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HepG2 *in vitro* Model to Predict Liver Toxicity

D3-PharmaChemistry Line

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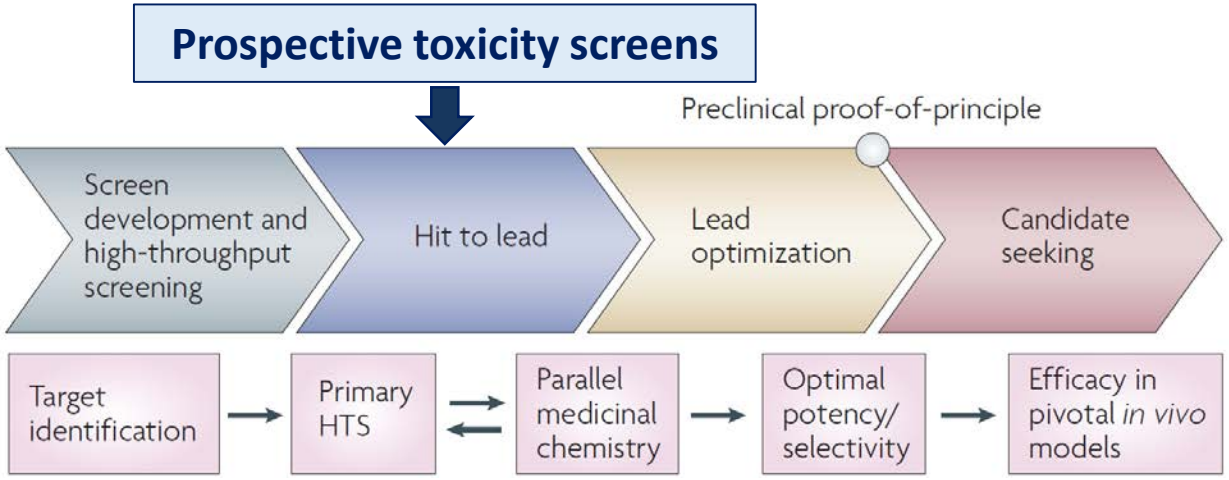
May 27th, 2024

Toxicity Assessment in Early Drug Discovery

Toxicity is a leading cause of **drug failure** at all stages of the Drug Development process (preclinical drug development, clinical phases, and post-market surveillance).

Approaches to identify "predictable" preclinical safety liabilities **earlier** in the Drug Development process could lead to the design and/or selection of better drug candidates that have increased probabilities of becoming marketed drugs.



Modified from Kramer et al.,Nat. Rev. Drug Discov. (2007)

Knowledge regarding the **toxicological liabilities** of the drug target and the chemical series.

Lead optimization to understand **Structure–Toxicity Relationships** (**STRs**), screen out development-limiting toxicities and minimize other adverse findings, thus delivering superior lead candidates into development.

Overview of some *in vitro* models to predict drug-induced liver toxicity

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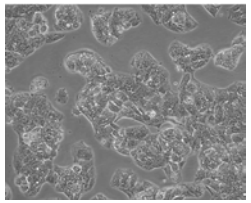
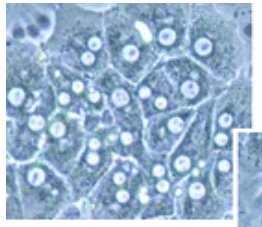
Traditional Liver 2D Models

