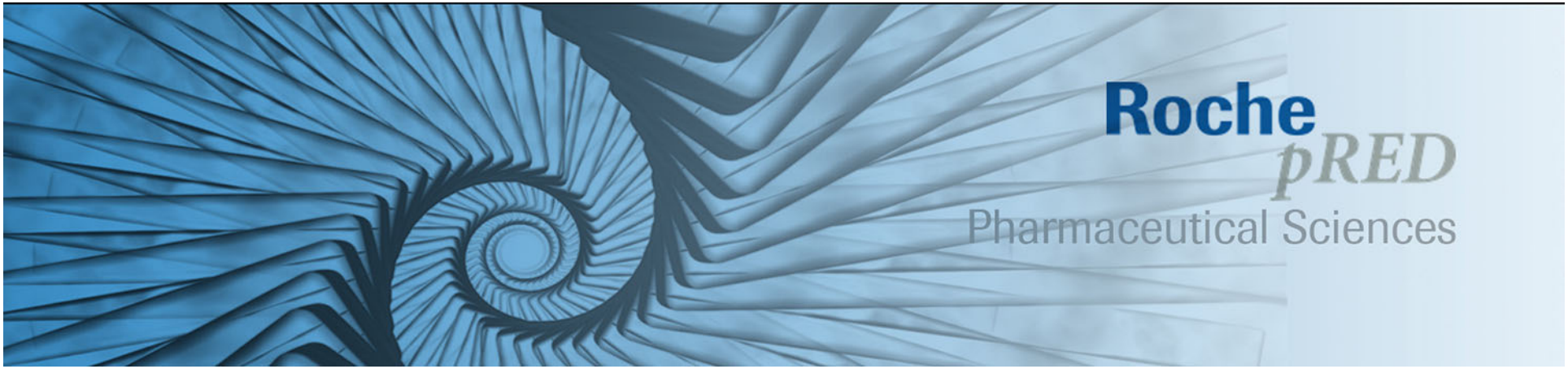
Advanced cell models, organs on a chip & microphysiological systems in drug development:

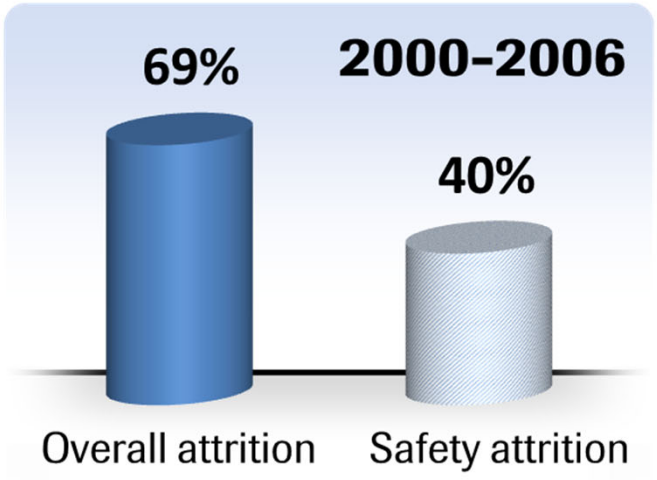
the need, the vision – and challenges to overcome

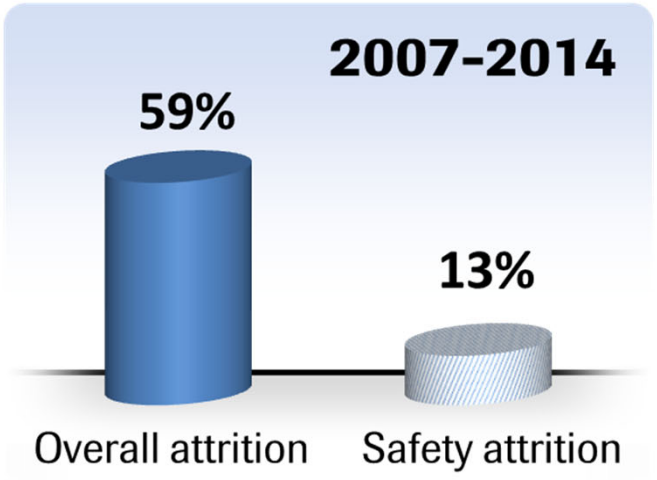
PD Dr. Adrian Roth
Head Mechanistic Safety
Dept DDS, Roche Innovation Centre Basel, Switzerland



Advanced cell models in pre-clinical safety

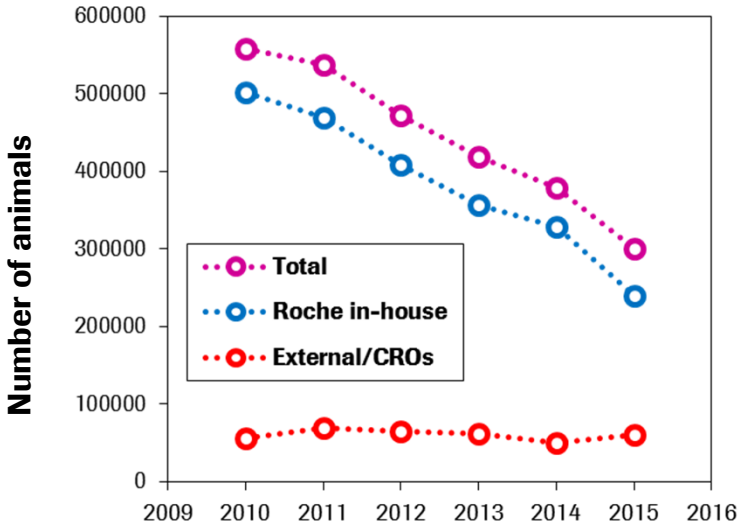
Reducing animal numbers - increasing patient's safety





→ **Human in vitro models to**

- reduce attrition rate due to species-specificities.
- reduce pre-clinical animal testing



Drug Safety assessment:

