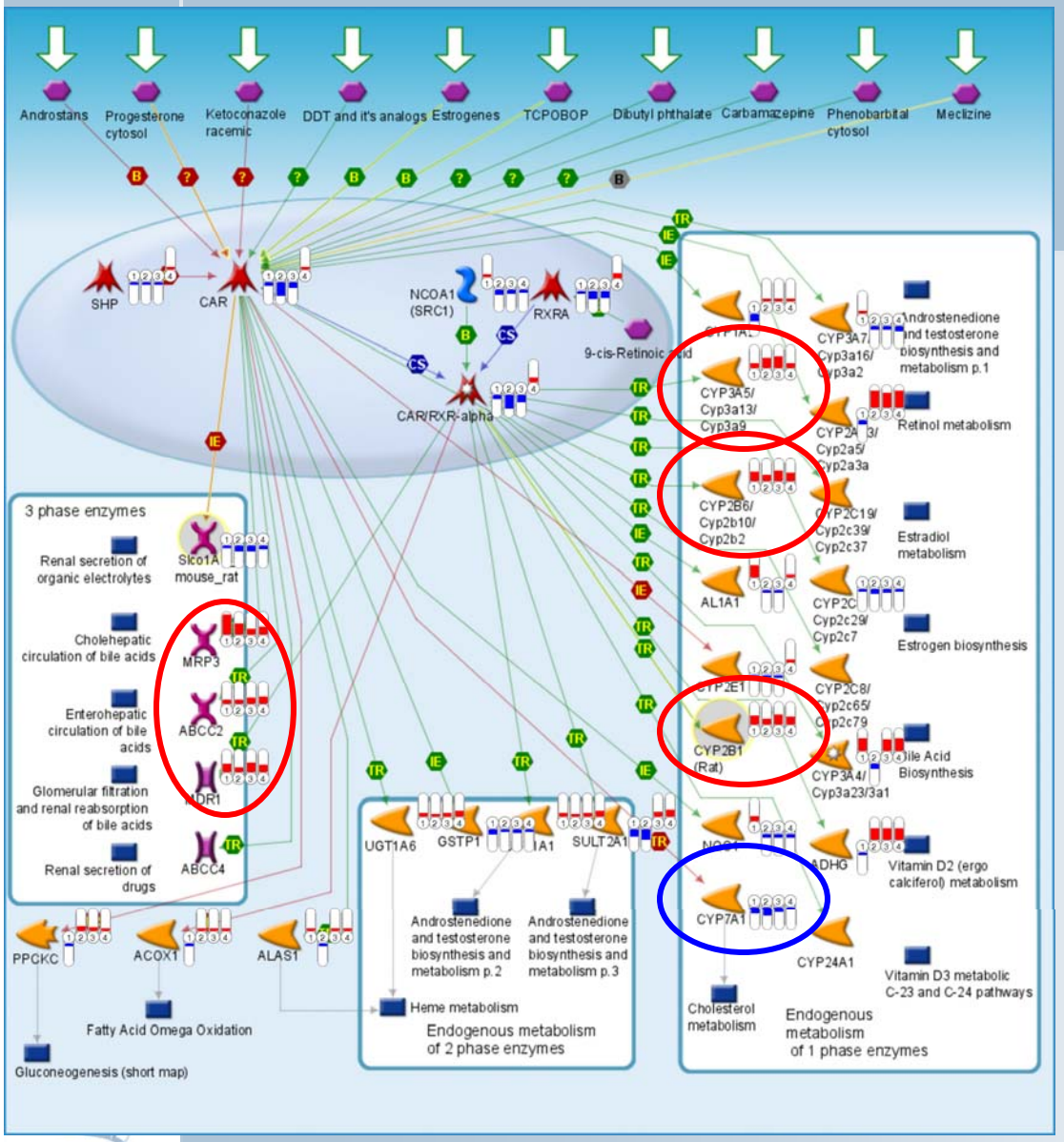


- 2D cultures also downregulate P450 genes but only in response to IL-6 at 24 hr
- Some P450s are upregulated, mainly by 2D cultures but not as much by 3D cultures

RegeneMed

Log10 ratios shown for P450genes on Affy Rat 230 2.0 GeneChip
Two dimensional hierarchical clustering
Sprague Dawley male rat
Rat 230 2.0 data processed with Mas5