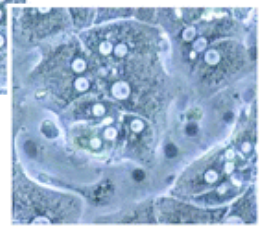
Cell lines



HepG2
HepaRG

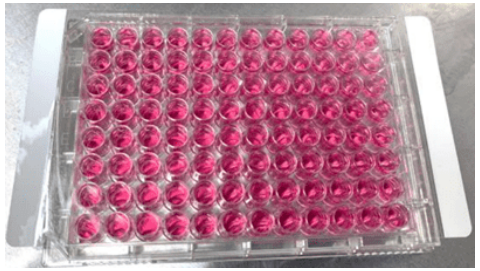
Primary Hepatocytes





Co-cultures of liver cells

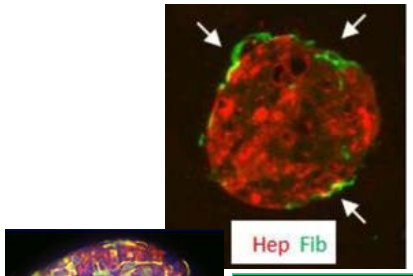
Long-term 2D co-cultures

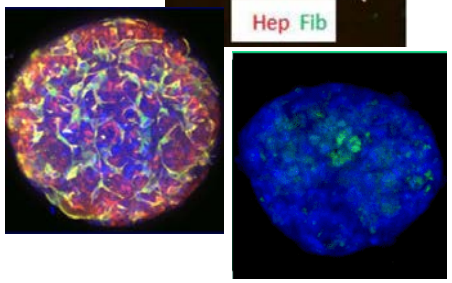


Primary Hepatocytes
+ Fibroblasts

Liver complex in vitro Models

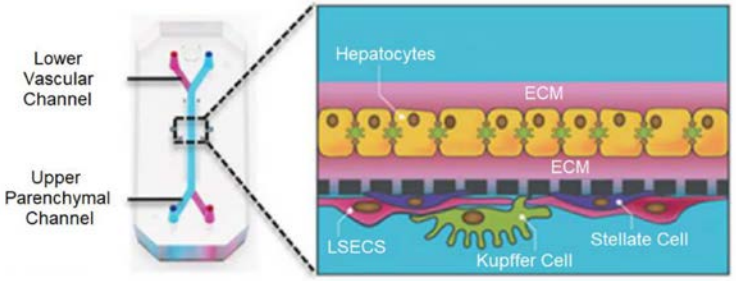
Long-term 3D co-cultures



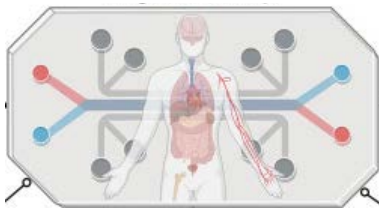


Spheroids
Organoids

Liver-On-A-Chip



Body-On-A-Chip

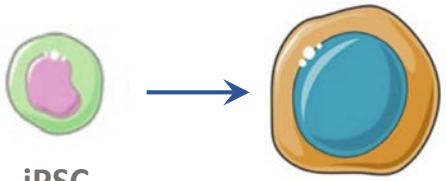


Simplicity
Reproducibility
Speed
Throughput

Complexity
Interplay of Different Cell Types
Physiological Relevance
Long-term Drug Exposure
Cost

Modified from Atienzar and Nicolas (2018). In: Chen, M., Will, Y. (eds) Drug-Induced Liver Toxicity. Methods in Pharmacology and Toxicology. Humana, New York, NY.

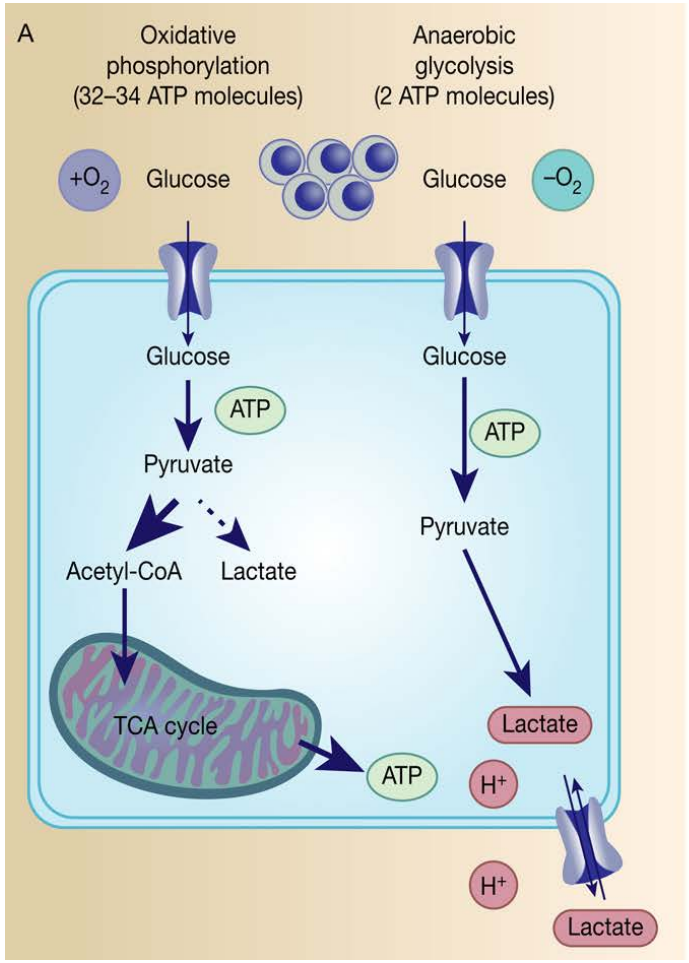
Advantages and Limitations of commonly used Cell Lines

		ADVANTAGES	LIMITATIONS
Immortalized Hepatic Cell Lines 	**HepG2**	• Highly proliferative • Readily available • Low cost • Easy to culture	• Altered metabolic function
	HepaRG	• Readily available • High metabolic activity • High albumin, ALP & AFP secretion • CYPs inducible	• Complicated differentiation & maturation conditions • Loss of proliferation following differentiation
	**Primary Human Hepatocytes (PHH)**	• Patient specific • Complete metabolic enzyme and transporters • Physiological function	• High cost • Limited source • Limited culture time • Rapid differentiation and loss of function
 iPSC	**iPSC-derived hepatocytes**	• Donor specific • Infinitely expandible	• High cost • Long time differentiation • Immature phenotype • Low CYPs expression

