Organs on a Chip: The Integrated Discrete Multiple Organ Co-culture System (IdMOC)

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Failure to Predict Human Drug Toxicity

- 25% of drug candidates failed in clinical trials due to toxicity
- Post-marketing withdraw or limited use of drugs due to adverse drug effects

Recently Withdrawn Drugs

- Vioxx (approved May, 1999; withdrawn September, 2004 due to association with cardiovascular risks)
- Propulsid (approved 1993; withdrawn 2000), gastric reflux, suspected 302 deaths due to cardiotoxicity
- Rezulin (approved 1997; withdrawn 2000), Type II noninsulin dependent diabetes, suspected 391 deaths due to liver failures
- Duract (approved 1997; withdrawn 1998), painkiller, suspect 68 deaths (17 liver failures)

Recently Withdrawn Drugs (contd)

- Posicor (approved 1997; withdrawn 1998), arrhythmia due to drug interactions, suspect 100 deaths
- Raxar (approved 1996; withdrawn 1997), arrhythmia, suspect 13 deaths
- Redux (approved 1996; withdrawn 1997), heart-valve damage, suspect 123 deaths
- Lotronex (approved 1997; withdrawn 1997), suspect 5 deaths and bowel surgeries

2003 and 2004 Safety Alerts (FDA MedWatch)

- Pergolide mesylate: cardiac valvulopathy
- Sildenafil-containing dietary supplements: fatal interaction with nitrates
- Risperidone: fatal cerebrovascular events
- ORLAAM: Severe cardiac-related events
- Repaglinide: Significant DDI with gemfibrozil
- Leflunomide: Rare fatal hepatic injurie
- Nevirapine: Severe, life-threatening hepatotoxicity

www.fda.gov/medwatch

Major Challenge: Accurate Prediction of Human Drug Toxicity

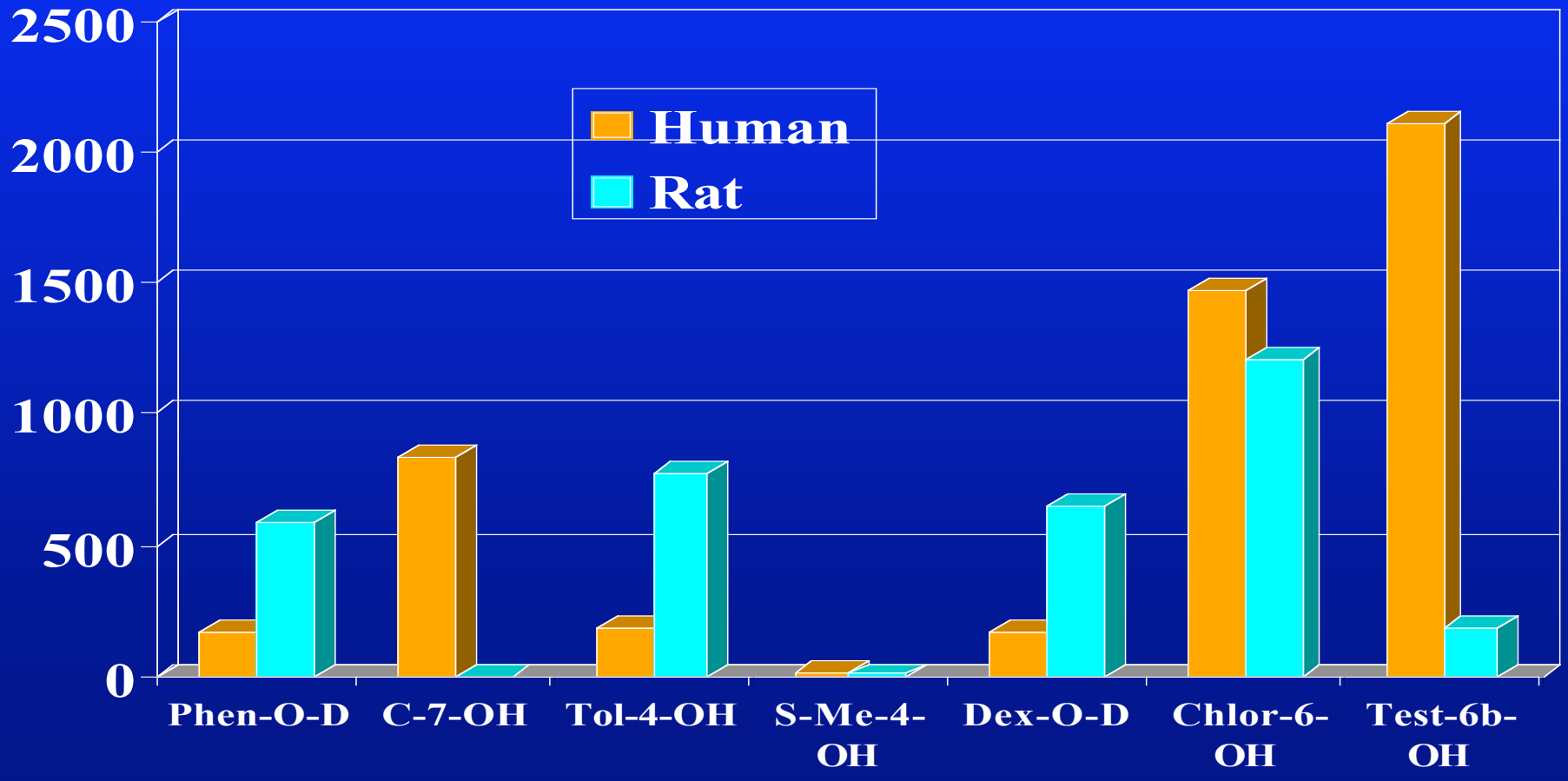
Limitation of Preclinical Safety Studies