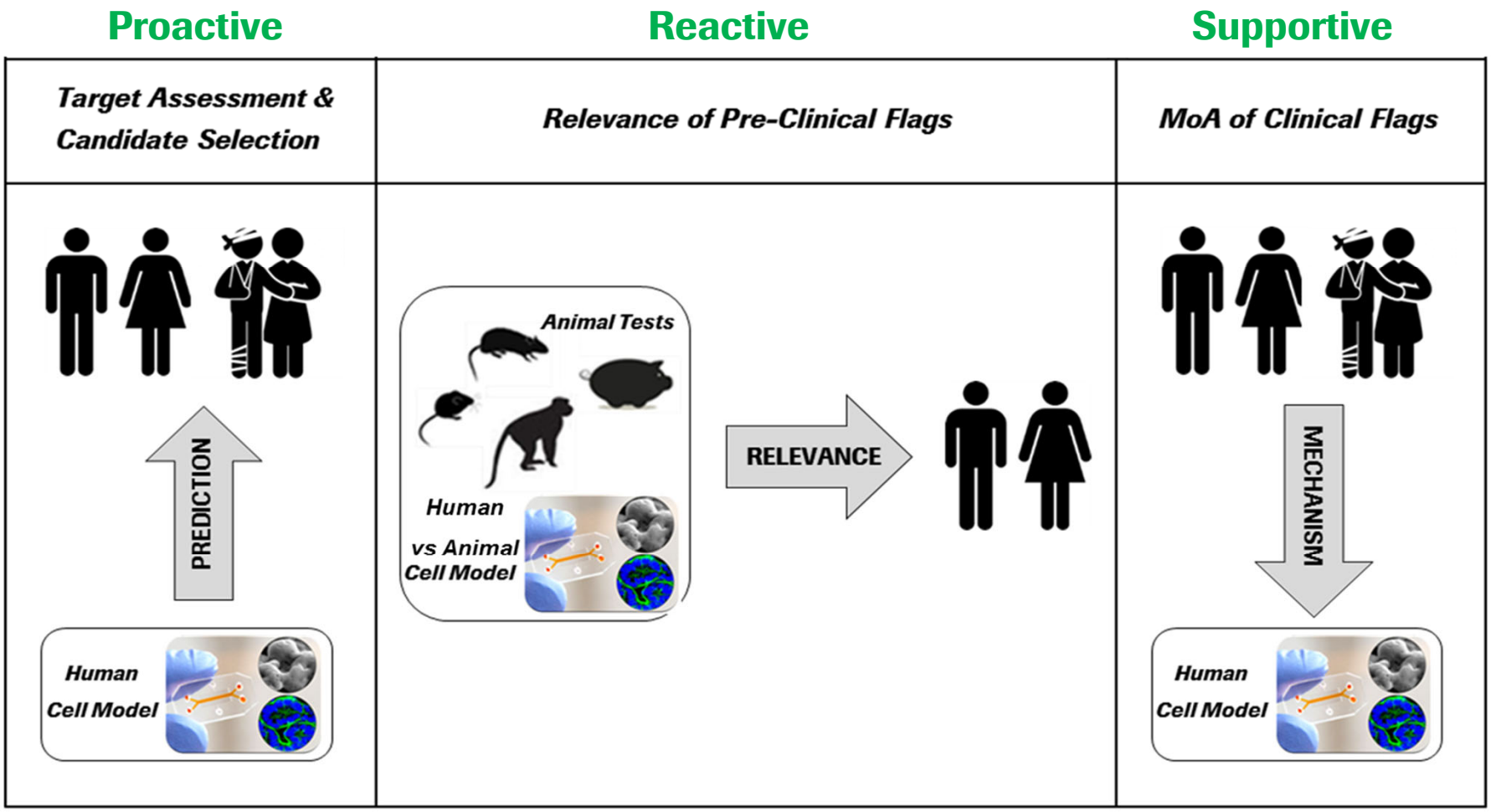
Why we need better in vitro models

Toxic Concentrations (µM) *in vivo* and *in vitro*

Drug	Plasma Concentration (Rat In Vivo)	2D Hepatocytes	3D Hepatocytes	Reference
Azathioprine	1	1	1	Wu et al., 2006
Chloroquine	2.5	5	2.5	Shen et al., 2008
Clozapine	4	30	5	Lu et al., 2008
Amiodarone	5.4	10	5	Shen et al., 2008
Rifampicin	12	600	12	Shen et al., 2008
Tetracycline	26	100	25	Shen et al., 2008
Isoniazid	30	1100	33	Shen et al., 2006

(Q. Meng ,Zhejiang Zhejiang University CHI)

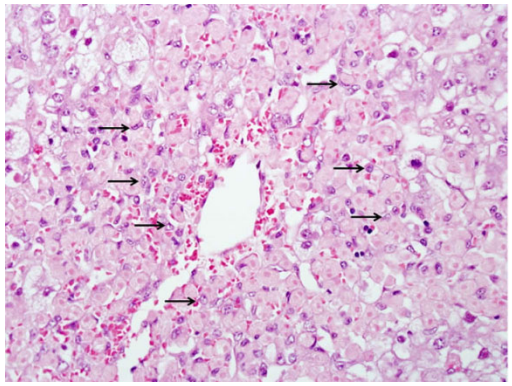
Where *in vitro* assays matter in drug safety today



1. **Predictive screens**
2. **Address human relevance of pre-clinical *in vivo* findings**
3. **Assess mode of action of clinical findings**

Major challenge for safety prediction:

Organ toxicity, long term effects, human relevance



- **Complex in nature – develops over longer time**
- **Involves multitude of factors & interplay of different cell types**
- **Often displays species dependency**

→ ***Difficult to address in vitro!***

ToxCast:

Initiative to predict in vivo endpoints of toxicants by use of high throuput screening assays

> 300 Chemicals

> 600 in vitro HT assays

"…..the overall predictive power of the in vitro assays was relatively low…."

Thomas et al., Tox Sci 128(2), 398–417 (2012)

«…These events are seldom recapitulated in molecular detail, kinetics, dynamics or cellular metabolic processing in simplified in vitro models (…) no in vitro model completely mimics all complexities of (…) organ toxicity in vivo…"

(Astashkinaa et al., Pharmacology & Therapeutics Volume 134, Issue 1, April 2012)

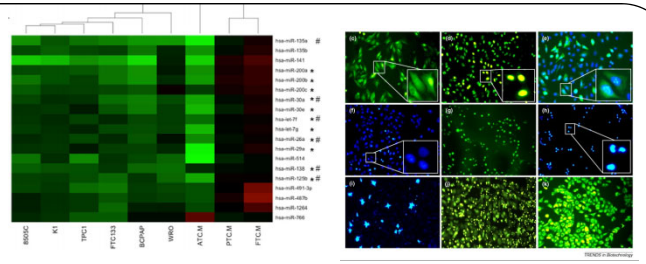
Improving in vitro prediction of safety liabilities

