The value of understanding

Human Stem Cell-Based Biomarker Assay for Screening of Developmental Toxicity

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ETS DART training course, Antwerp

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Animal testing for DART

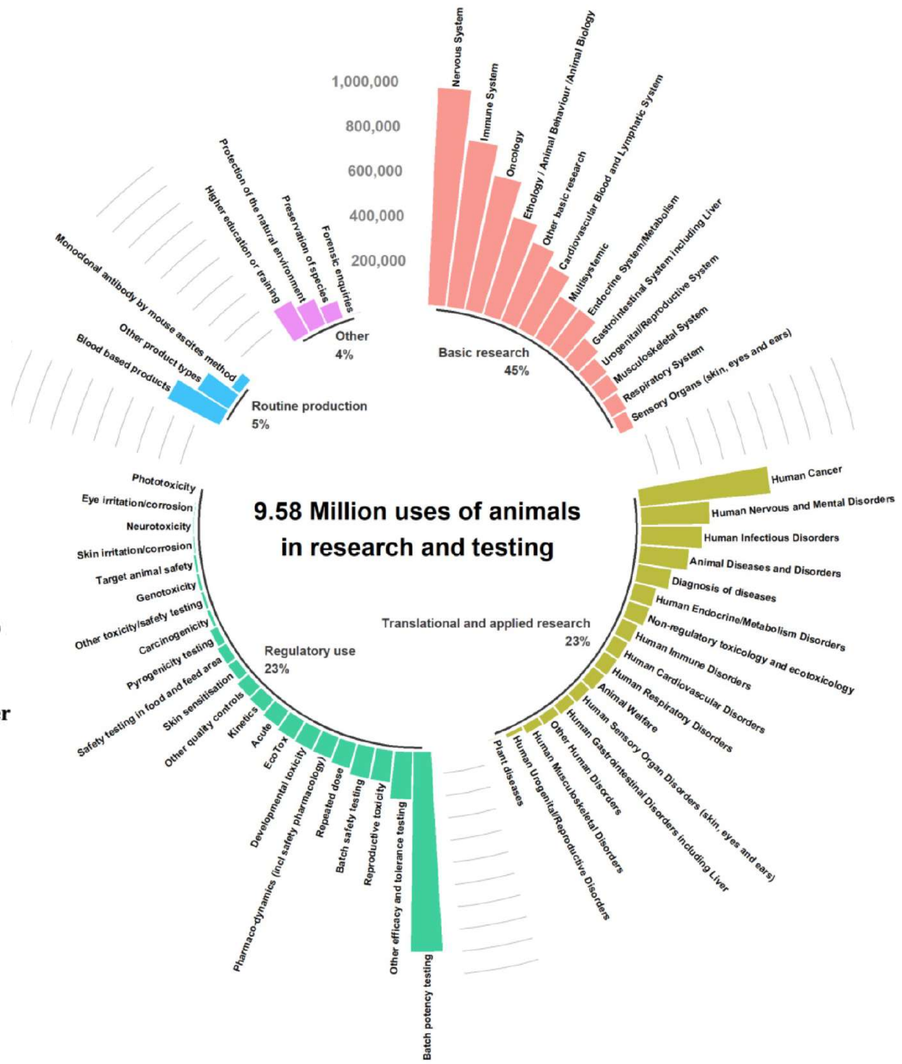
EUROPEAN COMMISSION

Brussels, 5.2.2020
COM(2020) 16 final

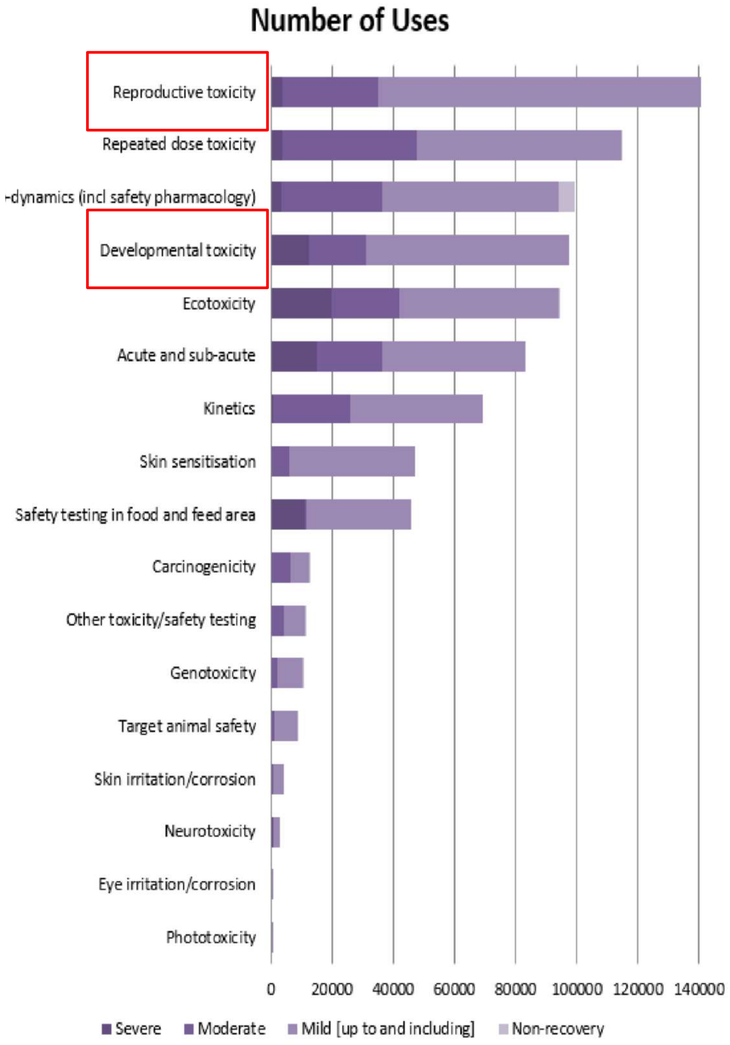
REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL

2019 report on the statistics on the use of animals for scientific purposes in the Member States of the European Union in 2015-2017

{SWD(2020) 10 final}



Uses of animals for research and testing in 2017



Toxicity and other safety testing by type and severity

Animal Alternatives for Developmental Toxicity Screening

“Any technology, methodology, approach, or combination that can provide information on chemical hazard and risk assessment without the use of animals”

Alternative methods:

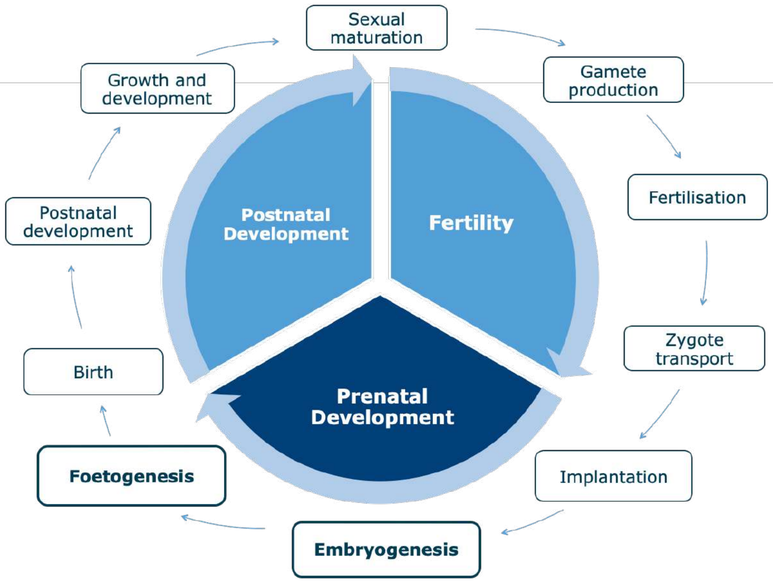
- Cell-based (mouse stem cell tests)
- Organ-based approaches (mini brains, liver, placenta)
- Whole embryo cultures (rat WEC, ZET, chicken embryo)
- *In silico* approaches (computer-driven predictive tools)

Advantages

- Time and cost efficient
- Insight into the key events and MoA
- Reduction in animal use