Phenobarbital *In Vitro* and *In Vivo* – CAR Activation



Experiments

1. (1) LR_InVivo_Rat_Male_Phenobarbital_100 mg/kg/day_72 hr_5
2. (2) LR_InVitro_Rat_Male_Phenobarbital_1 uM_24_11_144_2009
3. (3) LR_InVitro_Rat_Male_Phenobarbital_1 uM_48_11_147_2009
4. (4) LR_InVitro_Rat_Male_Phenobarbital_1 uM_72_11_149_2009

Cyp2b1(1371076_at)

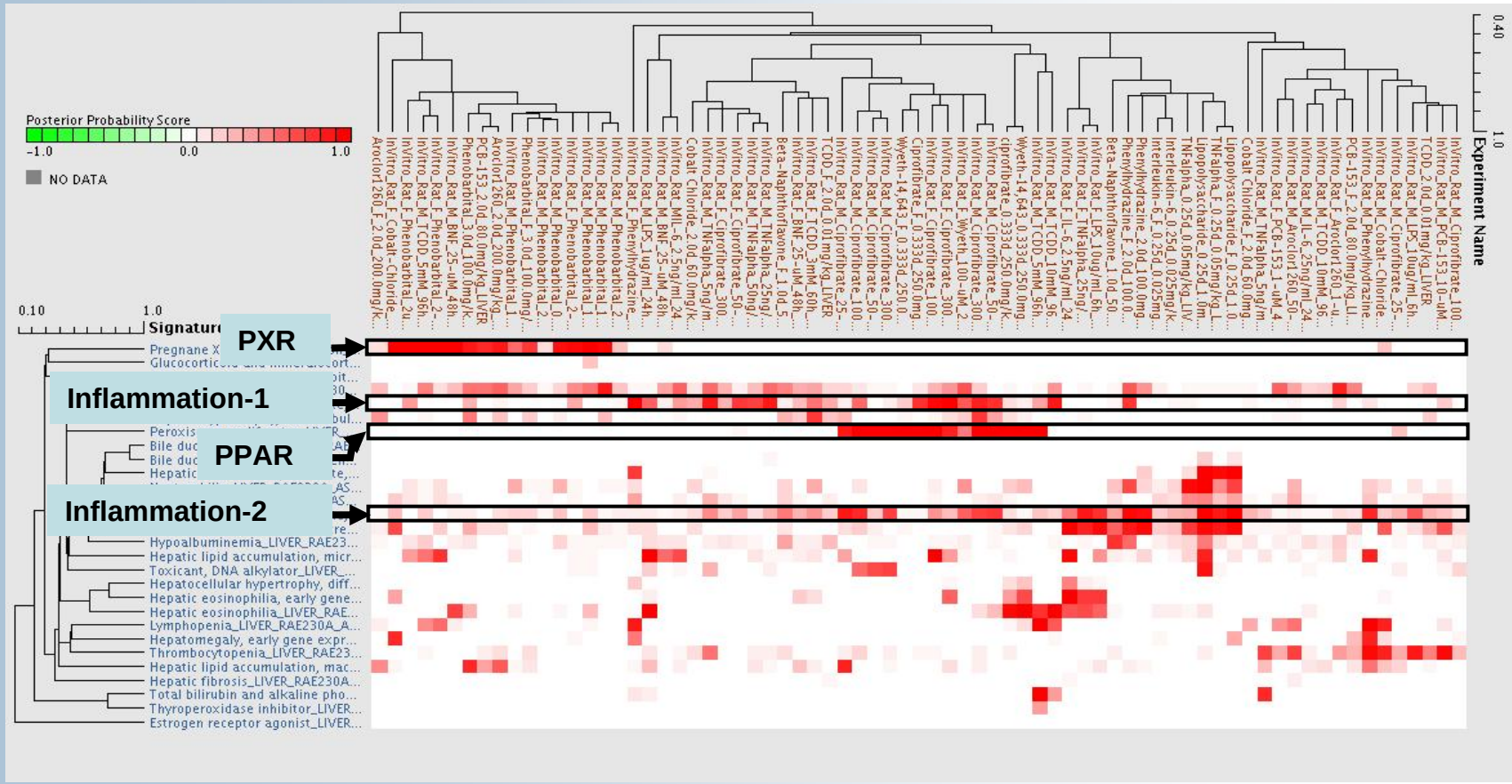
1: (1) LR_InVivo_Rat_Male_Phenobarbital_100 mg/kg/day_72 hr_5	0.67 [0]
2: (2) LR_InVitro_Rat_Male_Phenobarbital_1 uM_24_11_144_2009	0.44 [0]
3: (3) LR_InVitro_Rat_Male_Phenobarbital_1 uM_48_11_147_2009	0.81 [0]
4: (4) LR_InVitro_Rat_Male_Phenobarbital_1 uM_72_11_149_2009	0.65 [0]