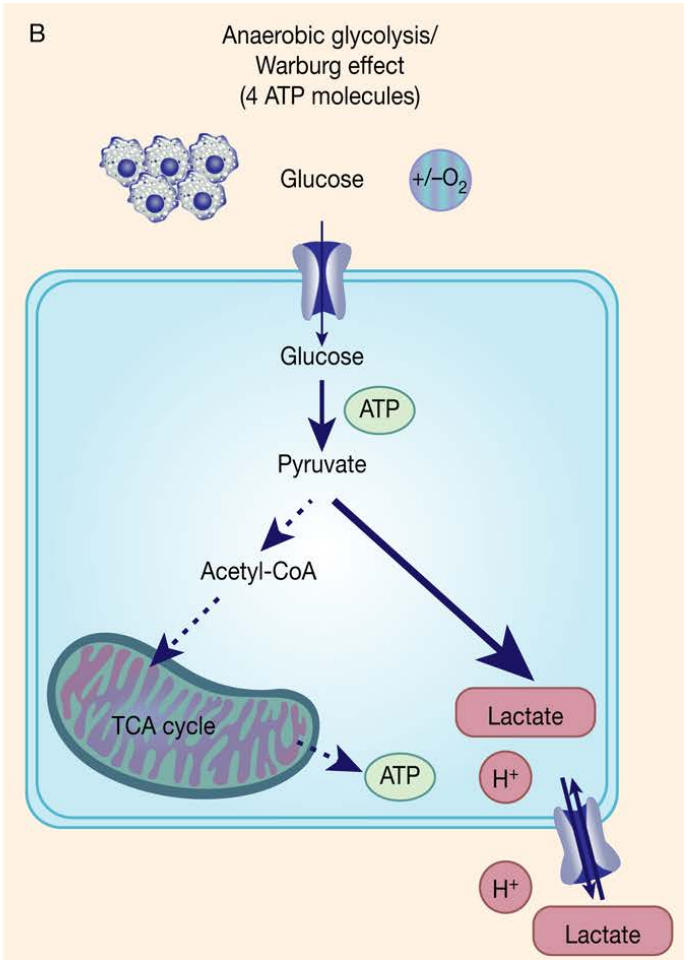
Modified from Yang et al., Cell Rep. Methods (2023)

The Warburg and Crabtree effects: Cancer Cell Energy Metabolism

Normal Cells



Tumor Cells



Unterlass and Curtin, Expert Rev. Mol. Med. (2019)

Warburg Effect

Enhanced **Glycolytic** Activity and
Impaired Oxidative Phosphorylation (**OXPHOS**)

↑ Tumor growth

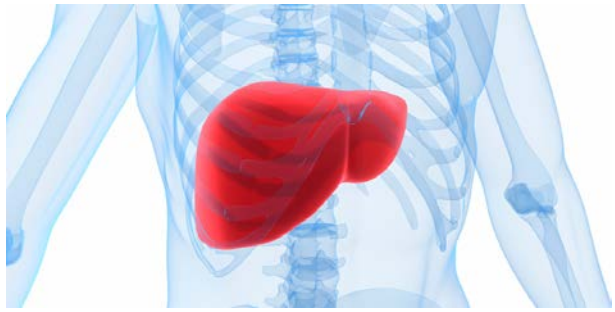
↑ Drug Resistance

Crabtree Effect

Some cancer cells, despite possessing functional mitochondria, can **switch** between glycolytic and oxidative metabolism in a **reversible fashion**

Drug-induced mitochondrial toxicity

HepG2 Model for Liver Toxicity Studies: Assay Principle



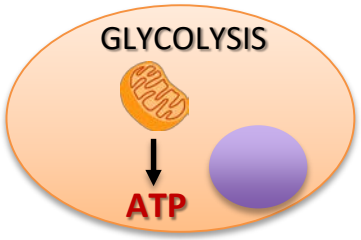
Liver heavily relies on **OXPHOS** for energy production

Investigate potential **mitochondrial liabilities** of new chemical entities to predict **hepatotoxicity**

Screening approach to discriminate **mitochondrial toxicity** from general cytotoxicity

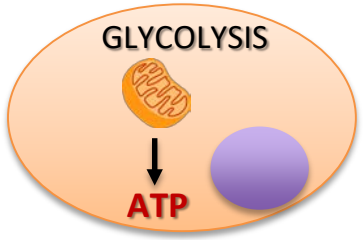
HepG2 Glucose/Galactose Model

Immortal Cell Line



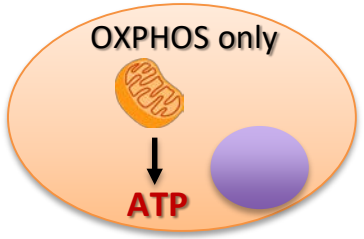
HepG2

GLUCOSE



Crabtree effect

GALACTOSE



Mitochondrial Toxicity Prediction
(ATP content + MTT)