Disadvantages

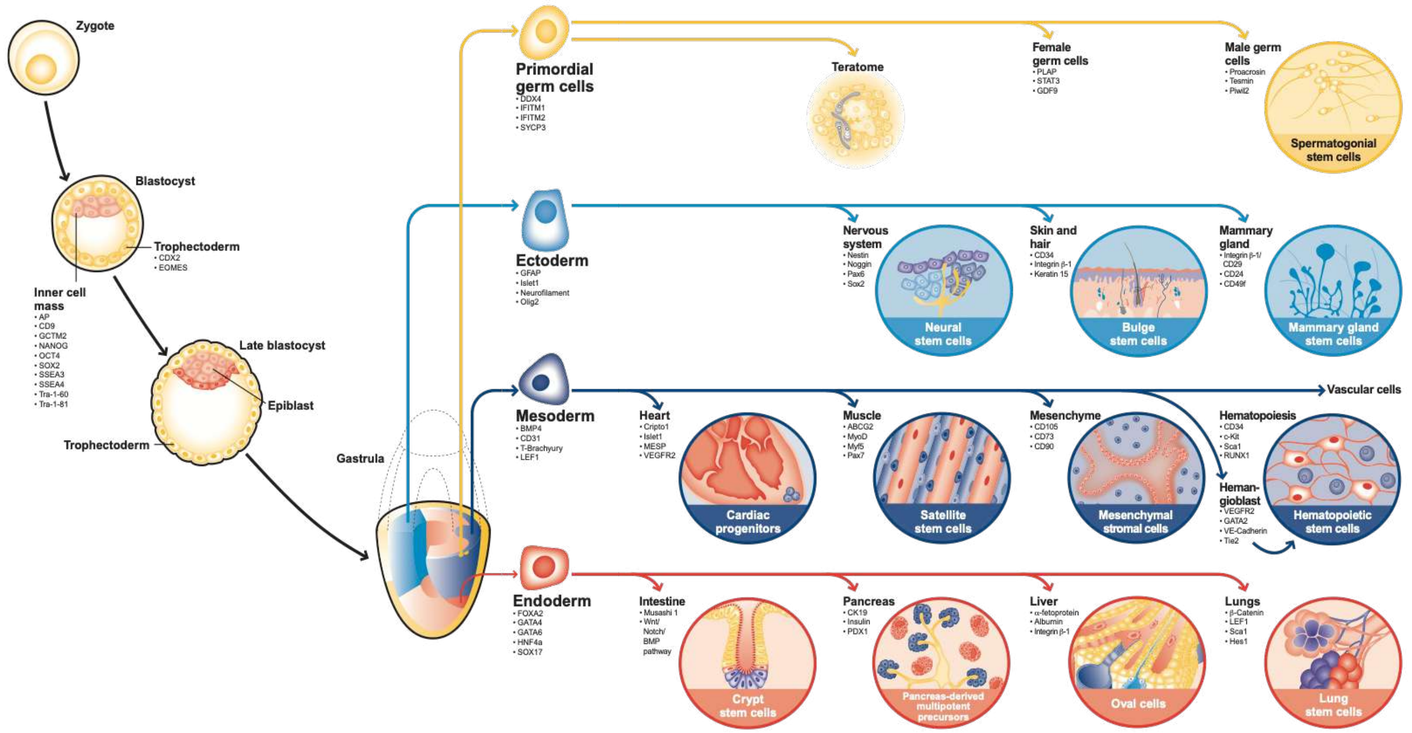
- Restricted duration of exposure
- Simplified biological system
- Interspecies differences



Reproductive cycle

Applicability of human induced pluripotent stem cells (hiPSCs)

- Overcoming the issue of species differences by using human material
- Potential to be differentiated towards specialized lineages and cell types
- Unlike human ESC, hiPSC have no ethical issues
- Principle: **adverse effects of chemical exposures on *in vitro* differentiation** are correlated to developmental toxicity



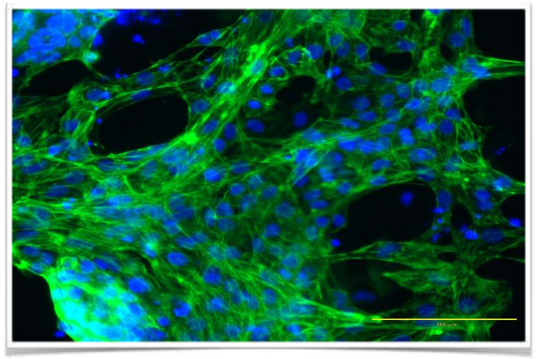
https://www.abcam.com/research/stem-cells

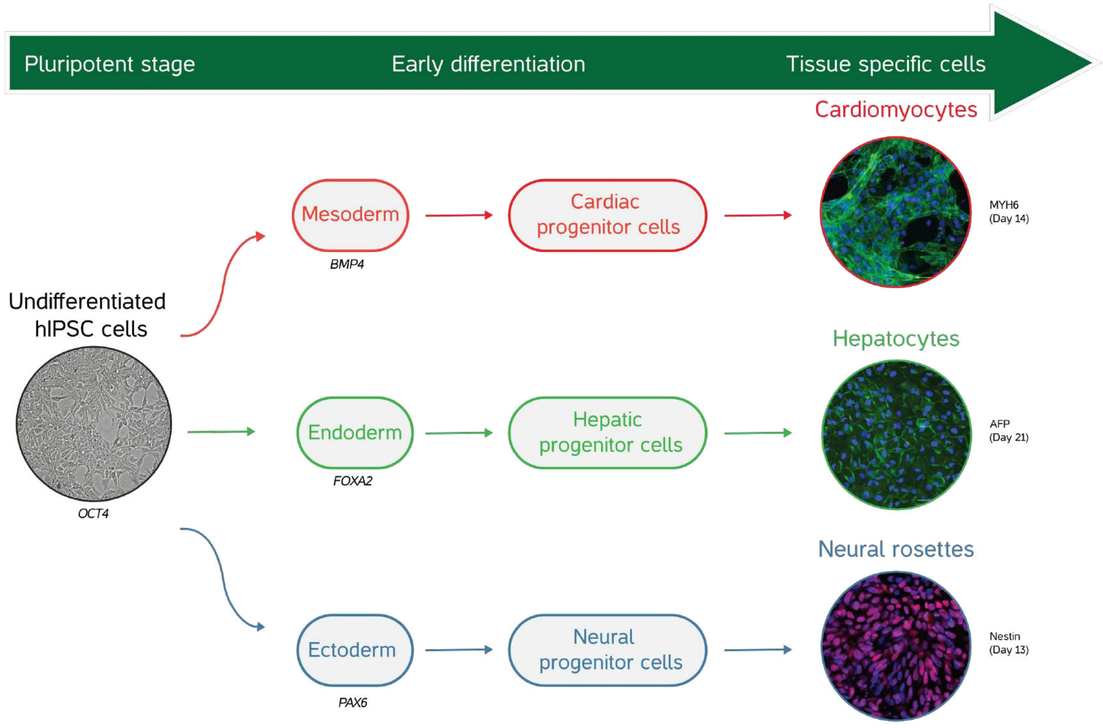
The ReproTracker® assay

Key features:

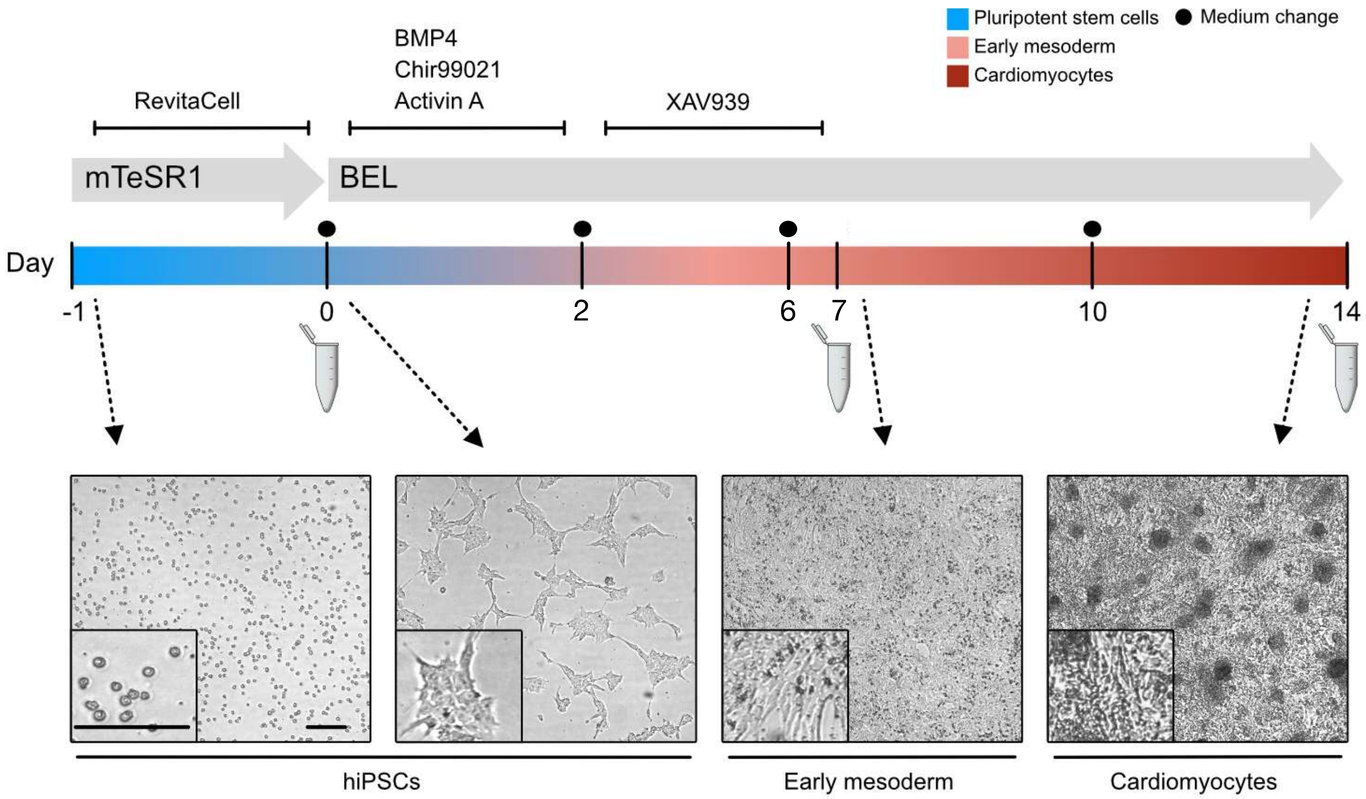
- Human test system
- *In vitro* development of functional heart, liver and neural tissues
- Visualization of the key cellular events of early embryonic development
- Detect disruption of developmental program based on morphological and molecular read-out