Areas of investment over the past decade

1) Apply molecular tools to *in vitro* tests

Pattern approach

Complex readouts which capture multiple/all genes, proteins, pathways

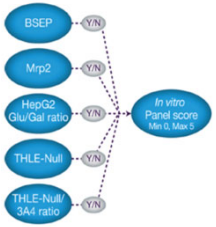


'Omics, High content imaging

2) Combine existing *in vitro* assays

Targeted approach

Combination of specific assay-data

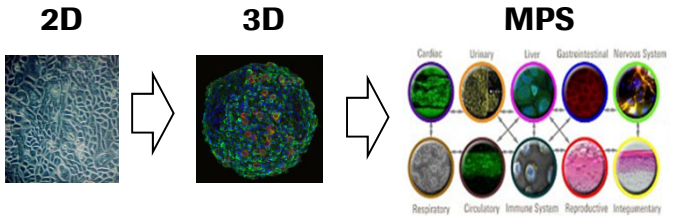


Integrated safety score

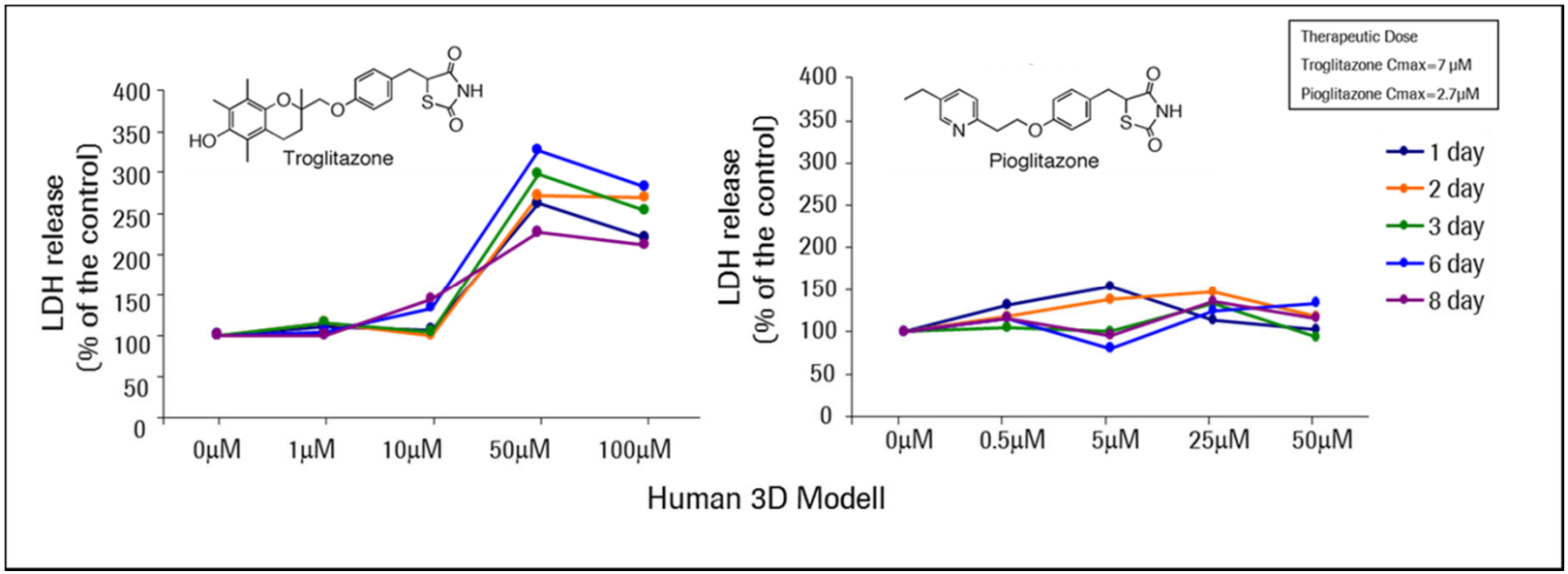
3) Improve cell models

Holistic approach

models which display in vivo-like functionality over prolonged time



Advanced models & drug-induced liver toxicity as an example



TOXICITY	DRUG	IN VIVO ISSUE	3DL RAT	3DL HUMAN	CORRECT ?
'Classic' hepatotoxicant	Ketoconazole	Rodent and human liver tox	Toxicity	Toxicity	✓
Metabolism-	Acetaminophenol	Nontoxic marketed drug	No effect	No effect	✓
related	Acetominophen	Nontoxic marketed drug, toxicity with overdose	Toxicity	Toxicity	✓
Species-	Fenofibrate	Rodent liver tox, tumors	Toxicity	No effect	✓
specific	Troglitazone	Human liver tox	No effect	Toxicity	✓
toxicity	Pioglitazone	Nontoxic marketed drug	No effect	No effect	✓
idiosyncratic	Trovafloxacin	Human liver tox	No effect	Toxicity	✓
idiosyncratic	Levofloxacin	Nontoxic marketed drug	No effect	No effect	✓
idiosyncratic	Entacapone	Nontoxic marketed drug	No effect	No effect	✓
idiosyncratic	Tolcapone	Human liver tox	No effect	Toxicity	✓
idiosyncratic	Perhexiline	Human liver tox	No effect	Toxicity	✓
idiosyncratic	Isoniazid	Human liver tox	No effect	Toxicity	✓

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

A long-term three dimensional liver co-culture system for improved prediction of clinically relevant drug-induced hepatotoxicity

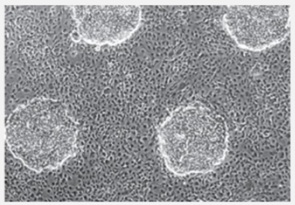
Radina Kostadinova [a], Franziska Boess [a], Dawn Applegate [b], Laura Suter [a], Thomas Weiser [a], Thomas Singer [a], Brian Naughton [b], Adrian Roth [a,*]

[a] Non-Clinical Safety, Hoffmann-La Roche Ltd, Grenzacherstrasse 124, Building 73 / Room 117b, 4070 Basel, Switzerland
[b] RegeneMed, 9855 Towne Centre Drive Suite 200, San Diego, CA 92121, USA

Advanced Liver Cell Models today

Different approaches – still room for improvement ?

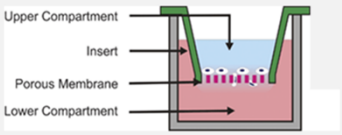
2D Micropatterned Co-Cultures



- **Ability for longterm Incubation increases Predictivity for DILI**

Khetani et al.; Toxicol Sci (2013) 132 (1): 107-117.

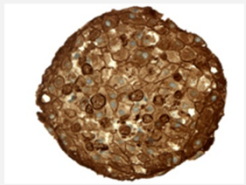
3D Transwell Co-Cultures



- **Different Cell Types contributing to toxic Effect**

Kostadinova et al.; Toxicol Appl Pharmacol. 2013 Apr 1;268(1):1-16

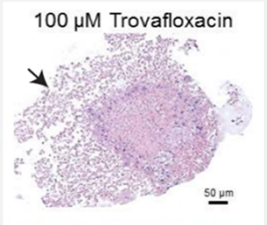
3D Spheroids



- **High Complexity in Semi-HT Format**

Messner et al.; Arch Toxicol. 2013 Jan;87(1):209-13

3D Bioprinted



- **Large tissue allows assessment of morphological changes**

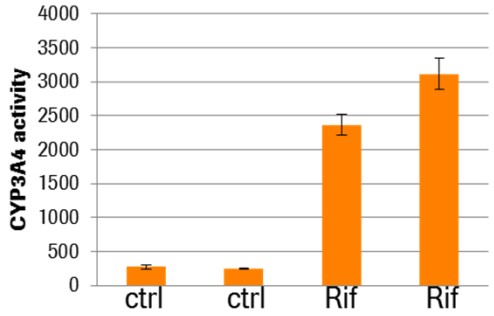
Ngyuen et al., PLoS One. 2016 Jul 7;11(7)

3D Models for Liver – Lessons learnt (1)

"Pushing" a cell system into specific direction may create a highly artificial model

Same lot of human hepatocytes used to measure CYP3A4 activity under 3 different conditions

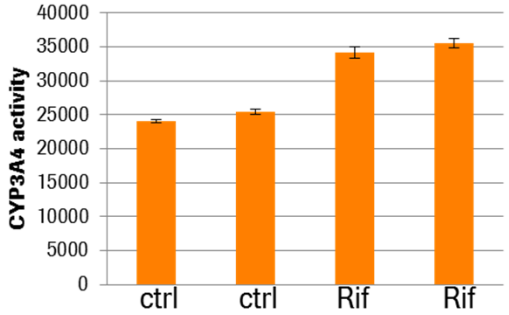
3D model Medium from Vendor 1



Low basal activity

Robust inducibility

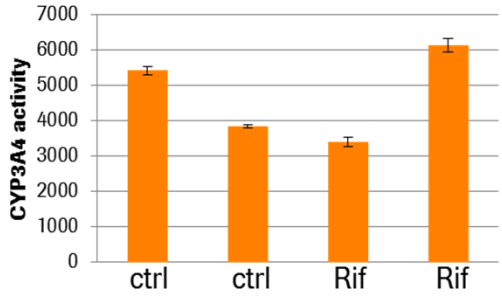
3D model Medium from Vendor 2



Very high basal activity

low inducibility

3D model William's E Medium



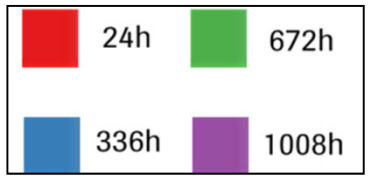
high basal activity

no inducibility

- 7 day old human hepatocyte culture
- CYP3A4 enzyme activity measured using Promega's P450-Glo assay

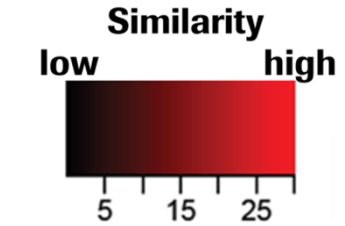
3D Models for Liver – Lessons learnt (2)

Longterm multicellular systems are dynamic



Comparison of gene sigantures at 1day, 2-, 4- & 6-weeks:

Benchmark against reference genes from human tissues



Roth & Singer, Adv Drug Deliv Rev. 2014 Apr;69-70:179-89

3D Models for Liver – Lessons learnt (3)

Microfluidic devices and non-specific binding

Drug binding to microfluidic device to assess likelihood of non-specific binding affecting drug clearance measurements

Compound	initial	inlet 24h		outlet 24h	
bufuralol	100	76	100	1	4
amitriptyline	100	33	64	BLQ	BLQ
benzydamine	100	46	77	BLQ	BLQ
nifedipine	100	8	11	3	2
trifluoperazine	100	6	7	BLQ	BLQ
dextromethorphan	100	70	100	BLQ	BLQ
raloxifene	100	8	10	11	12
amodiaquine	100	35	41	1	1
verapamil	100	26	23	BLQ	BLQ
chlorzoxazone	100	36	40	16	15
warfarin	100	84	118	114	80
tolbutamide	100	94	79	67	65
midazolam	100	53	29	BLQ	BLQ
S-mephenytoin	100	96	63	36	50
ethoxyresorufin	100	35	41	BLQ	1