- **Unfiltered data**

RegeneMed

3D in vitro treatments match DrugMatrix Drug Signatures

3D liver mimics *in vivo* transcriptional profiles



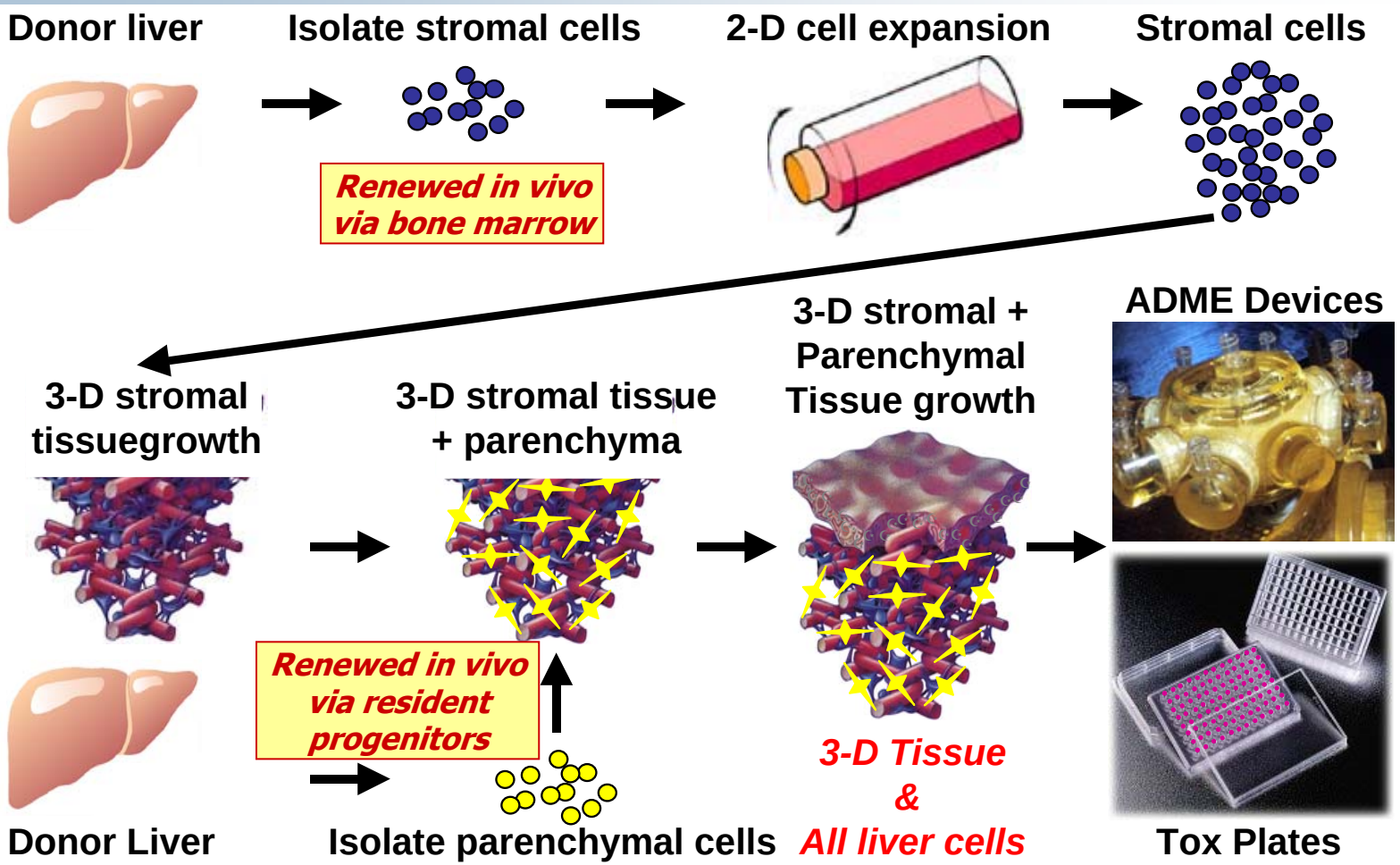
All RegeneMed 3D in vitro and in vivo experiments were scored against a panel of DrugMatrix Drug Signatures (biomarkers for specific pathologies). The posterior probability scores for each treatment and signature combination were clustered together. A score above 0.5 (red color) is considered a positive match.