REPROTRACKER®

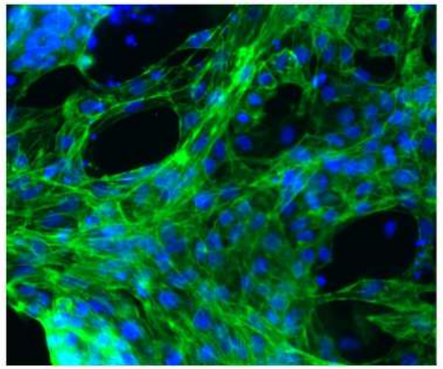




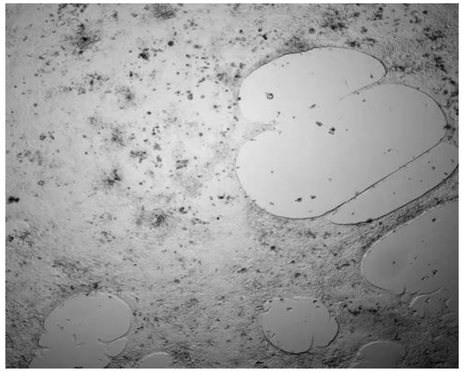
ReproTracker® assay – Cardiomyocyte differentiation

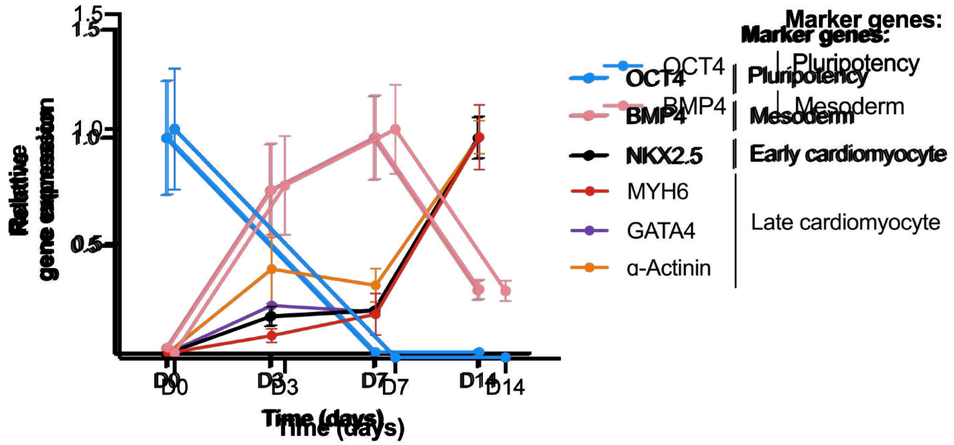


TNNT2



Cardiomyocyte beating

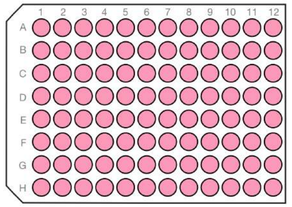




ReproTracker protocol

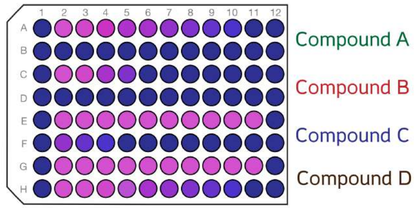
1. Dose range finding

Undifferentiated human iPSC

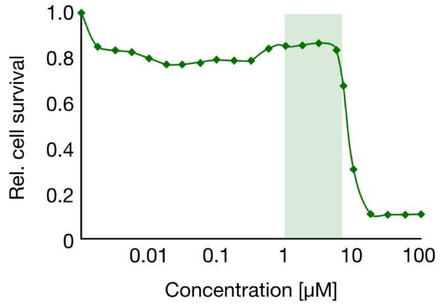


Expose hiPSC to 20 conc. for 7 days

Alamar Blue cell viability assay

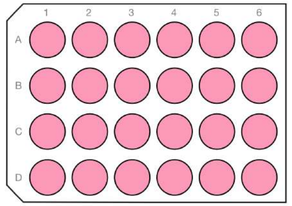


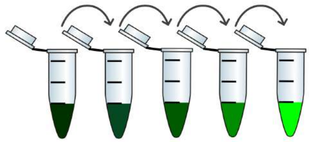
Dose selection



2. Stem cell differentiation

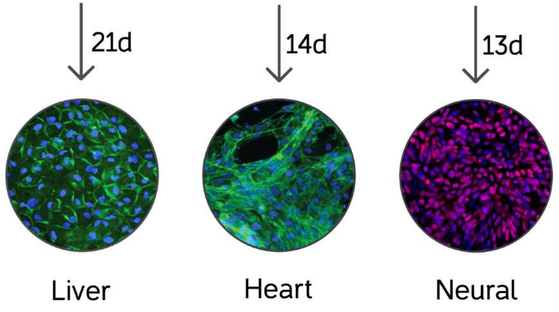
Undifferentiated human iPSC





Expose hiPSC to 5 conc.

Differentiate pluripotent stem cells



3. Biomarker analysis

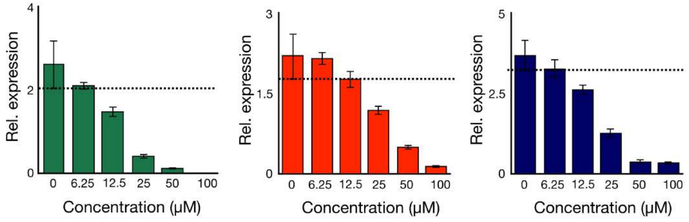
Morphological profiling

- Tissue morphology
- Beating cardiomyocytes
- Liver/neural marker expression
- Toxicity

Biomarker expression

- Multiple time points
- RNA isolation
- Quantitative real time PCR

Hepatocytes (AFP) | Cardiomyocytes (MYH6) | Neural rosettes (PAX6)

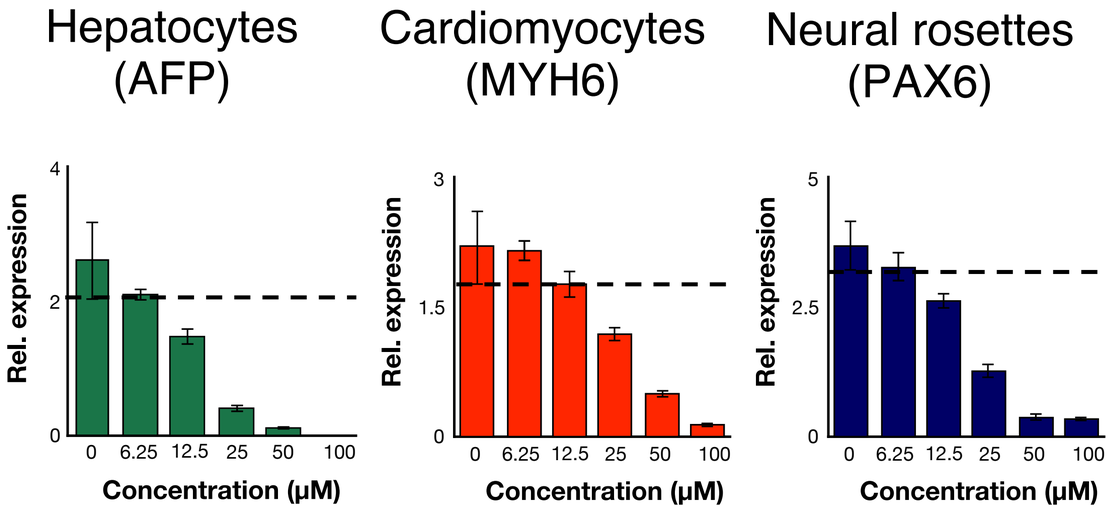


ReproTracker assay- Criteria for teratogenicity assessment

Adverse effects determined based on:

1. Reduced biomarker expression

2. Morphological/functional read-out
   - Morphology of liver and neural tissues
   - Heart: Contracting cardiomyocytes

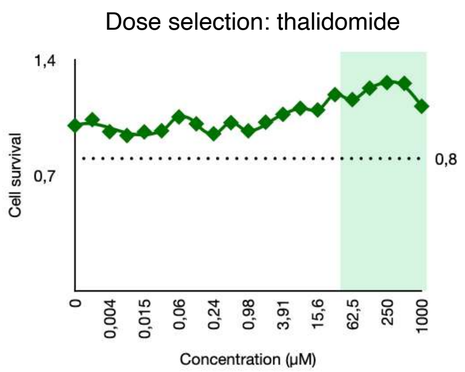


Teratogen classification

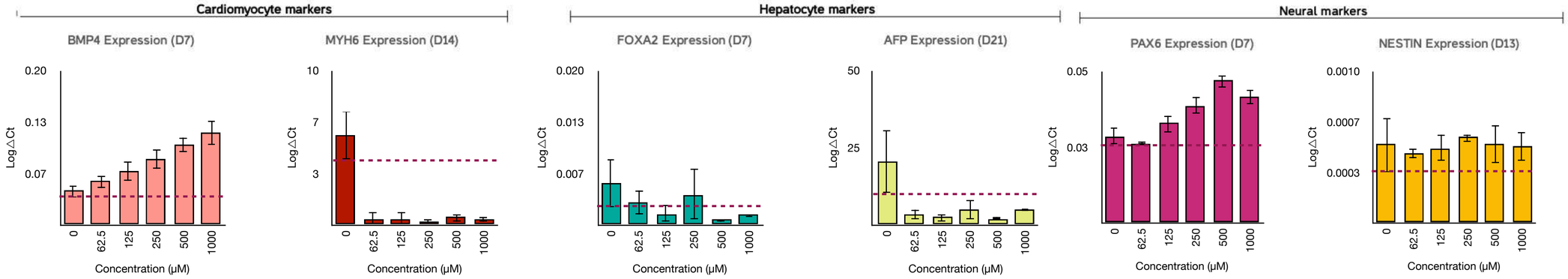
- Reduced expression of the biomarker genes below the set threshold level
- Dose response at 2 consecutive non-cytotoxic concentrations
- Validity of the assay confirmed by morphology and functionality of the control cultures