- Compounds submitted in duplicate to inlet wells of microfluidic device.
- Concentrations of remaining substance in inlet chamber and that which flowed through to outlet chamber assessed 24h later
- **BLQ=Below Limit of Quantitation**

N Kratochwil / S Fowler

Almost all of the test compounds showed high non-specific binding which needs to be overcome before device can be used for DMPK applications

3D Models for Liver – Lessons learnt (4)

Improved physiological relevance does not automatically lead to improved predictivity

Reference Drug Pairs tested in 2D vs 3D (rat & human)

DRUG	CLASS	2D rat	2D human	3D rat	3D human
AMAP	Non-tox	>100	>100	>100	>100
APAP	«Tox»	>100	>100	>100	>100
CLOZAPINE	Rare Tox	38.9	42.5	29.6	30.4
LEVOFLOXACIN	Non-Tox	>100	>100	>100	>100
TROVAFLOXACIN	Tox	>100	>100	71	85.8
CIPROFLOXACIN	Non-Tox	>100	>100	>100	>100
TROGLITAZONE	Tox	72.3	73	>100	3.1
PIOGLITAZONE	Non-Tox	>100	>100	>100	>100
AMODIAQUINE	Idiosyncr Tox	25.4	27.1	9.3	4
CYCLOPHOSPHAMIDE	Idiosyncr Tox	>100	>100	>100	>100
METHOTREXATE	Rare Tox	>100	>100	>100	>100
KANAMYCIN	Non-Tox	>100	>100	30.3	>100
ENTACAPONE	Non-Tox	13.5	>100	>100	65.4
AZATHIOPRINE	Tox	3.8	8.6	9.3	1.9
CARBOPLATIN	Non-Tox	62.5	>100	31.9	>100
DEBRISOQUINE	Non-Tox	37.4	22.2	73.2	5.5
FLUCLOXACILLIN	Idiosyncr Tox	>100	>100	>100	>100
ISONIAZID (RIMIFON)	Idiosyncr Tox	>100	>100	>100	>100
LOSARTAN	Rare Tox	>100	>100	>100	>100
PUROMYCIN	Non-Tox	2.1	2.1	6.9	1.0

IC50 LDH (uM)

2D hepatocyte cultures: 48h , 2x treatment

3D cultures: 8days, 5x treatment

F Boess, Hoffmann-La Roche

- **3D not always an improvement**
- **Species specificity not always reflected**
- **Tox sometimes seen in all in vitro systems – and sometimes in none**

3D and other advanced cell models in Drug Development: What is important ?

- **Thorough Validation addressing key aspects**
  - Unspecific Drug Binding (!)
  - Key Functions of organ to be represented in vitro
  - Stability of the model over time
  - Gain in predictivity vs price for complexity

- **General challenges of *in vitro* systems & safety prediction remain**
  - In vitro conc & clinical exposure
  - Drug-related factors vs patient-related factors
  - Acute effects vs rare clinical events (idiosyncartic)

Our approach

The question defines the choice of the cell model

Ease of use & throughput
Compound ranking
Candidate selection

Complexity & functionality
Unknown MoT
Longterm effect

Tissue cross-talk, PK/PD aspects (?)
Unknown MoT
Known, complex MoT
Metabolites
Organ-Organ interaction

Address specific known mechanism

Generate hypothesis - resolve unexplained issue

Where advanced tissue models can win

- **<u>«General» target organs of toxicity</u>**
  - Liver, Cardiac, Neuro…
- **<u>Barrier systems</u> (Vascular, Kidney, Gut, Retina, BBB)**
  - Barrier intergity – leaktightness
  - Directional Transport , Disposition
- **<u>Connecting/combining organ systems</u>: 2,3,4,….Body on a chip**
  - Liver+: Liver-Kidney, Liver-Gut, Liver-Bone marrow
  - Vascularized tissue: Endothel-Cardiac, Endothel-gut
  - Tumor/Non-tumor: Tumor killing vs off-tumor killing
  - …..
- **<u>Incoporate immune component</u>**
  - Non-parenchymal cells in Liver
  - «Blood»-tissue co-culture
  - Tissue infiltration of immune cells
  - …..

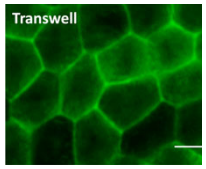
Example: Gut Models

From Transwell to 3D to Microfluidic "Gut on a chip"

CACO2 culture on Transwells

⊕ Well-characterized colonocyte cell line with brush border formation and transporter expression. Form a tight epithelial barrier on Transwell filte

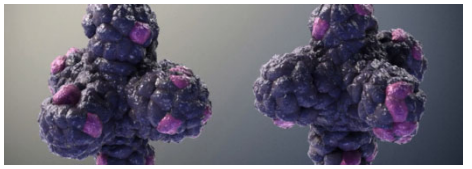
⊖ Limited physiological relevance of cell line



"Mini-gut" Organoids

⊕ Intestinal stem cells expand and form a polarized epithelium comprising all cell types

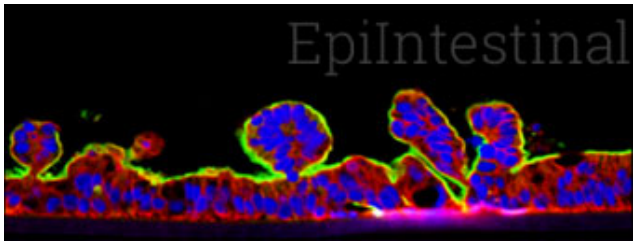
⊖ 'closed' lumen - static



Primary cells in 3D

⊕ Incorporates enterocytes, paneth cells, M cells, tuft cells and intestinal stem cells. Off the shelf product

⊖ Static model, cannot culture with e.g. PBMCs

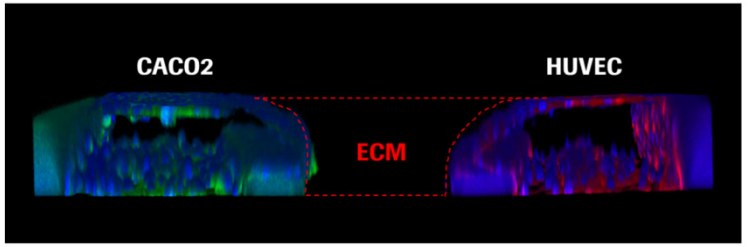


MatTek EpiIntestinal

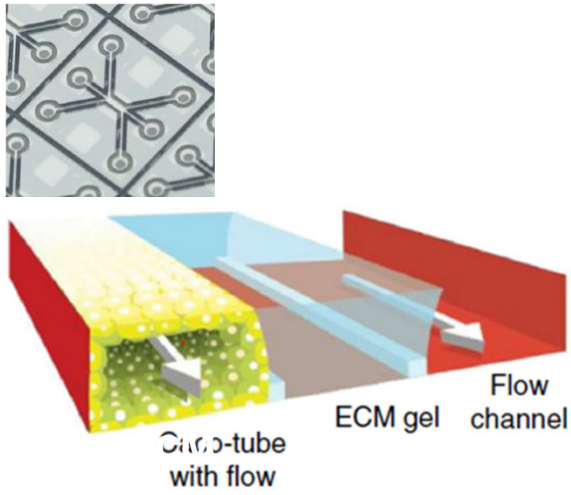
Multicellular, 3D microfluidic system

⊕ Possibility to administer drug to intestine apically in 'lumen' or baso-laterally via 'blood vessel' (or cell-free channel)