RegeneMed

Stem Cells for 3-D Liver Tissue

Liver Tissue Co-Culture Growth Process

Progenitor and Stem Cell Sourcing for Every Cell Type of adult phenotype with full cell function



30 pharma equivs * 100 systems/day * 260 days/year = 780,000 systems/yr

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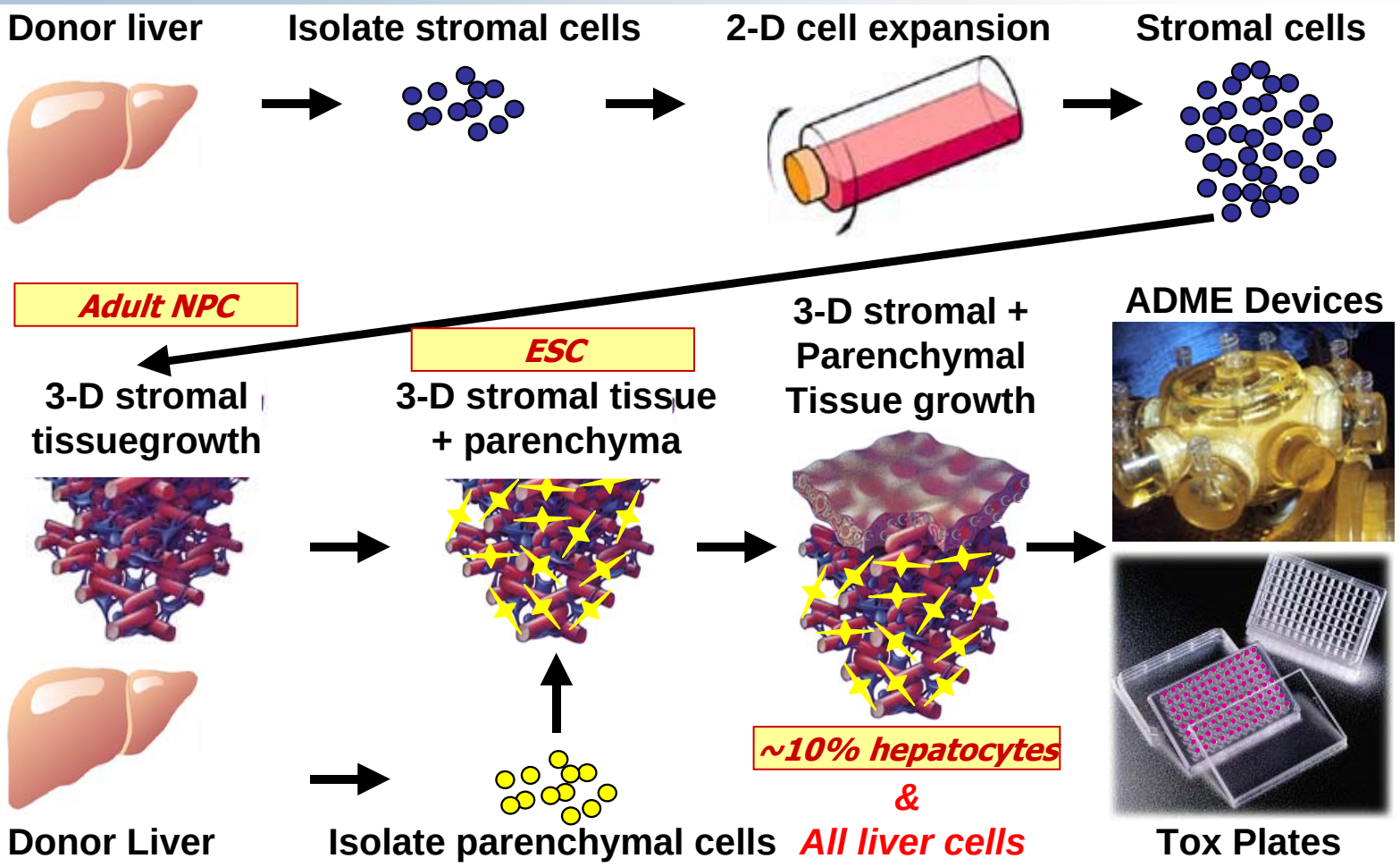