ReproTracker® examples - Teratogens

Dose selection

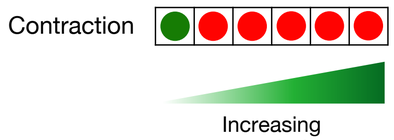


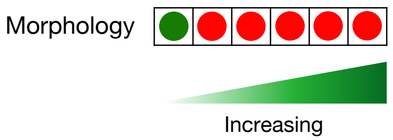
Biomarker analysis

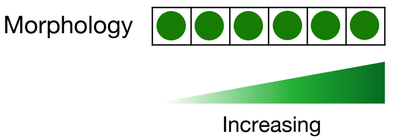


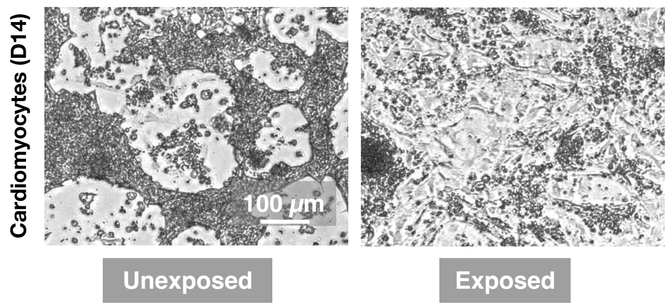
Clinically relevant concentration is between 1-6 µM

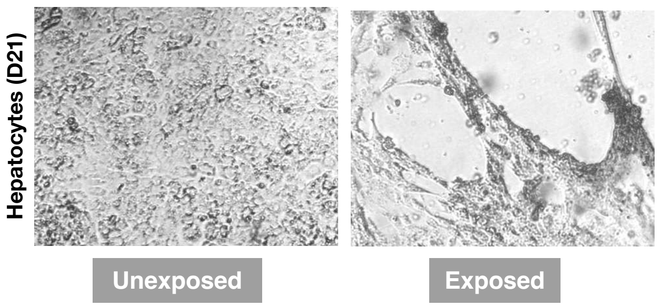
Morphology analysis

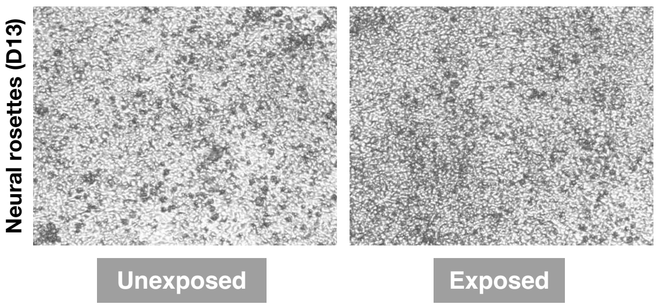






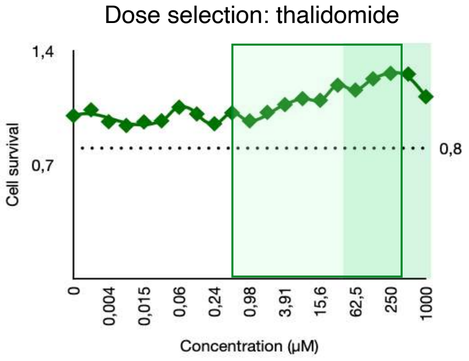




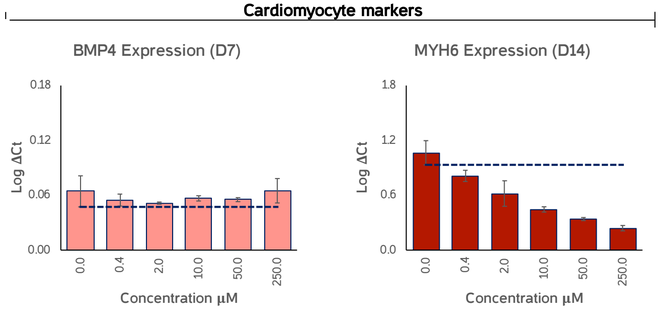


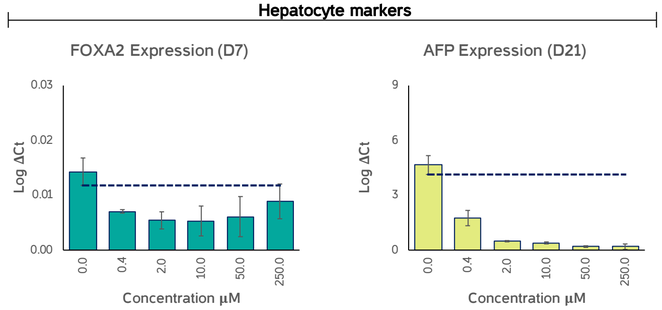
ReproTracker® examples - Teratogens

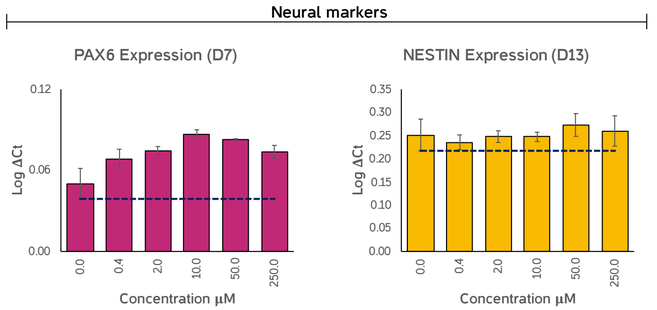
Dose selection



Biomarker analysis

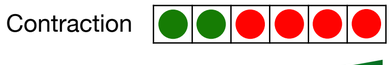


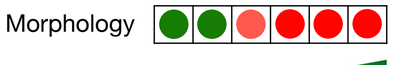


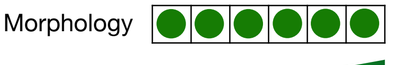


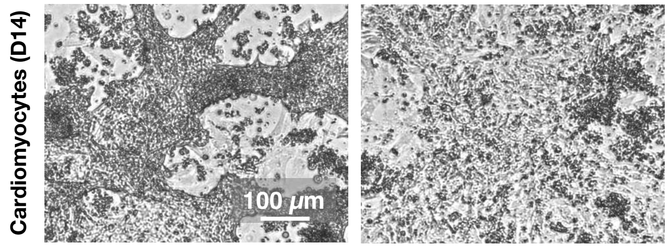
Clinically relevant concentration is between 1-6 µM