⊖ Thickness of ECM matrix



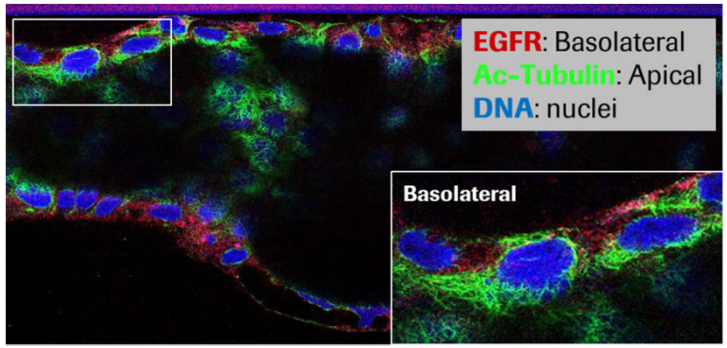
Gut Models:
Microfluidic approach



nature COMMUNICATIONS

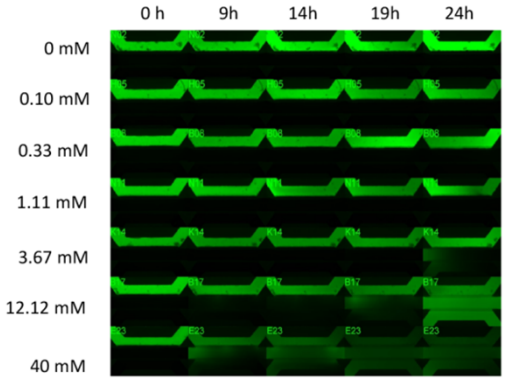
Membrane-free culture and real-time barrier integrity assessment of perfused intestinal epithelium tubes

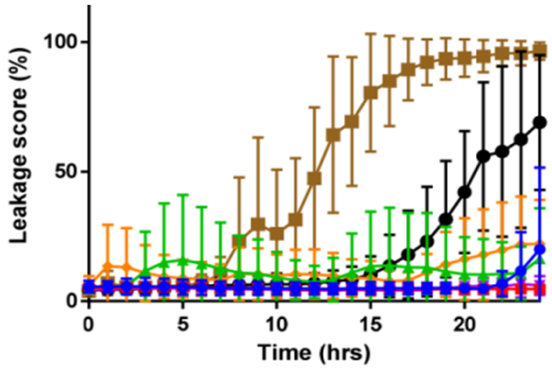
Sebastiaan J. Trietsch[1], Elena Naumovska[1], Dorota Kurek[1], Meily C. Setyawati[1], Marianne K. Vormann[1], Karlijn J. Wilschut[1], Henriëtte L. Lanz[1], Arnaud Nicolas[1], Chee Ping Ng[1], Jos Joore[1], Stefan Kustermann[2], Adrian Roth[2], Thomas Hankemeier[3], Annie Moisan[2] & Paul Vulto[1]

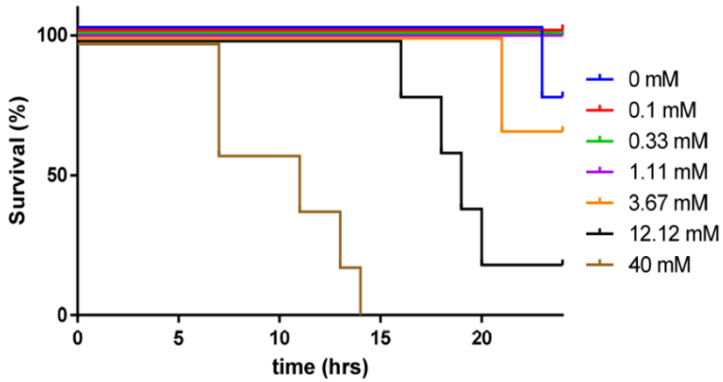


- **40 leak-tight tubules on single plate**
- **5d continuous culture**
- **glucose and MRP2 transporters**

Aspirin-induced leakage in organoplate



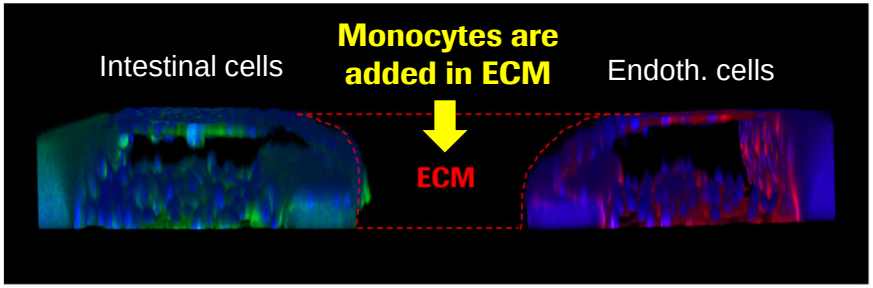


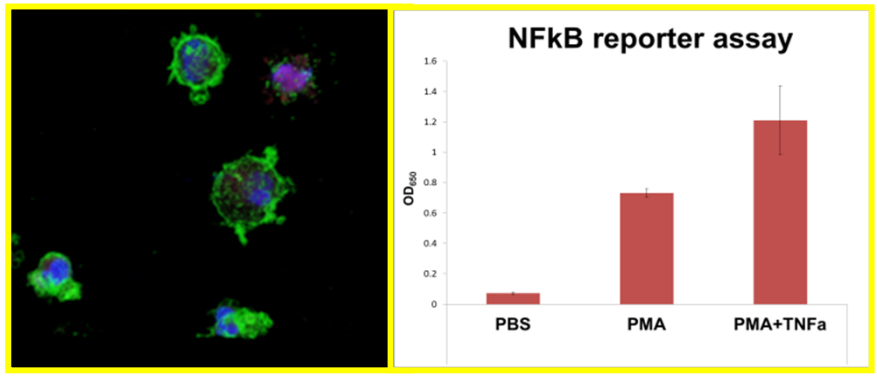


Next step for “Gut on a chip”

Inflamed, vascularized gut

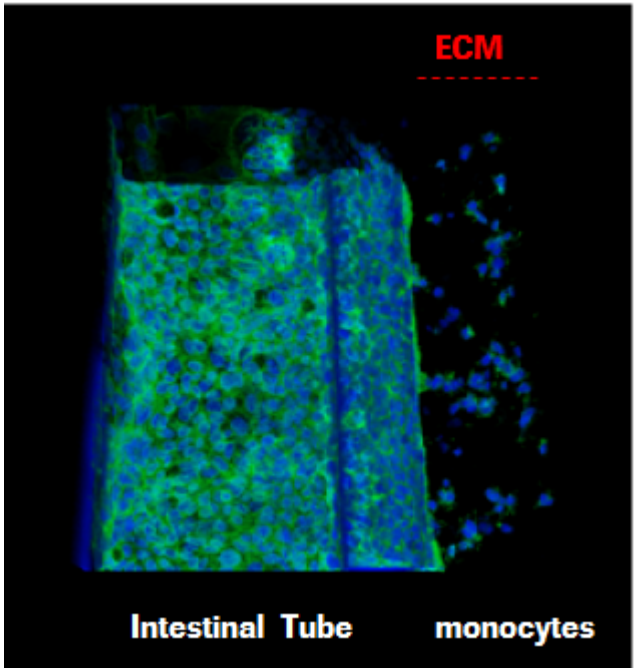
- Inflammation and dysfunctional vascularization are risk factors associated with drug-induced adverse events in the gut (*e.g.* with anti-VEGF therapies).
- Real time imaging of injury, healing and immune cell migration.