Cytotoxicity Evaluation: A Multiple Endpoints Approach

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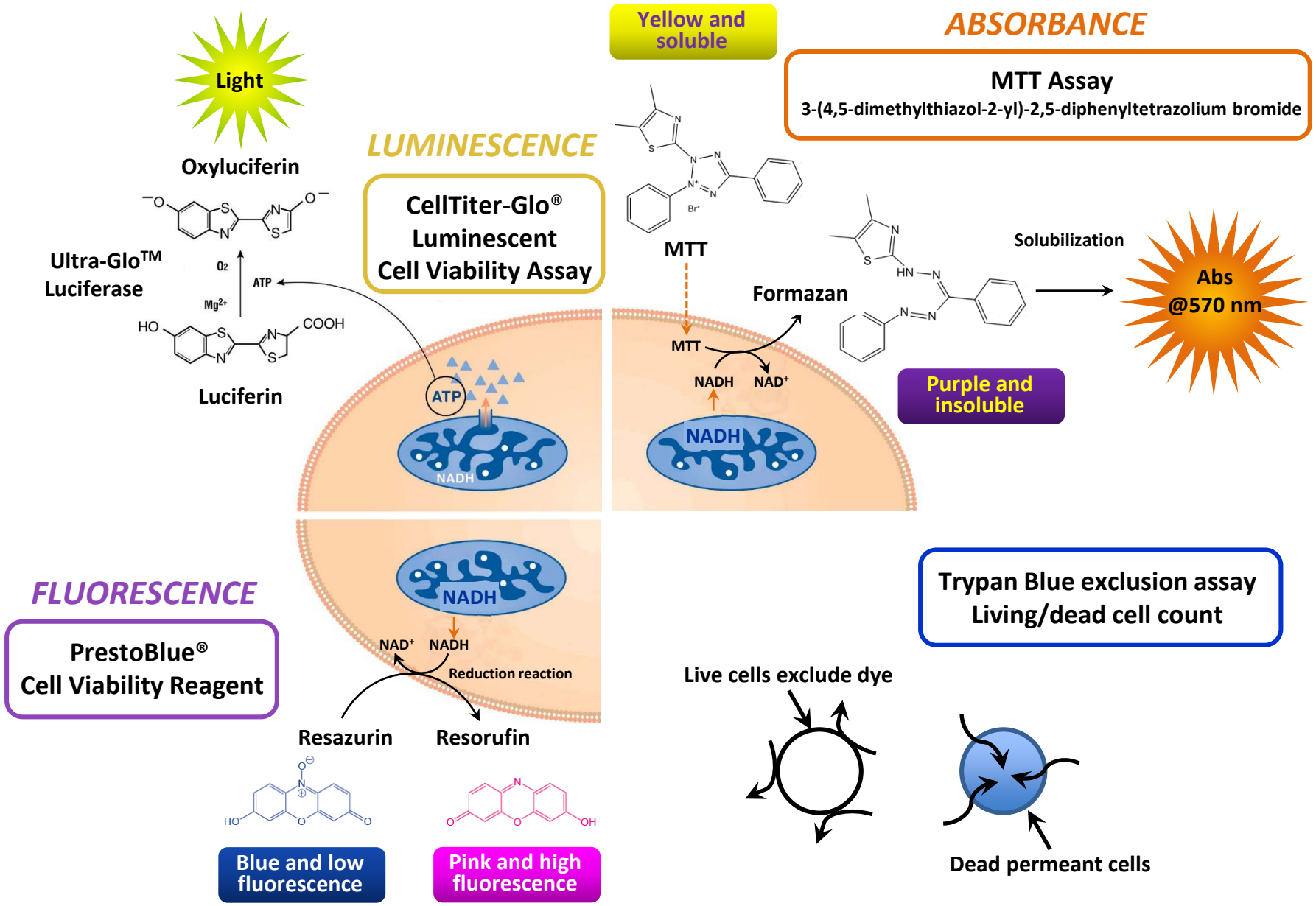


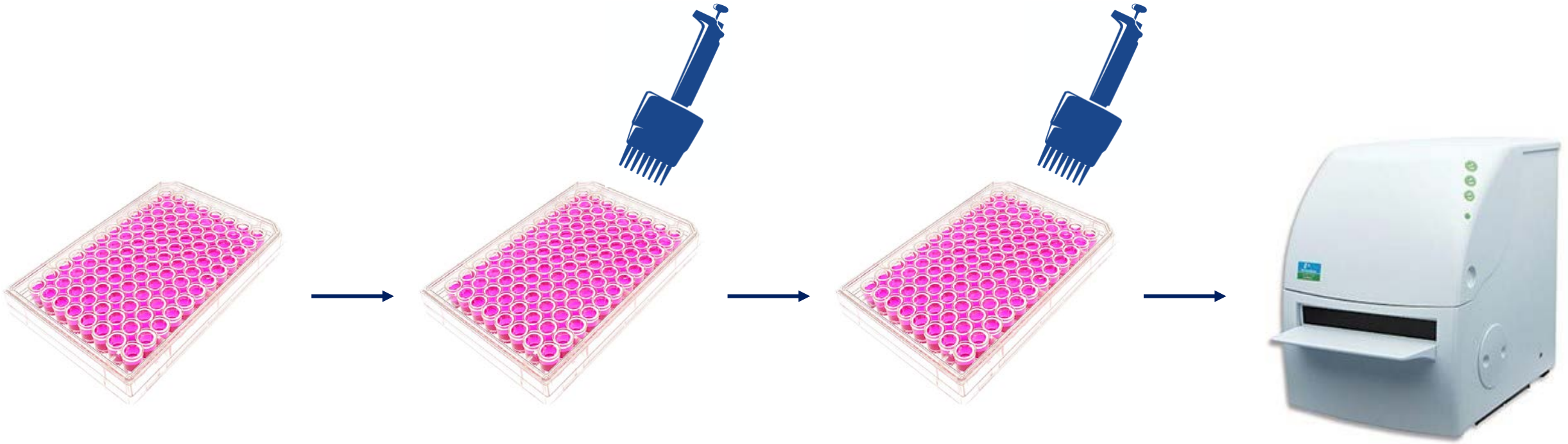
HepG2 Model for Liver Toxicity Studies: Assay Execution

HepG2 Glu/Gal 96-well plates

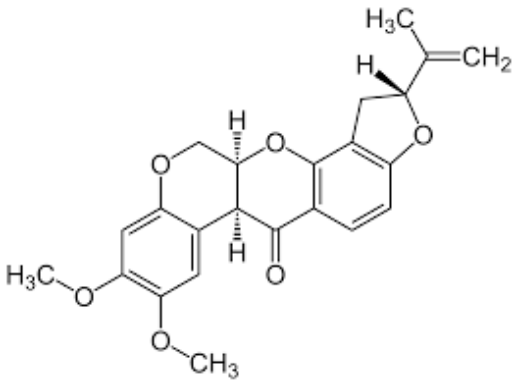
Compounds' Treatment (24hr)