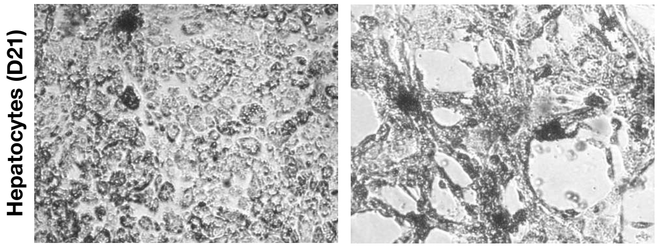
Morphology analysis

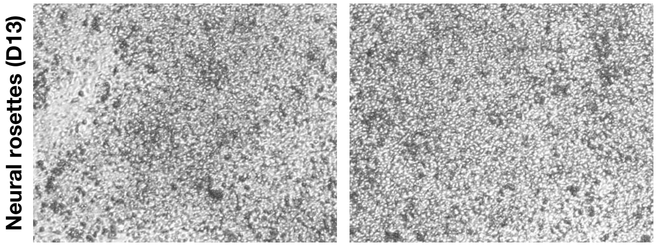






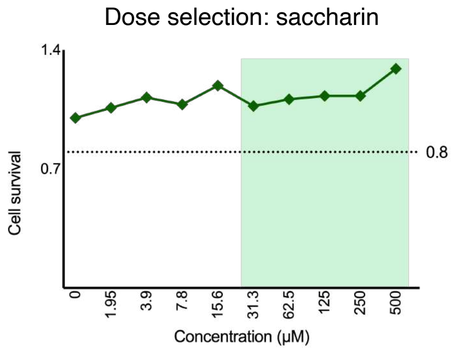




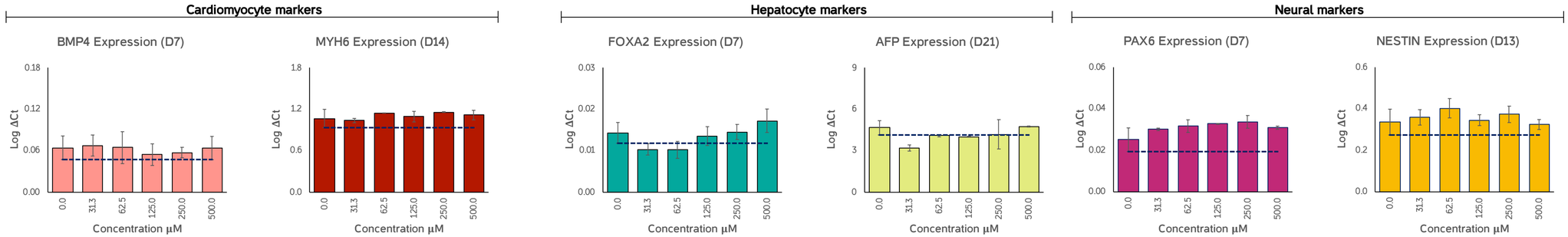


ReproTracker® examples – Non-teratogens

Dose selection

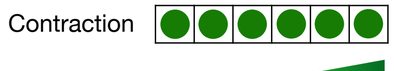


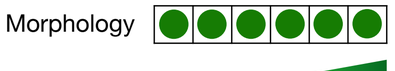
Biomarker analysis

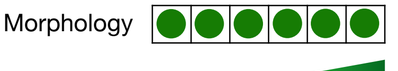


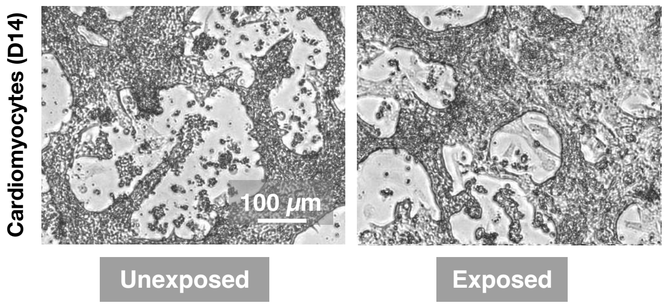
Morphology analysis

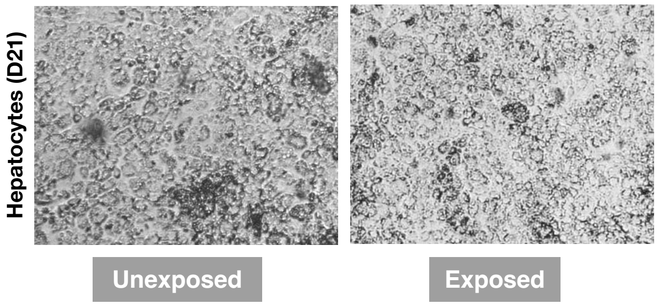
Clinically relevant concentration is 1.5 µM

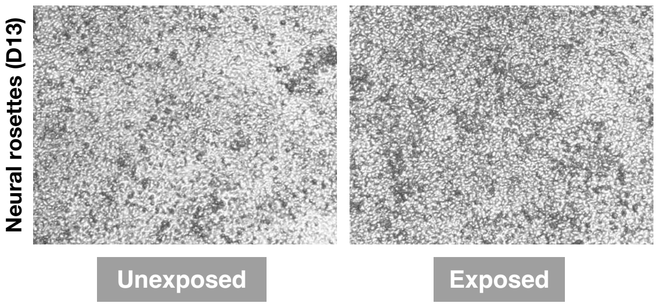












Extended validation of the ReproTracker assay

- > 100 compounds have been tested so far
- Validated with ICH S5 and EURL ECVAM-suggested libraries of teratogens and non-teratogens

Model system	Model accuracy (%)	Reference
ReproTracker	85%	A. Jamalpoor et al., 2022
Mouse EST	78%	A. Seiler et al., 2011
Whole Embryo Culture	68%	K. Augustine-Rauch et al., 2010
Micromass	70%	I. Wilk-Zasadna et al., 2009

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

29 August 2017
EMA/CHMP/ICH/544278/1998
Committee for Human Medicinal Products

ICH S5 (R3) guideline on reproductive toxicology: detection of toxicity to reproduction for human pharmaceuticals

ReproTracker® Validation

Chemical group	Compound	In vivo DART	ReproTracker classification	ReproTracker in vitro responses		
				Liver	Heart	Neural
Channel modulator	Carbamazepine			X	X	✓
	Diltiazem			X	X	X
	Trimethadione			✓	✓	✓
DNA modifiers	Busulfan			✓	✓	X
	Cisplatin			✓	✓	X
	Thiotepa			✓	✓	✓
Enzyme modulator	Aspirin			✓	X	✓
Hormone modulator	Dexamethasone			✓	✓	X
Kinase modulator	Tacrolimus			X	X	X
Nucleoside modulator	Methotrexate			X	✓	X
Transcription modulator	Acitretin			X	X	✓
Antibiotics	Clarithromycin			✓	✓	X
Anticonvulsant	Valproic acid			X	X	✓
	Diphenylhydantoin			X	✓	X
Antifungal	Bitertanol			X	✓	X
Other	Monobutyl phthalate			✓	✓	✓
	Methoxyacetic acid			X	✓	✓
	Thalidomide			X	X	✓
	Triadimenol			X	✓	X
	Retinoic acid			X	✓	X

Chemical group	Compound	In vivo DART	ReproTracker classification	ReproTracker in vitro responses		
				Liver	Heart	Neural
Channel modulator	Hydrochlorothiazide			X	X	✓
Enzyme modulator	Vildagliptin			✓	✓	✓
Receptor modulator	Cetirizine			✓	✓	✓
	Diphenhydramine HCl			X	✓	X
Vitamins	Folic acid			✓	✓	✓
	Thiamine			✓	✓	✓
Hormone	Progesterone			✓	✓	✓
Antibiotics	Amoxicillin			✓	✓	✓
	Penicillin G			✓	✓	✓
Other	Saccharin			✓	✓	✓
	Acrylamide			✓	✓	✓
	Dimethyl phthalate			✓	✓	✓
	Acetaminophen			✓	✓	✓

Accuracy	Sensitivity	Specificity
85%	85%	84%

Teratogen

Non-teratogen

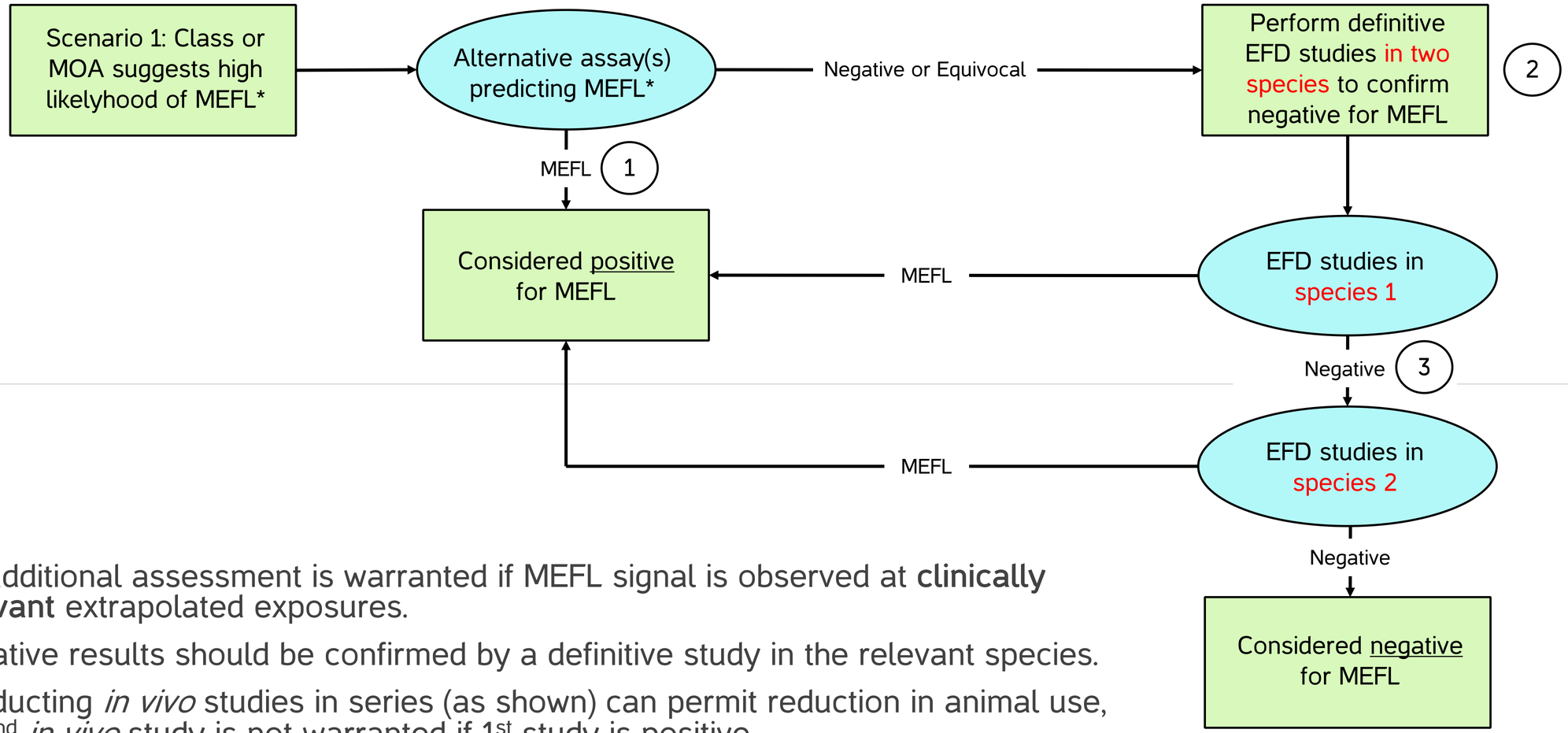
ReproTracker as a late phase verification test for animal testing outcomes

Compound	Therapeutic Cmax (μM)	FDA label	Humans	Rodent	Rabbit	mEST	WEC	True classification	ReproTracker classification
Sitagliptin	1	B				n.d.	n.d.		
Warfarin	25	X					n.d.		
Imatinib	2-4	D	n.d.			n.d.	n.d.		
Bosentan	2	X	n.d.			n.d.	n.d.		