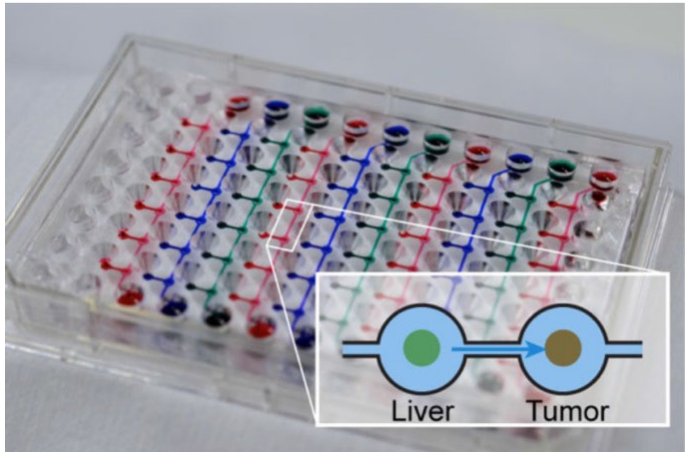


Activated monocytes in ECM

Live / **Dead** / **F-actin**



Connecting Organoids



nature COMMUNICATIONS

ARTICLE

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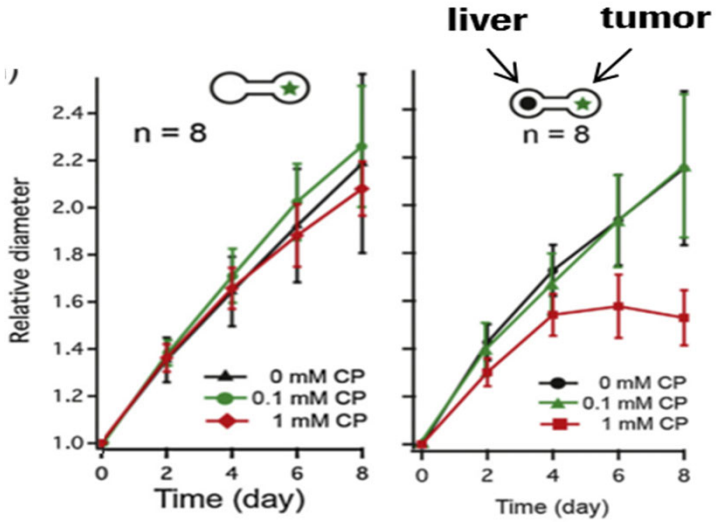
Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis

Olivier Frey[1], Patrick M. Misun[1], David A. Fluri[2], Jan G. Hengstler[3] & Andreas Hierlemann[1]

Cyclophosphamide metabolism and tumor killing

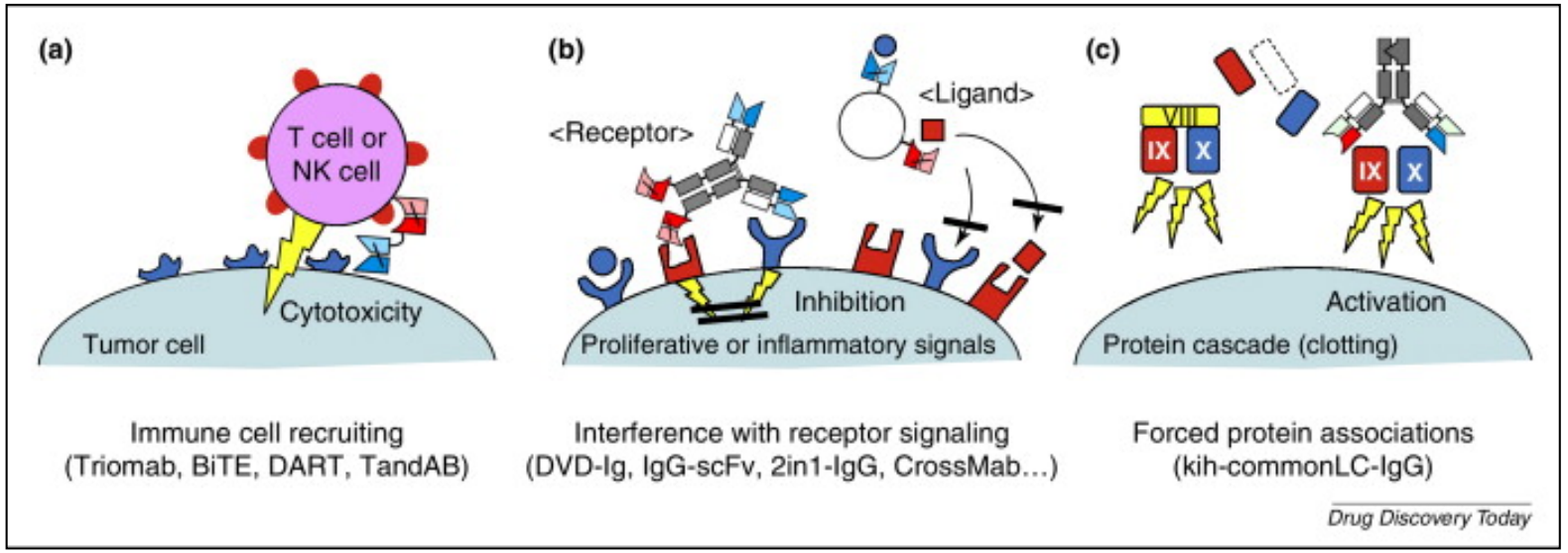


- **Liver-Tumor: 2-compartment approach to study Drug-effect on target tissue after undergoing liver metabolism**
- **Liver-Kidney; Liver-Gut, Liver-Bone Marrow....**

Incoporation of Immune Component

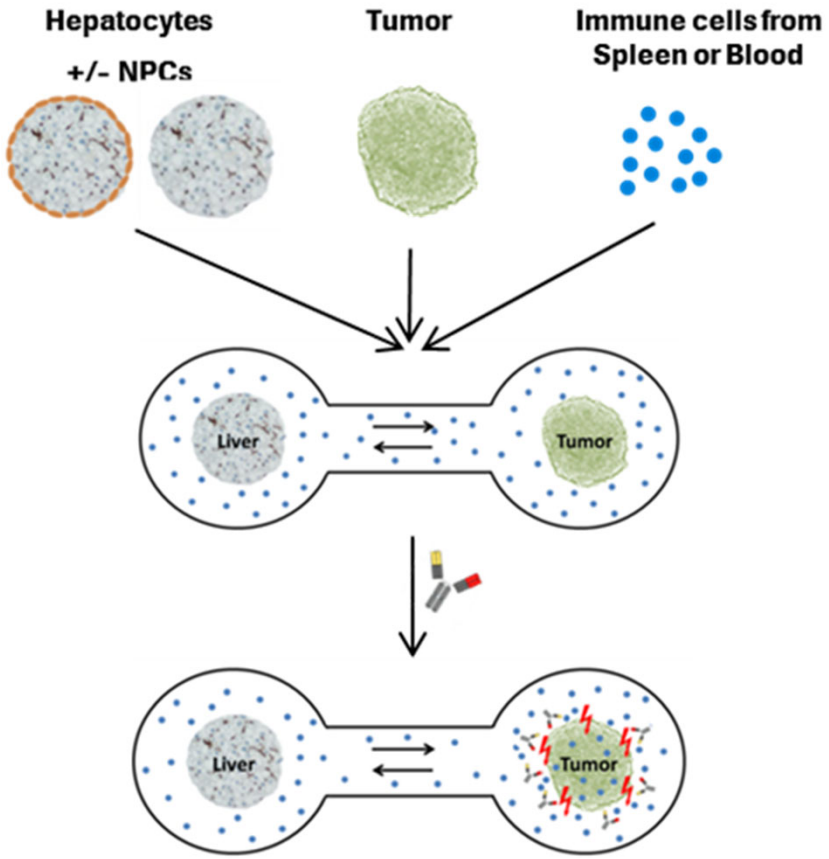
Oncology drug Development: Cancer Immuno-Therapy

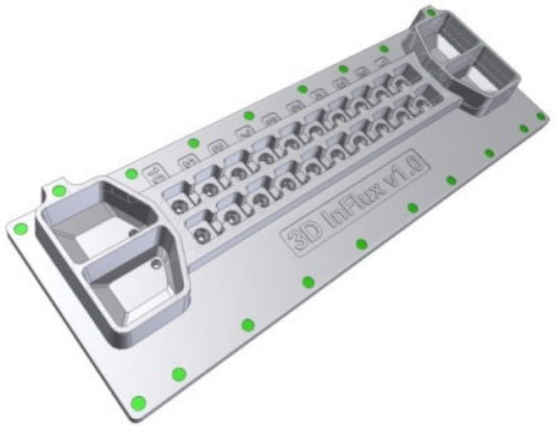
Can we model Tumor – Immune cell Interaction in vitro ?