Cytotoxicity Reagents Addition (MTT/CTG) and Incubation

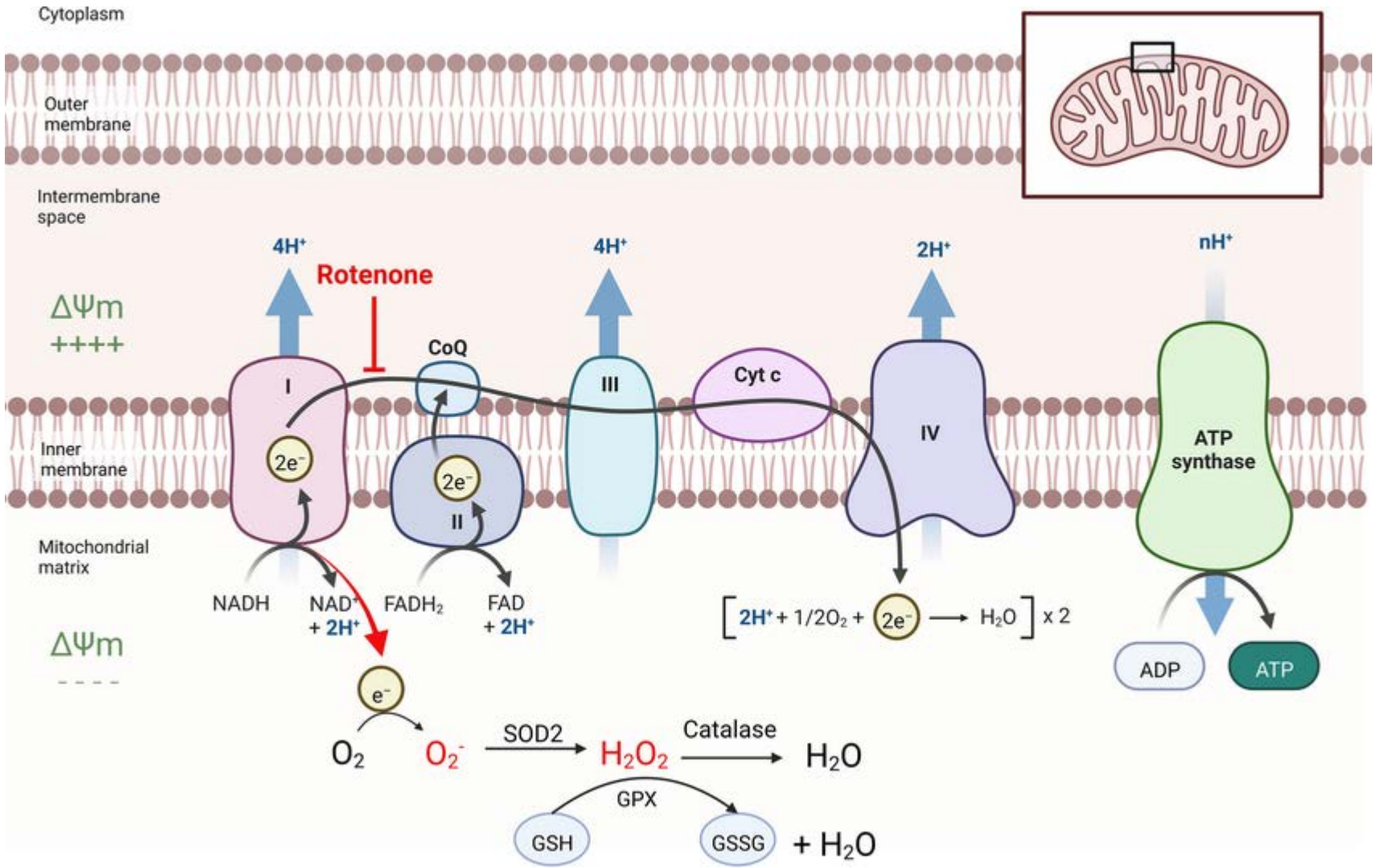
Measure Absorbance/Luminescence



HepG2 model for liver toxicity studies: Reference Compounds for Validation



ROTENONE
(*mitochondrial toxicant*)