- ReproTracker utilizes human material (hiPSCs) and hence can be more predictive of responses in humans.
- ReproTracker can resolve the outcome differences in animal testing.

ReproTracker: a fit within ICH S5 regulatory framework

Pharmaceuticals expected to be embryo-fetal toxicants (EFD toxicants)



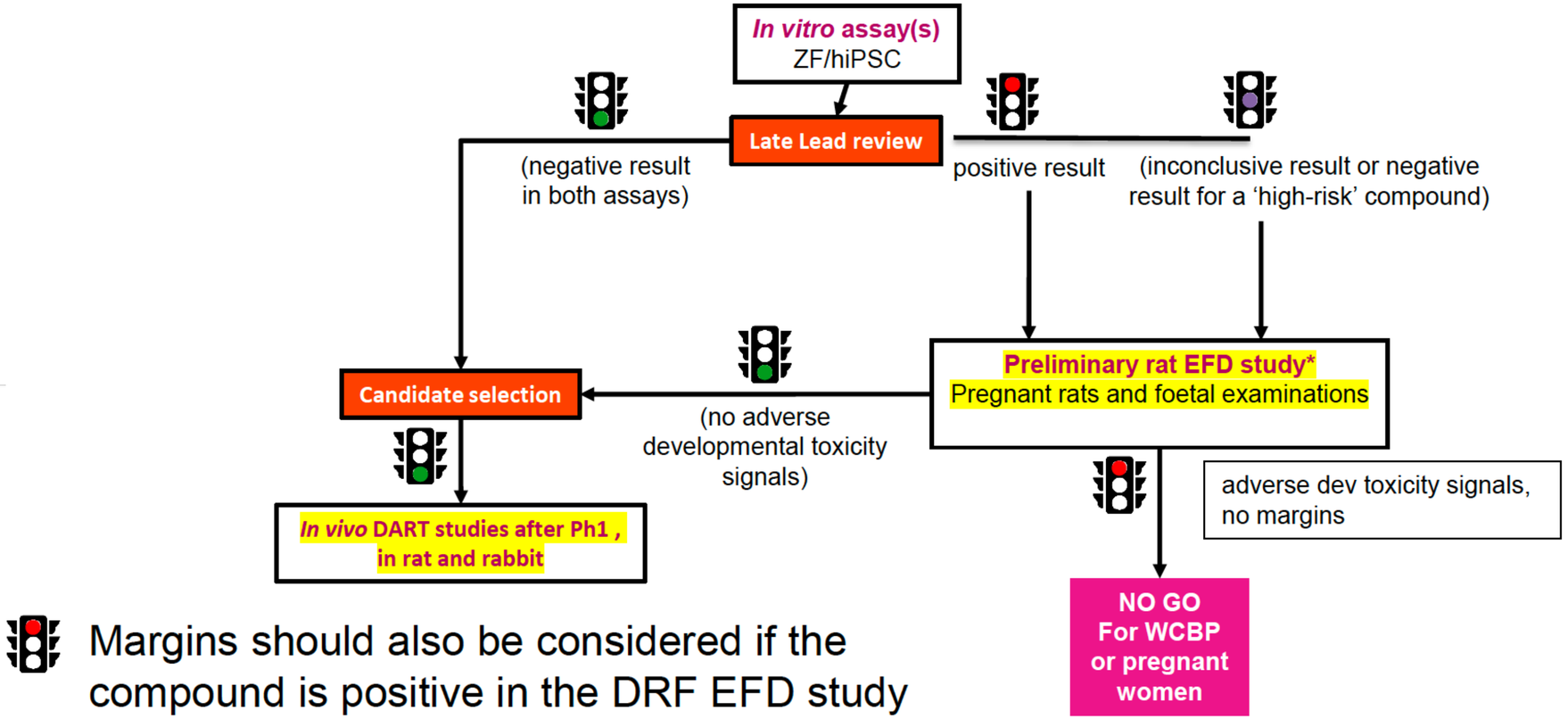
1) No additional assessment is warranted if MEFL signal is observed at **clinically relevant** extrapolated exposures.
2) Negative results should be confirmed by a definitive study in the relevant species.
3) Conducting *in vivo* studies in series (as shown) can permit reduction in animal use, as 2nd *in vivo* study is not warranted if 1st study is positive.

* Malformation of embryo-fetal lethality

Adapted from https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s5-r3-guideline-detection-reproductive-developmental-toxicity-human-pharmaceuticals-step-5_en.pdf

Integrating ReproTracker into the MMV screening strategy

Front Loading EFD studies early into drug development

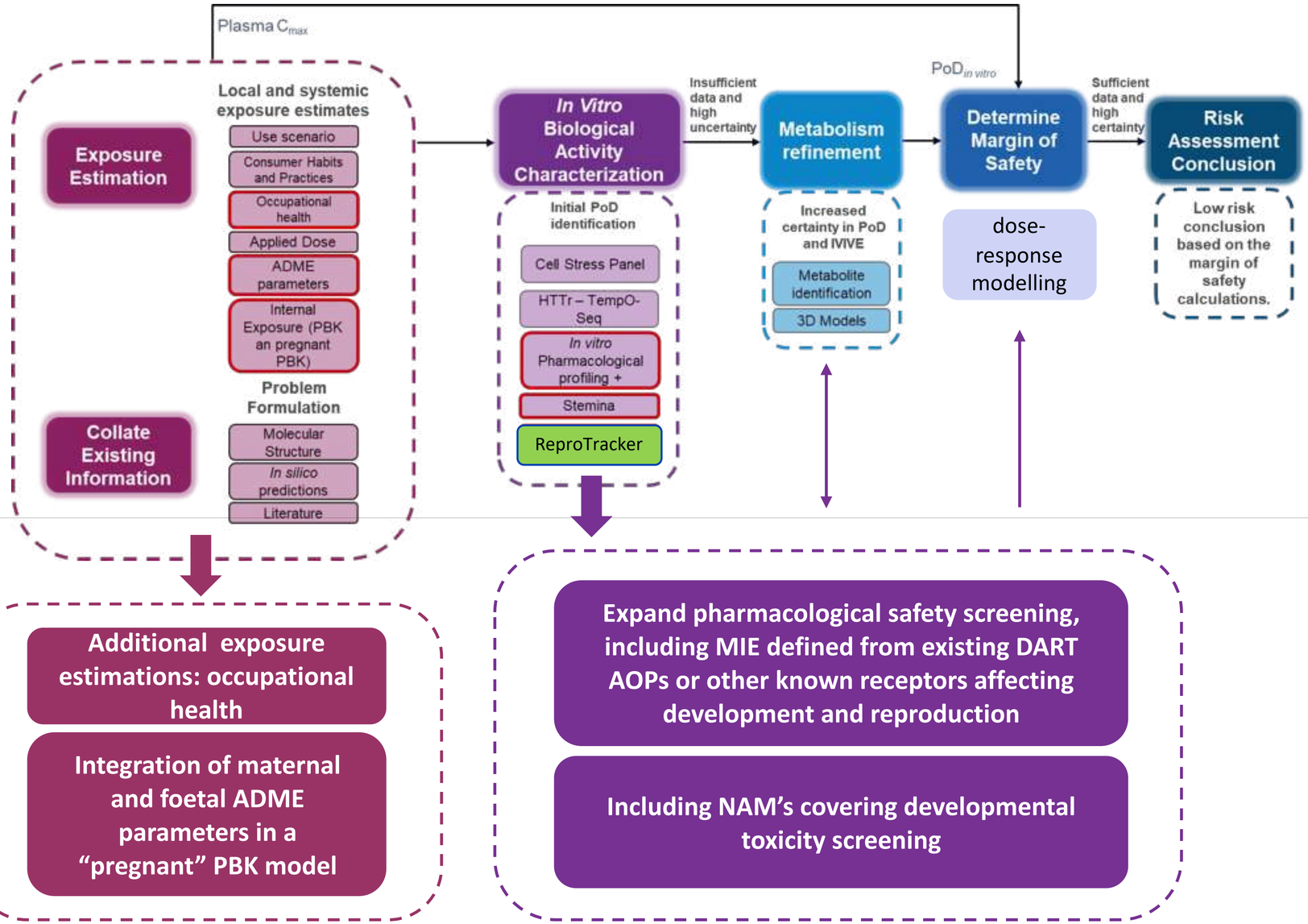


Margins should also be considered if the compound is positive in the DRF EFD study

Demarta-Gatsi C, Jamalpoor A, Hendriks G, Tornesi B. Integration and application of new approach methodologies in assessing the developmental hazards. Birth Defects Res. 2023 May 23. doi: 10.1002/bdr2.2190.