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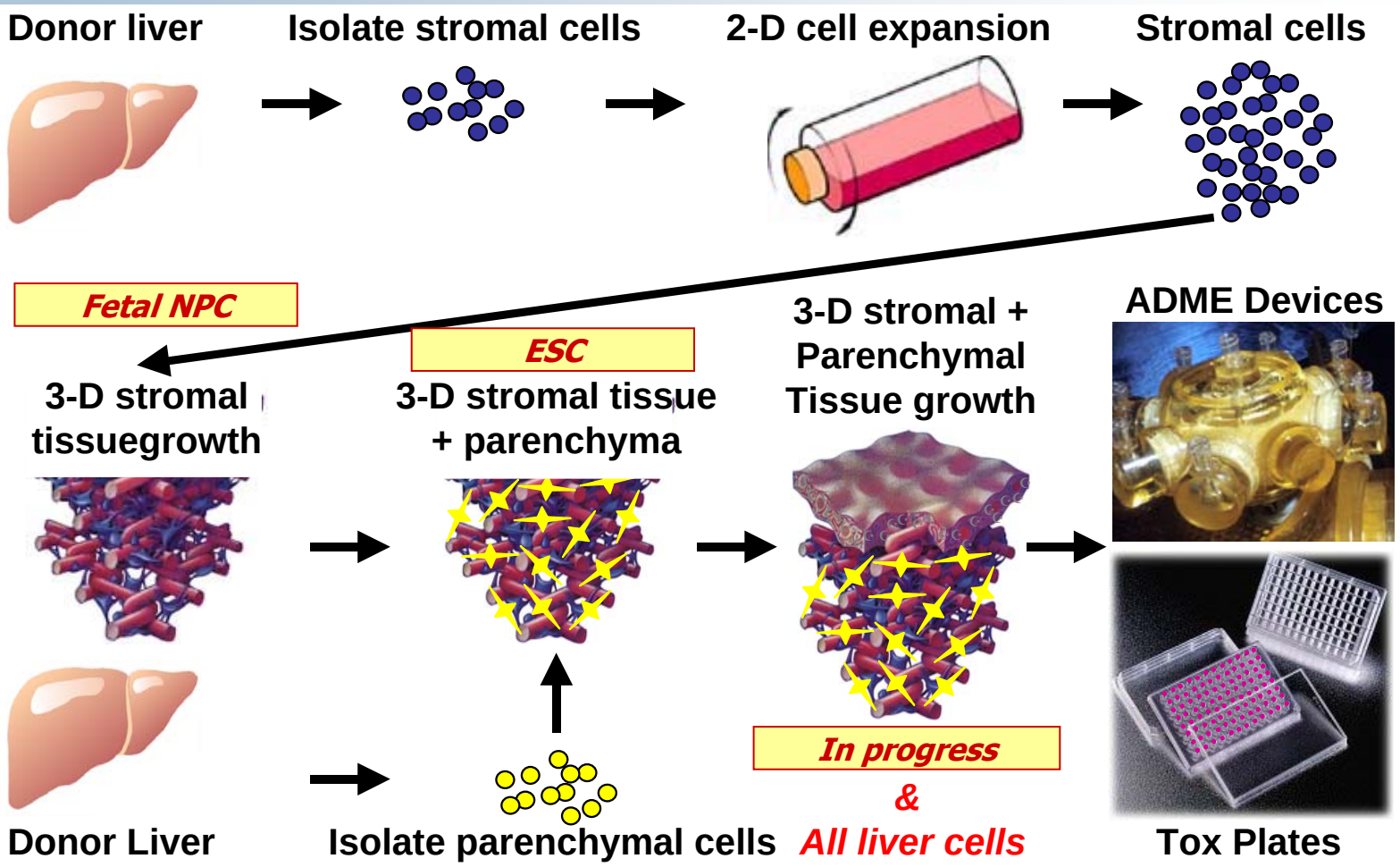