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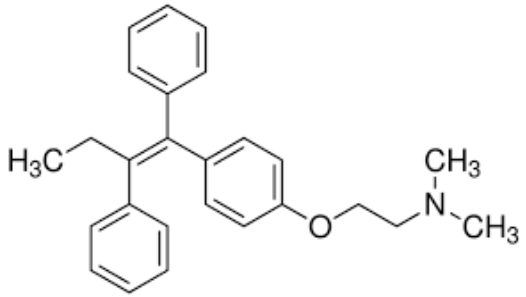
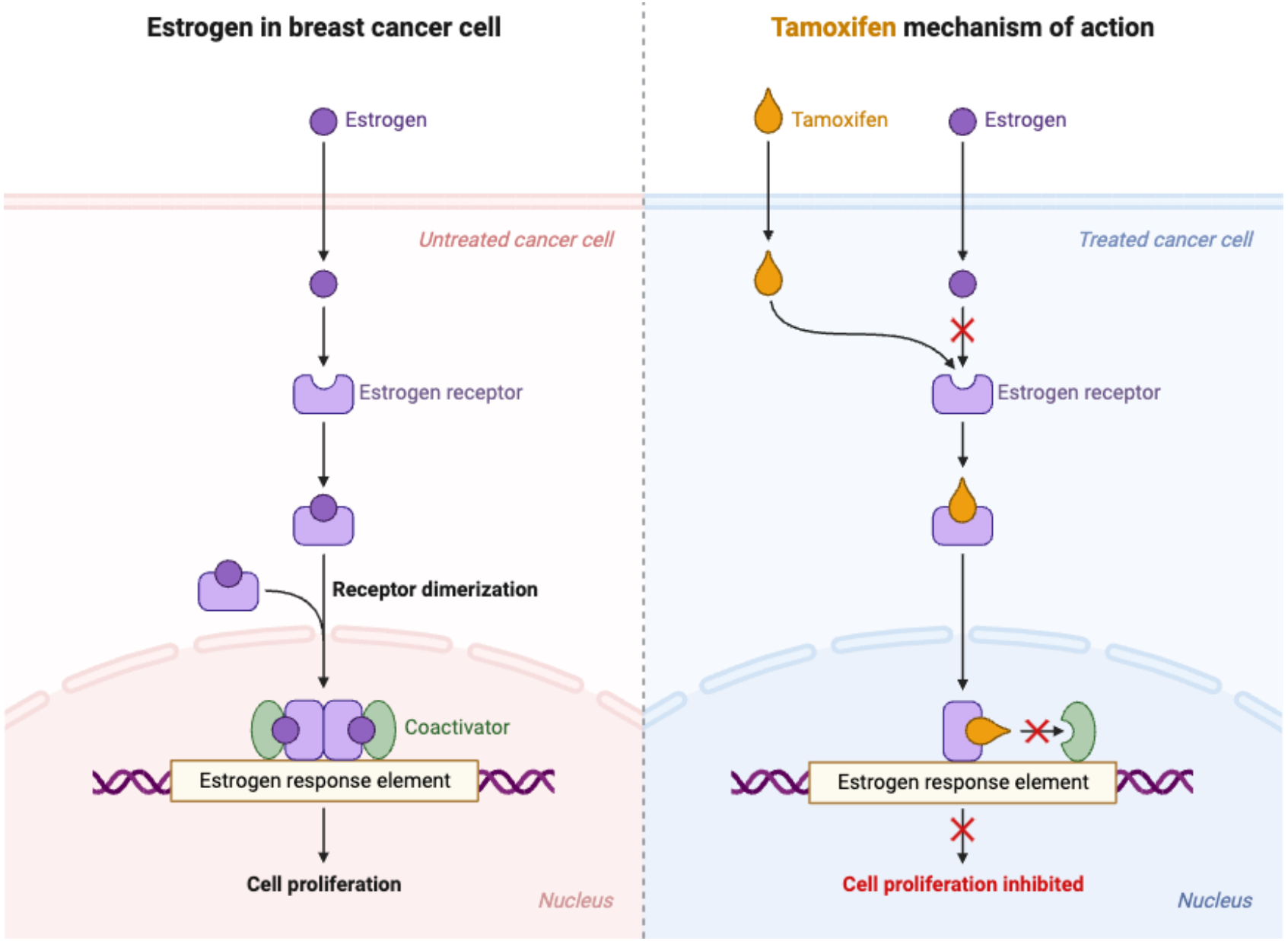


TAMOXIFEN
(*non-mitochondrial toxicant*)



HepG2 model for liver toxicity studies: Assay Validation

TASK FORCE for Cystic Fibrosis

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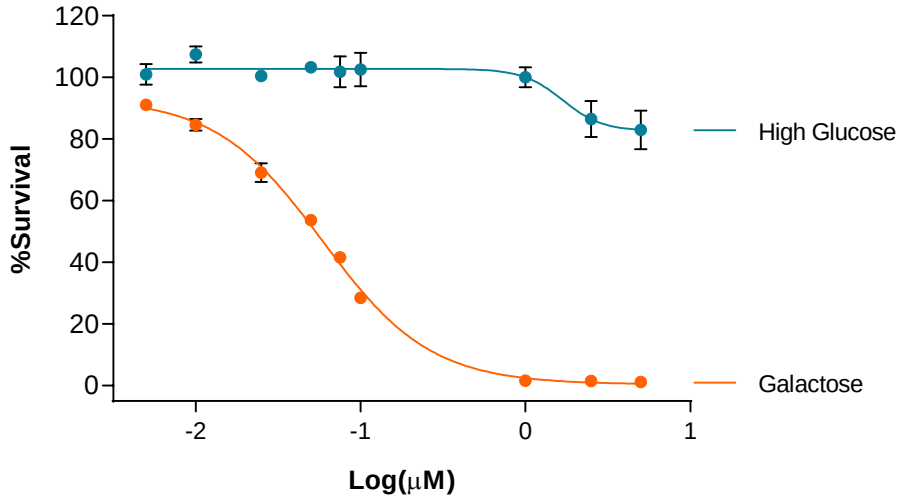
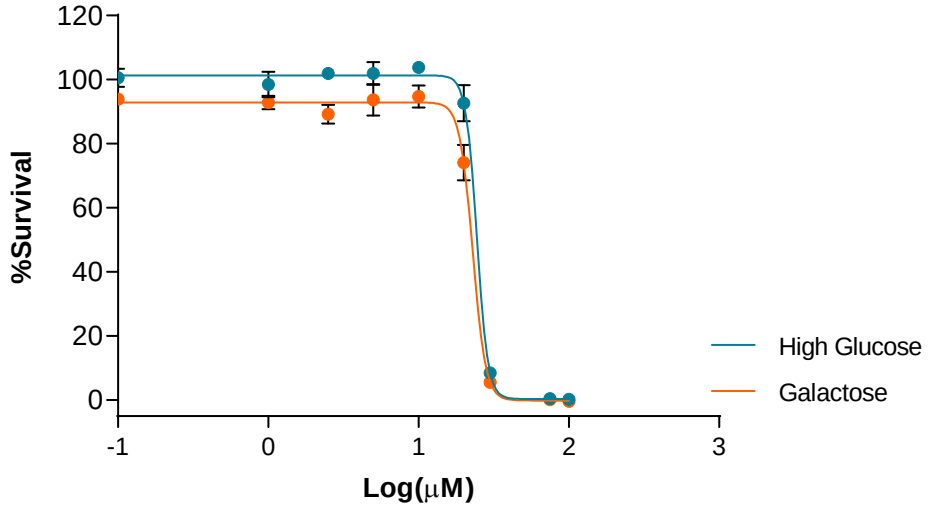
ROTENONE