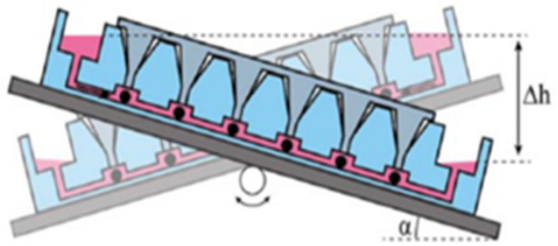


Liver – Tumor- Blood on-a-Chip

Towards a 3-dimensional microfluidic in vitro model to assess efficacy & safety for immuno-modulatory drugs







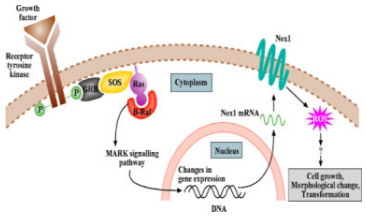
Cell models in drug safety today

where do assays currently drive/support decisions

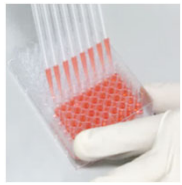
Roche

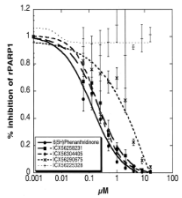
Target Selection & Hit identification → **Lead identification & optimisation** → **Pre-Clinical Development** → **Clinical Development**

Support target assessment, benchmark to competitors



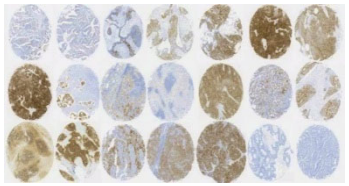
Run early safety tests to allow candidate selection





De-risk preclinical in vivo findings, address human relevance

Support mode of action identification of clinical flags



Strong focus on optimizing candidate selection process before moving into animal testing phase

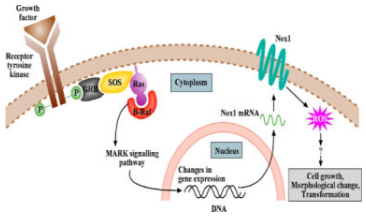
Cell models in drug safety today

where there's gaps

Roche

Target Selection & Hit identification → **Lead identification & optimisation** → **Pre-Clinical Development** → **Clinical Development**

Support target assessment, benchmark to competitors



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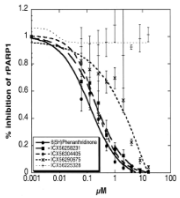

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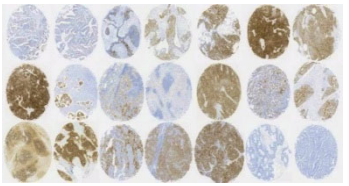


low predictivity – unclear *in vitro* to *in vivo* translation

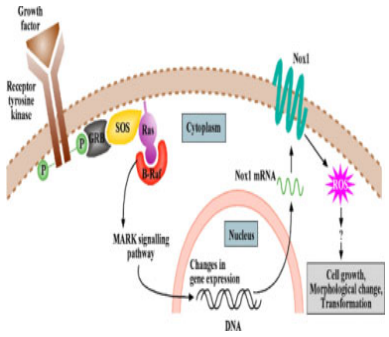
Cell models in drug safety tomorrow

where there's opportunities

Target Selection & Hit identification → **Lead identification & optimisation** → **Pre-Clinical Development** → **Clinical Development**

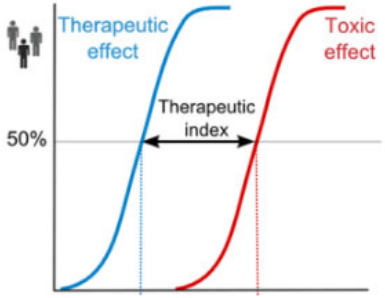
Use disease-relevant human *in vitro* model to study pharmacological MoA , Target ID

- Primary patient cells
- iPS
- Inflamed/healthy
- Immune-competent



Use complex model allowing generation of "*in vitro* therapeutic index"

- On target-tumor vs on target-off tumor killing
- Ability of innate immune system to mount response to bacterial challenge when repressed

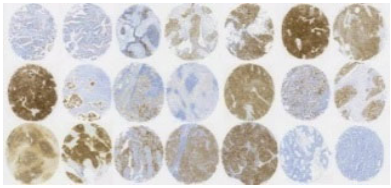


Assess key questions for tox assessments in MPS

- target organs in MPS – retire rodent pilots ?
- Replace/Refine 2yr carc studies ?
- Potency & safety studies *in vitro* (new EMA FIH Guideline)
- Prepare for cyno *in vivo*

Enable EIH , support MABEL

- Use *in vitro* human TI to support MABEL
- Assess key safety questions *in vitro* when target/pathway not expressed in pre-clin *in vivo*
- *Biomarker Development*



Cell models in drug safety tomorrow

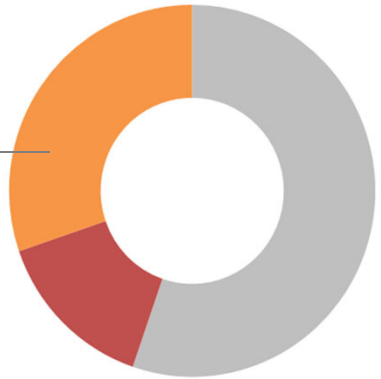
where there's opportunities

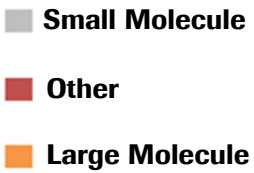
- **Aim for repalcing animal studies – not just increasing number of 'supportive' tests**
- **Adding even more stringent filters early on for established areas (small molecules) may not significantly improve candidate selection process**
- ***Use MPS/OoaC for integrated pharmacology/toxicology assessment (potency, in vitro TI)***
- ***Focus on areas where there's a lack of tools (Biologics, no x-reactive in vivo species)***

Outlook: A shift in the pharma portfolio calls for more advanced cell models

– 2013

Today





- **ca ⅓ “complex” (non-IgG)**
- **often no crossreactive pre-clinical species !**

More simple experiments

- “ATP” Assays
- 2D (3D) cell cultures
- Typical Question:

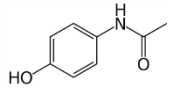
  Ranking/Prioritization of Small Molecule Leads

Sophisticated Cell Models, Complex Readouts

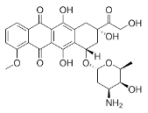
- Live Imaging, Metabolomics, Cytokines, ….
- 3D/Microfluidic/Organ on a Chip
- Typical Questions:
  - unintented immune cell activation, tissue infiltration & damage
  - Dissect effects of multiple drug combos
  - Effect of disease background

Outlook: A shift in the pharma portfolio calls for more advanced cell models

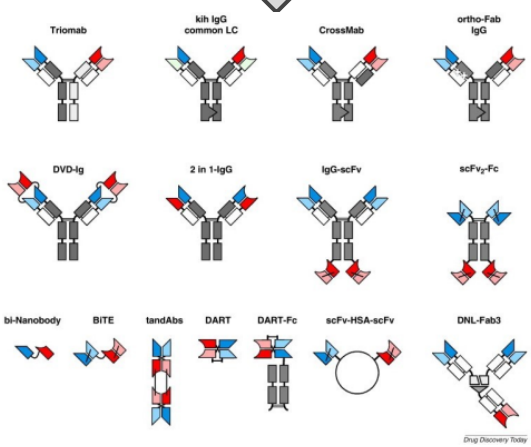
Drug Molecules

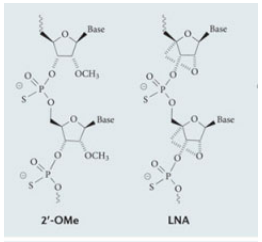


Acetaminophen



Doxorubicin





& combinations

Pharmacology

e.g.

- Selective binding to GPCR
- Inhibition of Ion Channel

e.g.

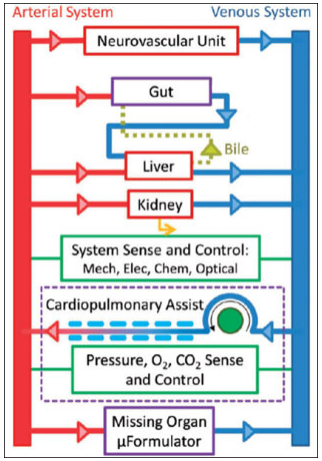
- Binding to T-cell target and activation after concomittant engagement to tumor environment specific 2nd target
- Combination of drugs leading to immune cell depletion and/or modulation

Questions addressed by pre-clinical safety

- Overt organ otxicity
- Metabolites
- Biomarker?
- Can go to in vivo testing ?