Integrating ReproTracker into Unilever's NGRA framework

Assessment of developmental toxicity of cosmetics



Rajagopal et al., Front. Toxicol., 07 March 2022 https://doi.org/10.3389/ftox.2022.838466

The future field of toxicology

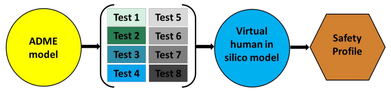
- Current *in vitro* developmental testing approaches do not consider the exposure of chemicals or drugs to mother and fetus – hampering adequate extrapolation *of in vitro* findings to relevant clinical dosing scenarios

Expectations for future toxicology field

- Combining assays to improve predictability: battery approach

Dose range finding

- PBPK models*
- Placental transfer
- Cell viability



iPSCs differentiation

- Hepatocytes
- Cardiomyocytes
- Neural rosettes
- Osteoblasts

REPROTRACKER®

Mechanistic insight

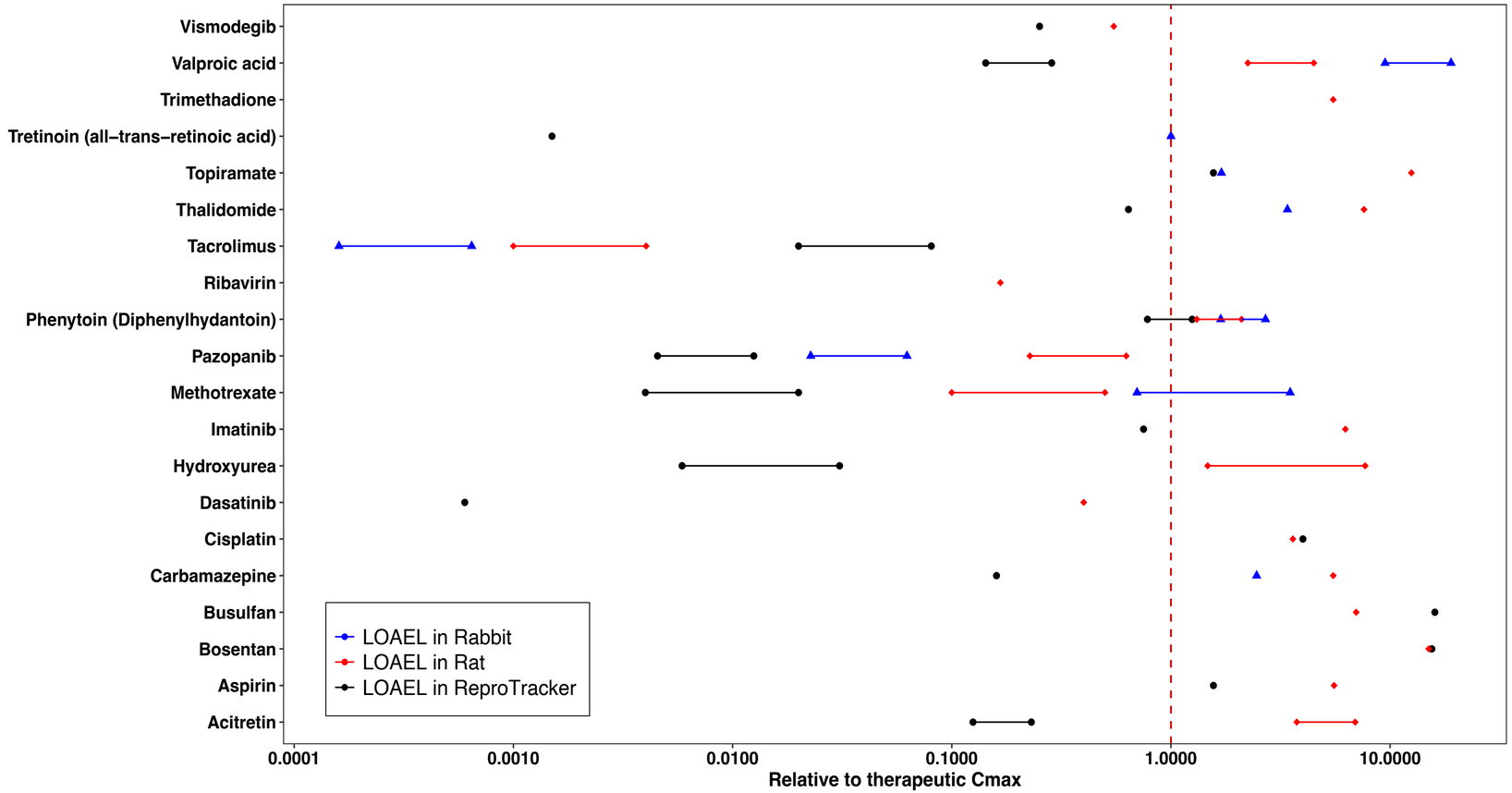
- Omics technologies
- POD
- IVIVE
- AOP mapping
- Complex mechanistic modelling

Impacts

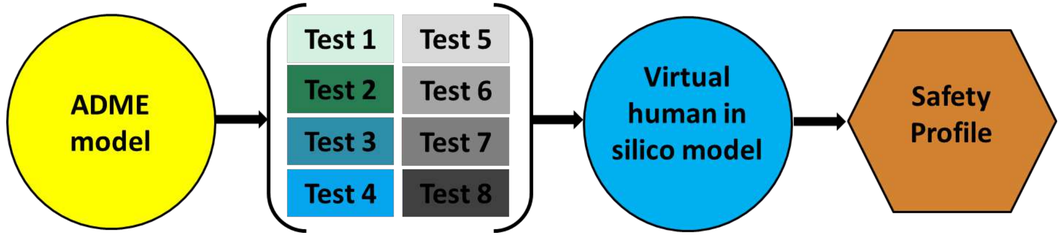
- **Reduction** or **replacement** of 2nd animal species
- Increased confidence in **predictive value** of exposure-informed
- Increased experience among industry and regulatory bodies

*Marjory Moreau, Amer Jamalpoor, John Carter Hall, Jeffrey Fisher, Sabine Hartvelt, Giel Hendriks, Andy Nong. Animal-free assessment of developmental toxicity: Combining PBPK modeling with the ReproTracker assay. Toxicology, Volume 500, 2023, 153684, ISSN 0300-483X, https://doi.org/10.1016/j.tox.2023.153684.

ReproTracker identifies teratogens at therapeutic plasma concentrations



- Use *in vitro* to *in vivo* extrapolation (IVIVE) → Predicting human equivalent dose (HED)
- Improving safety assessment of chemicals without animal testing



Conclusion

- Human stem cell-based test system
- Combines functional/morphological profiling and expression pattern of selected biomarker genes
- Biomarker based approach – a way to understand biological responses
  - Insight into the molecular mode of action and key events
  - Time-window sensitive gene-biomarkers

Predictability of ReproTracker assay

- Sensitive enough to predict compounds' adverse effects on early embryonic development
- Potency ranking

Applicability

- As part of early drug development phase
- Alternative for animal-free teratogenicity testing of chemicals
- Investigate the mode-of action of teratogenic compounds
- Extrapolate animal-derived results to humans

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The value of understanding

Thank you!