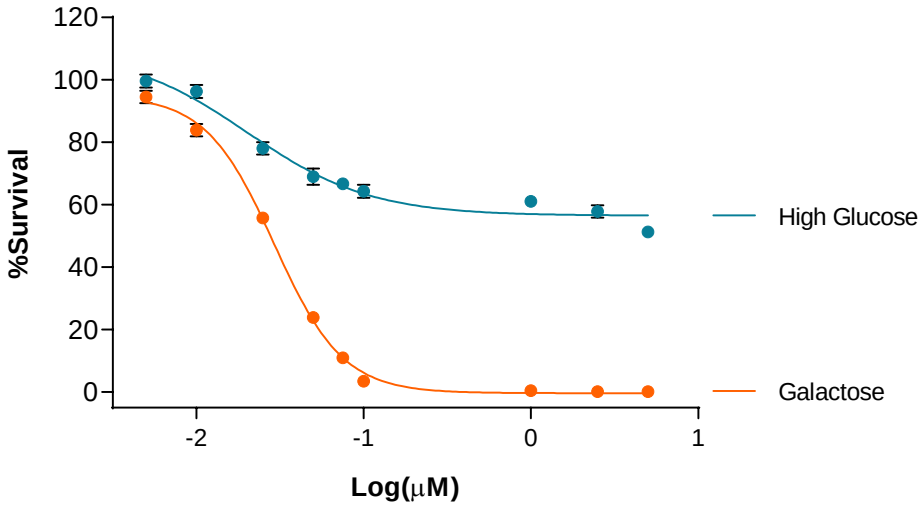
TAMOXIFEN

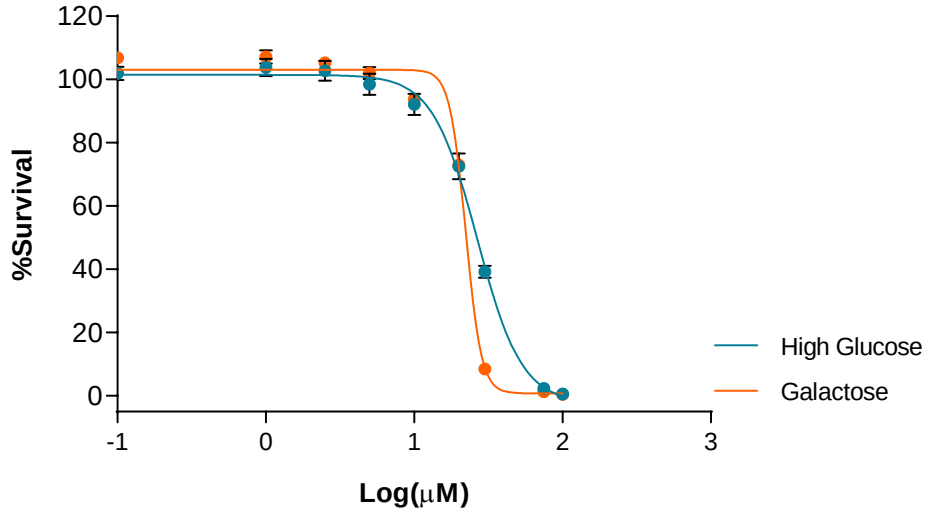
MTT ASSAY





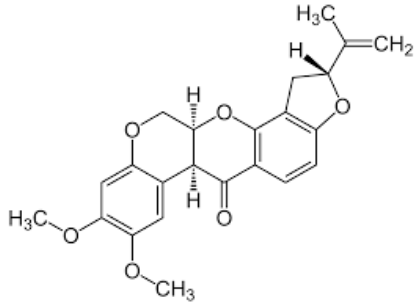
CTG ASSAY





HepG2 model for liver toxicity studies: Assay Validation

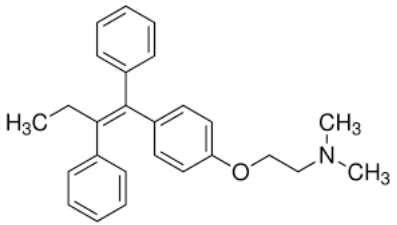
TASK FORCE for Cystic Fibrosis



ROTENONE
(mitochondrial toxicant)

Compound	Medium	% Survival at the Highest Dose (5 µM)
Rotenone	Galactose	2,26 ± 1,06
	High Glucose	80,54 ± 6,76

****p<0,0001*



TAMOXIFEN
(non-mitochondrial toxicant)

Compound	Medium	IC50 (M)
Tamoxifen	Galactose	19,87 ± 4,06
	High Glucose	24,21 ± 1,22