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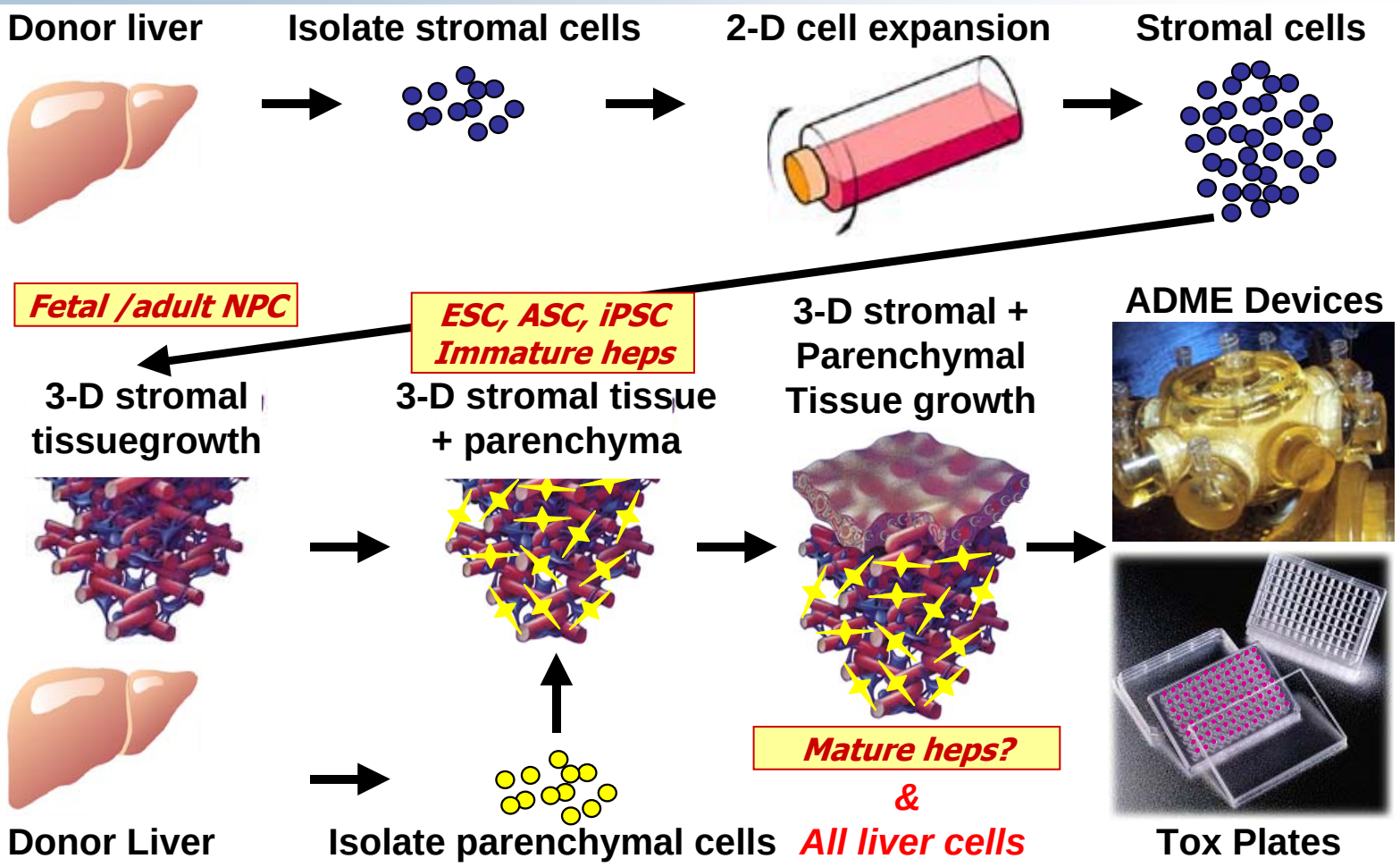
RegeneMed

Stem Cells for 3-D Liver Tissue

Progenitor and Stem Cell Sourcing for Every Cell Type of adult phenotype with full cell function

3D for cell expansion & maintenance of differentiated function



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Summary

3D *in vitro* human predictive models

- **Multicellular cell/tissue systems (with ECM, structure)**
- **Support primary human cell system optimization; stem cells next gen**
- **Acceptance of cell/tissue variability vs chem/bio assays**
  - ToxCast FDA pres: 19 human primary cell assays were more predictive than 100,000 chem/bio/transformed cell-based assays
- **Real-time, minimally invasive, in vivo-translated assays**
- **Complex endpoints (pathways, signatures, GWAS/epi)**
- **New approaches/assays (chronic, disease progression, mechanism)**
- **Better integration of databases (industry, NIH, FDA, etc.)**
- **More work on translation of animal to human data**
- **Insilco models with human primary cell/tissue data**
- **Balance academic complexity with industrially deployable**
  - Static vs flow; multiwell plate vs chips; standard workflow integration
- **Design for industry state-of-the art for rapid adoption**
- **Predictive focus before lower cost, higher throughput**
  - 10% of pharma expenditures for R&D, 10% of that for preclin dev.
  - $1M/day lost opportunity cost due to clinical toxicity failures
- **Industrial Leaders/1st Adopters OUS, how to change**
- **Regulation integration (ICCVAM, ECVAM, OECD, FDA, Reach) and accelerated pathway to animal replacement**

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