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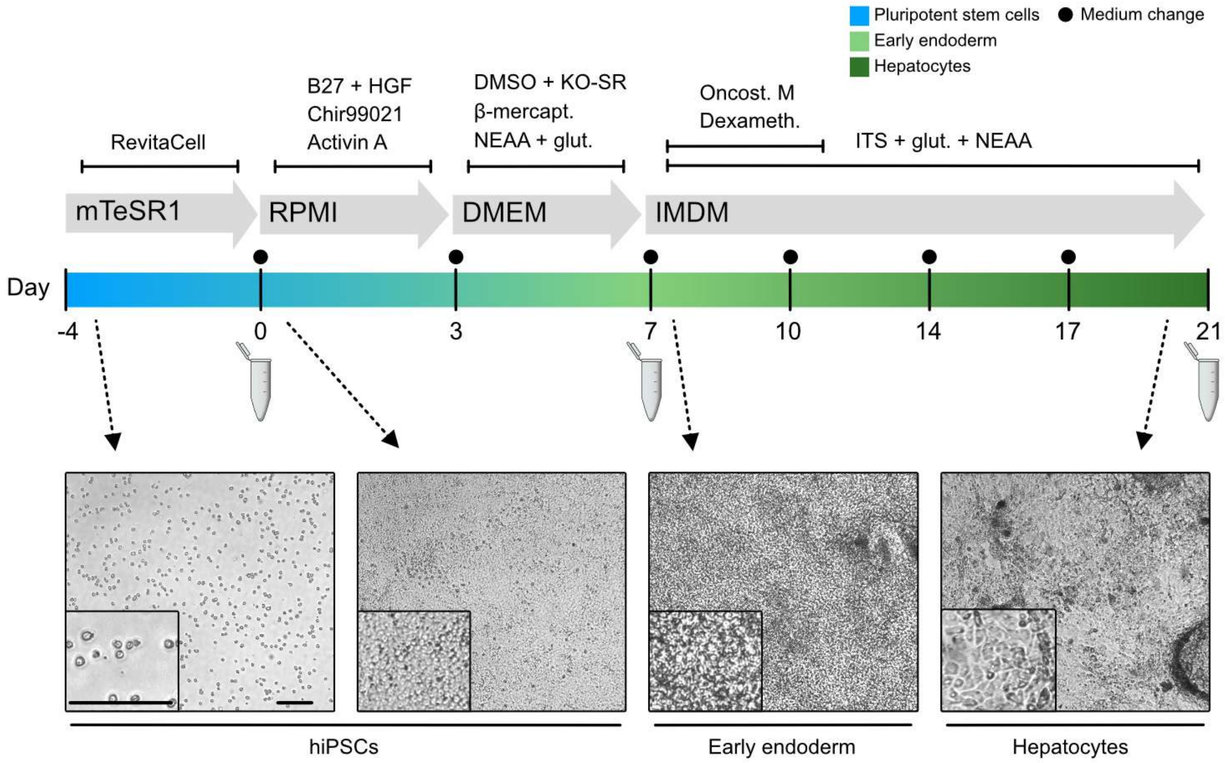
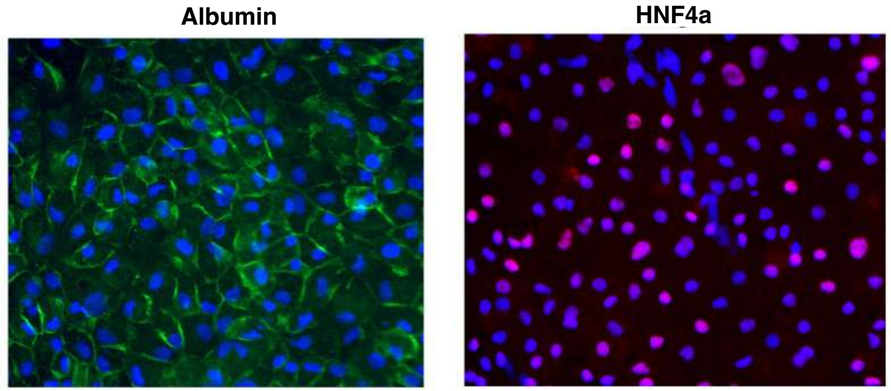
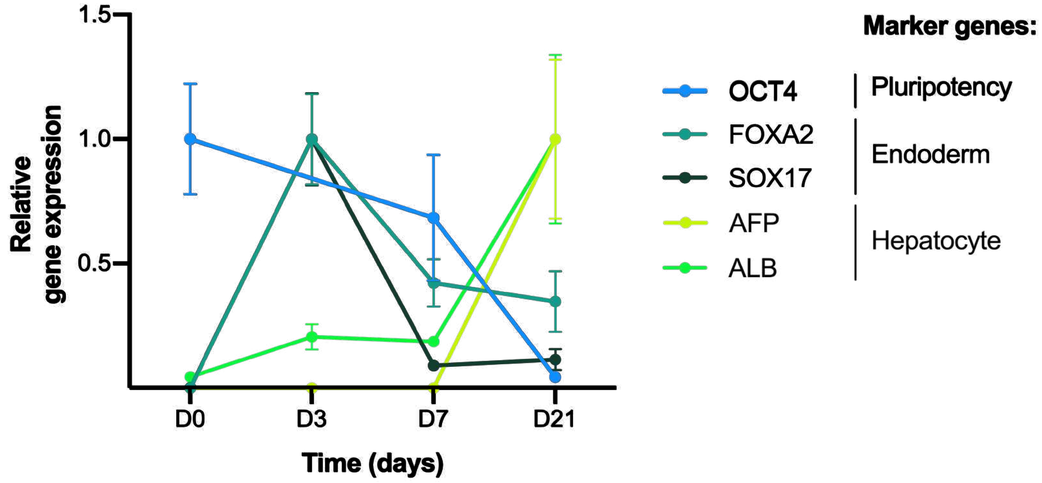
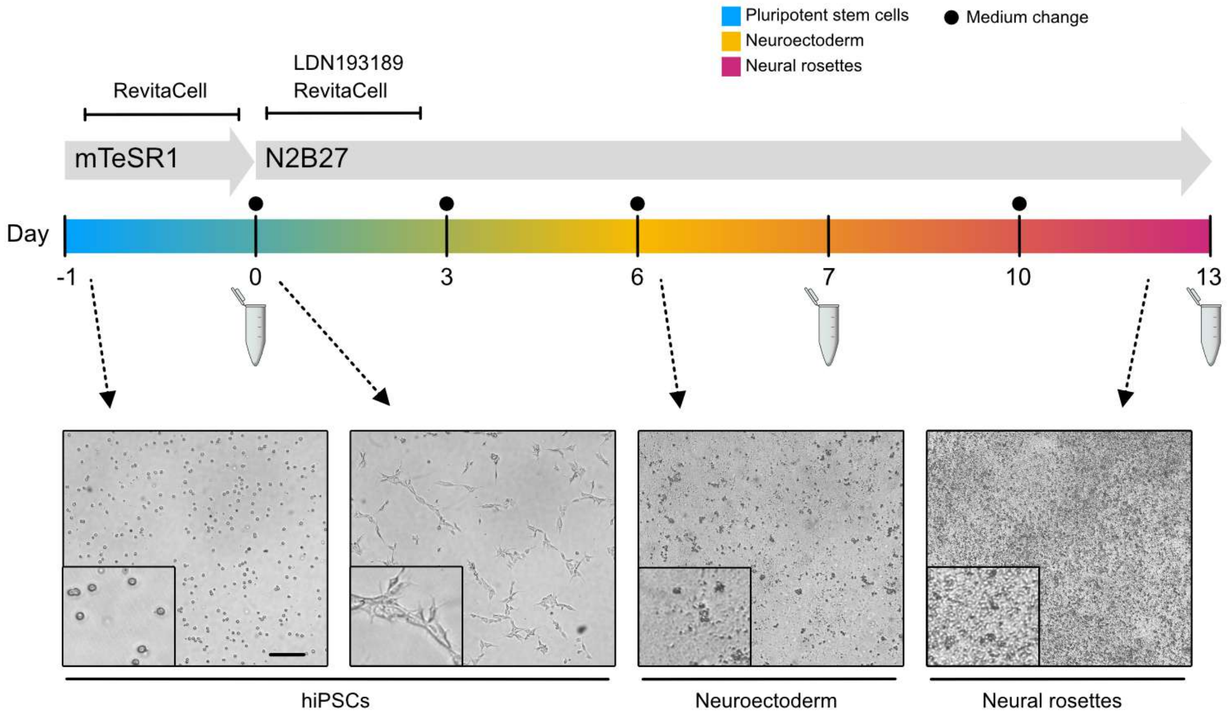
ReproTracker® assay – Hepatocyte differentiation

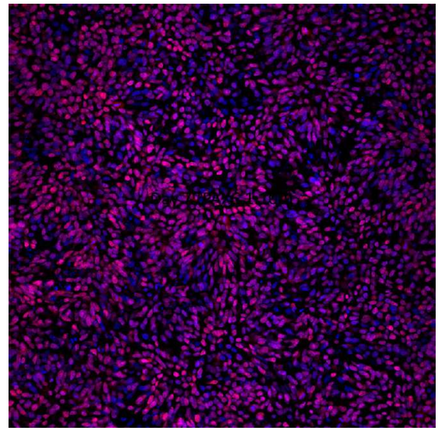


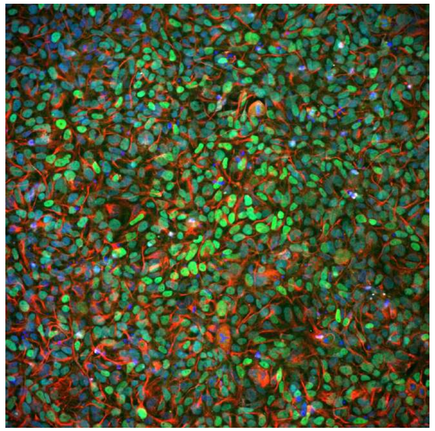


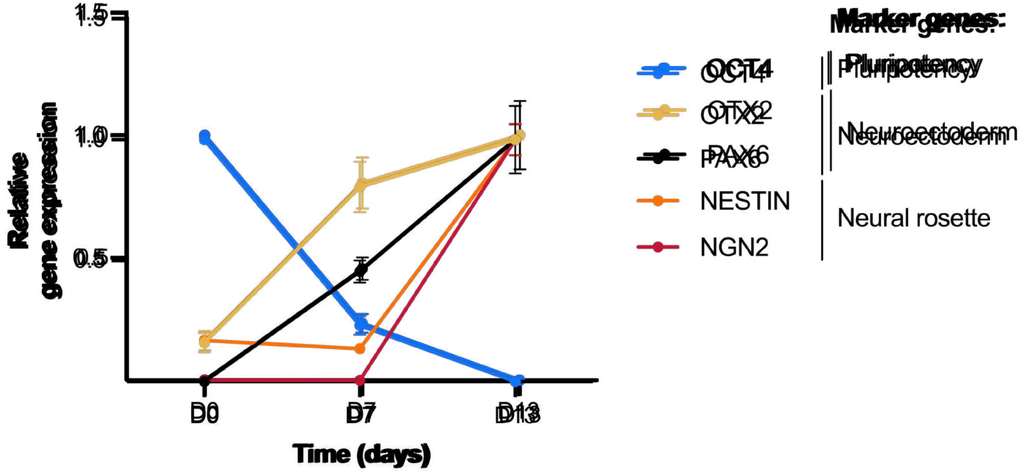


ReproTracker® assay – Neural differentiation









The ReproTracker® assay - practical facts

Compound requirement

- Top concentration 1 mM or 1 mg/ml
- 20-100 mg

Turn around time

- 8-10 weeks

Throughput

- Testing up to 20-25 compounds per run

Type of solvents

- DMSO
- PBS
- Water

Protocol

- Two biological exposures
- Five compound concentrations
- Multiple control cultures
- Positive and negative control compounds
- Biomarker analysis in three technical repeats