- **No crossreactive pre-clinical in vivo model – all in vitro ?**
- On tumor vs off tumor target mediated cell killing
- Can e.g. treatment with immunosuppressant drug lead to impaired innate immune response ?

How



John P Wikswo, 2014, Experimental Biology and Medicine

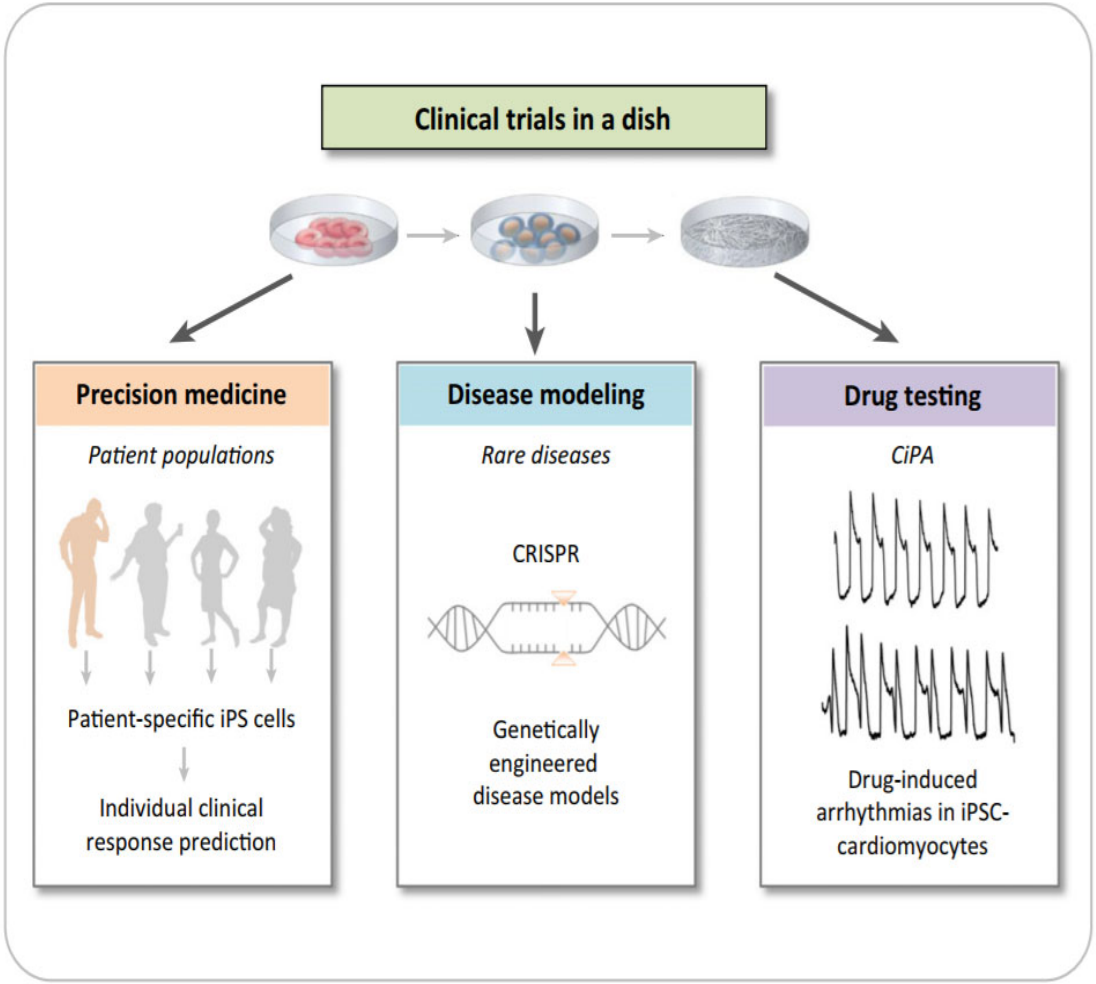
Cellular approaches for efficacy & safety become key

Regulatory authorities promoting application of innovative in vitro tools

CellPress
Trends in Pharmacological Sciences
January 2017, Vol. 38, No. 1

"Clinical Trials in a Dish"

David G. Strauss and Ksenia Blinova
(US Food and Drug Administration)



The path forward

Opportunities & challenges for «organs on a chip» & MPS

OPPORTUNITIES

- **Combine disease-pharmacology & safety *in vitro* (*«in vitro therapeutic index»*)**
- **Support internal decision making – reduce animal tests - aim for replacing regulatory studies**
- **Support EiH (e.g. MABEL) and clinical (Combos)**
- **Strive for more disease-population specific, more personalized testing**

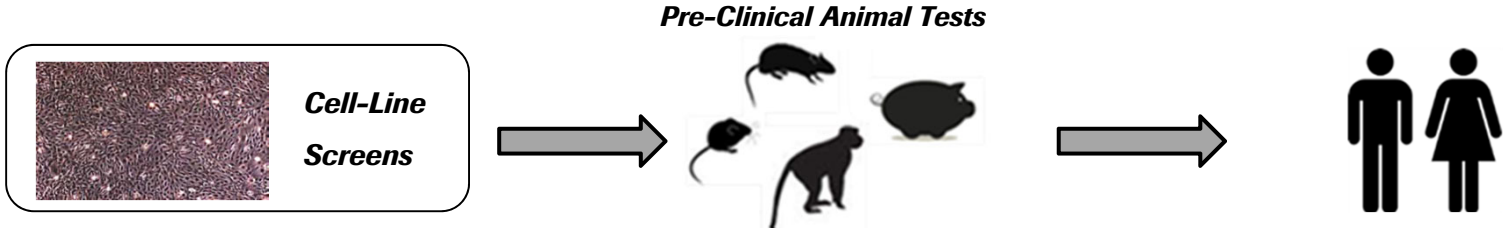
CHALLENGES

- **Demonstration of physiological relevance will be increasingly difficult with increasing complexity**
- **Price for increase in relevance versus increase in technical complexity needs to be assessed**
- **Sourcing of (primary) animal and human cells is central – can be very challenging if different cell types from same human donor needed**
- **IVIV translation remains key issue – most models 'semi'-validated**

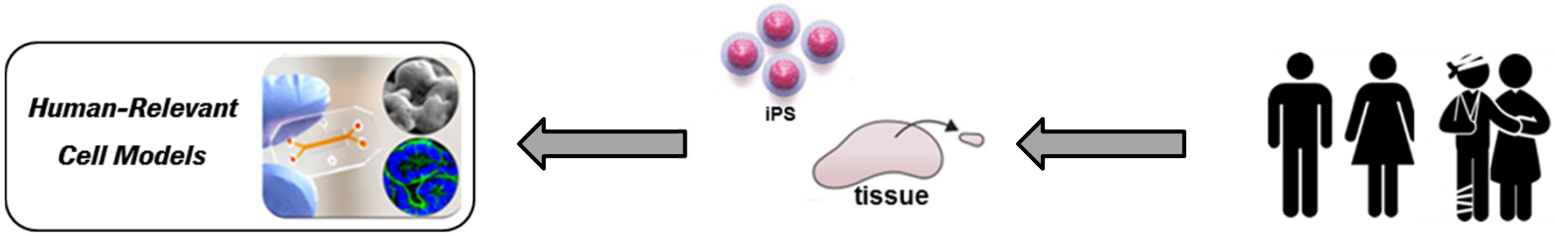
Our Vision for the Future

A Shift in Drug Testing using Pre-clinical Models

Predictive ModelsToday



Predictive Models Tomorrow



more human relevant – more «personalized»

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- David Kaplan, Tufts
- J Hickmann, U'Florida
- Emulate
- Uwe Marx & TissUse

Doing now what patients need next