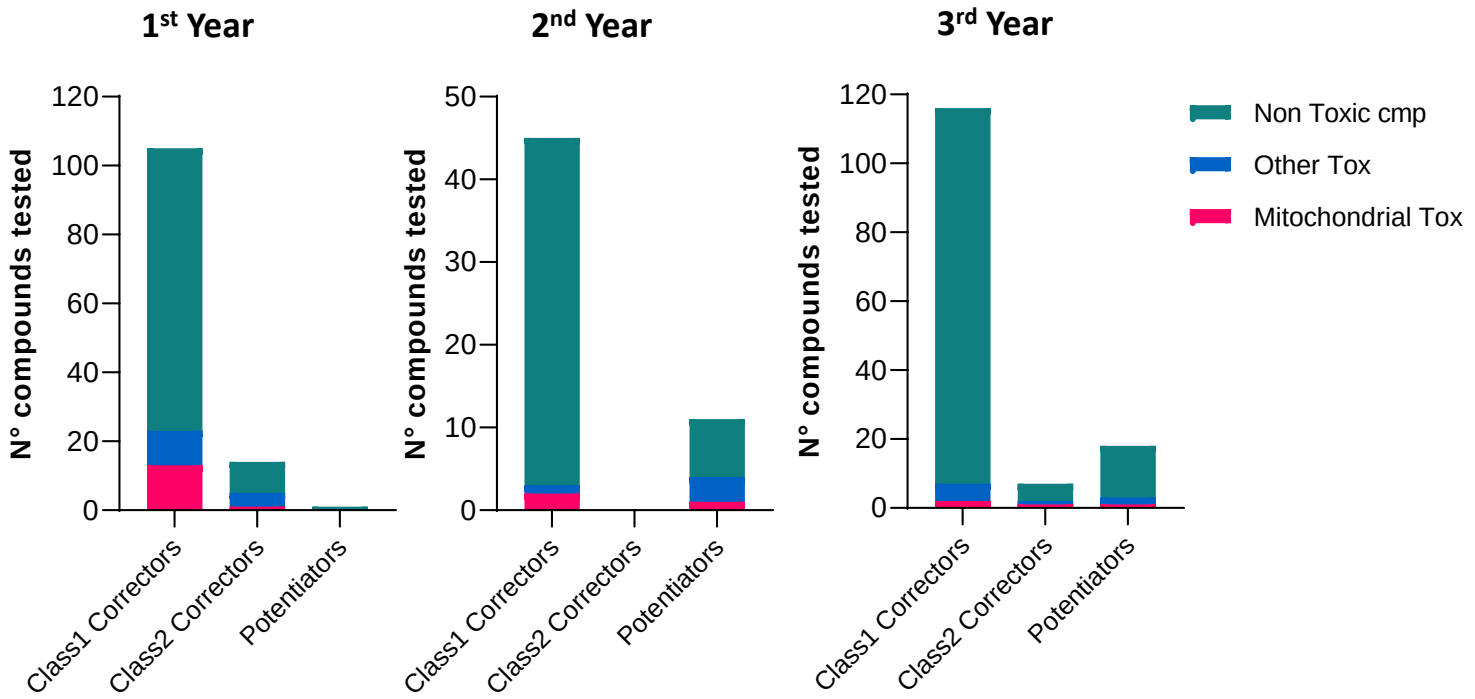
n.s.

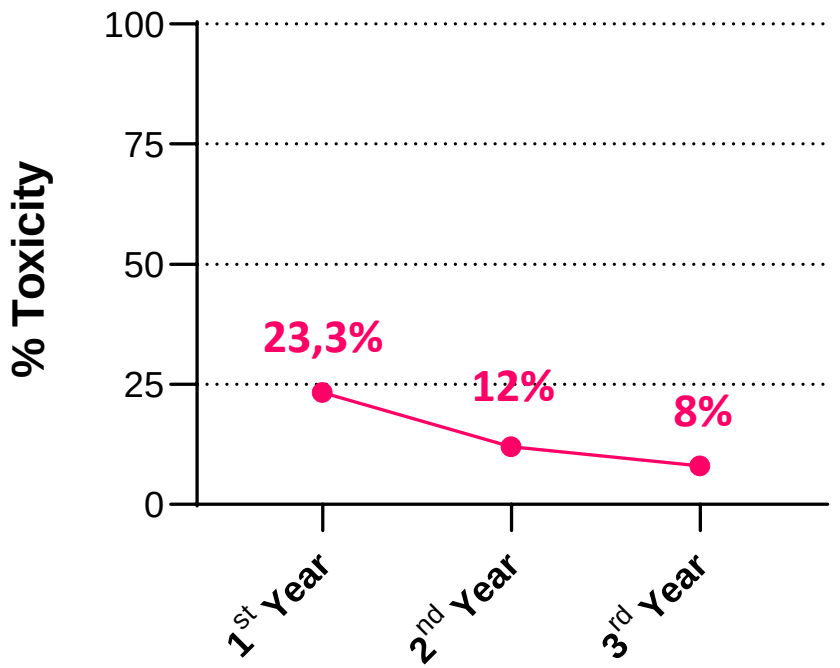
Average values ± SD of three independent experiments, each performed in three technical replicates

One-way ANOVA followed by Bonferroni's post-hoc test

HepG2 model for liver toxicity studies:
Compounds' screening within the TASK FORCE for Cystic Fibrosis Project

Assay	Assay Format	Readout	N° compounds tested	Compound Class
ATP-content	96 well plate	LUMINESCENCE	**317** cmps	**266** Class 1 Correctors
MTT		ABSORBANCE		**21** Class 2 Correctors
				30 Potentiators





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