- Known human-laboratory animal differences
  - Metabolic differences
  - Sensitivity of target organs to toxicants

Laboratory animal results do not always translate to man

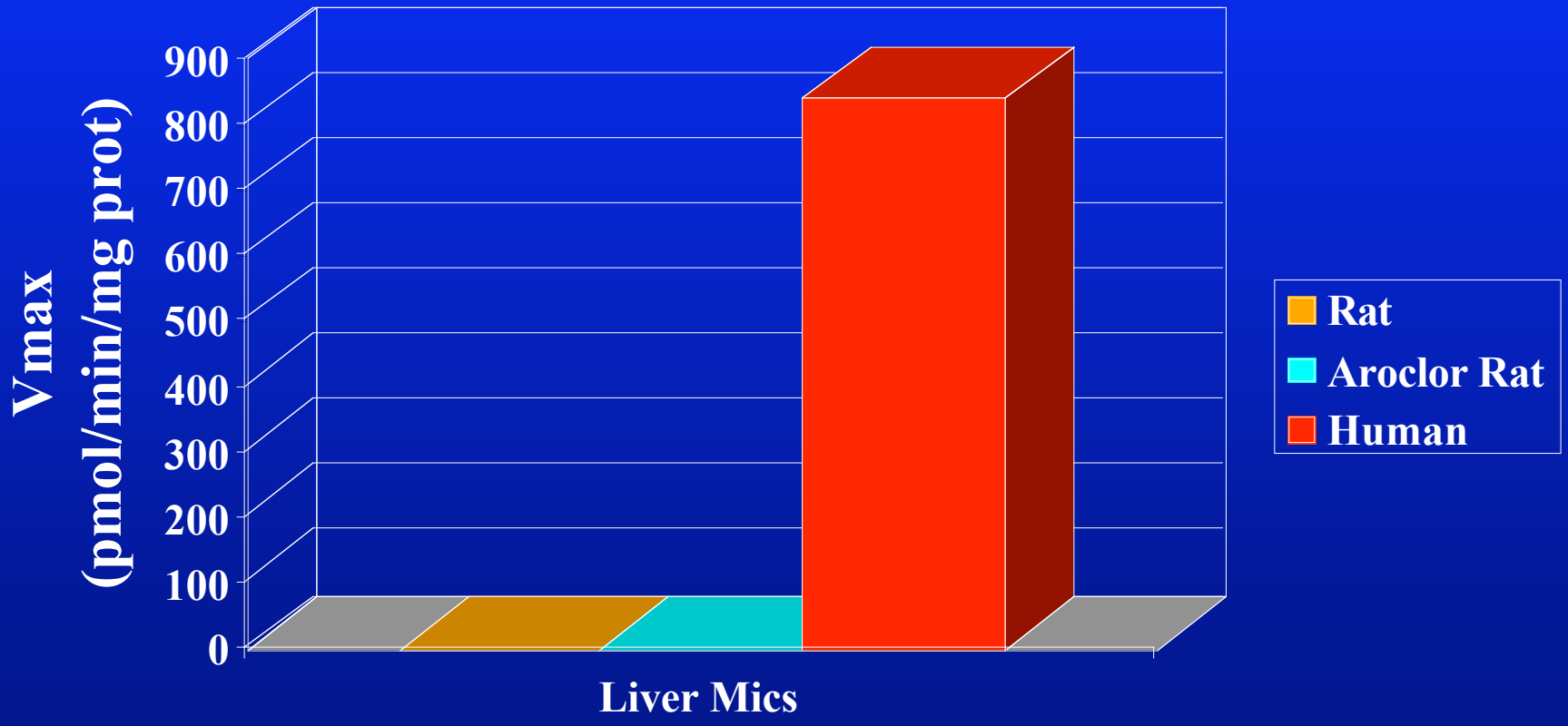
Comparison of Human and Rat Liver Microsomes in Xenobiotic Metabolism

(Easterbrook et al., CBI, 2001)

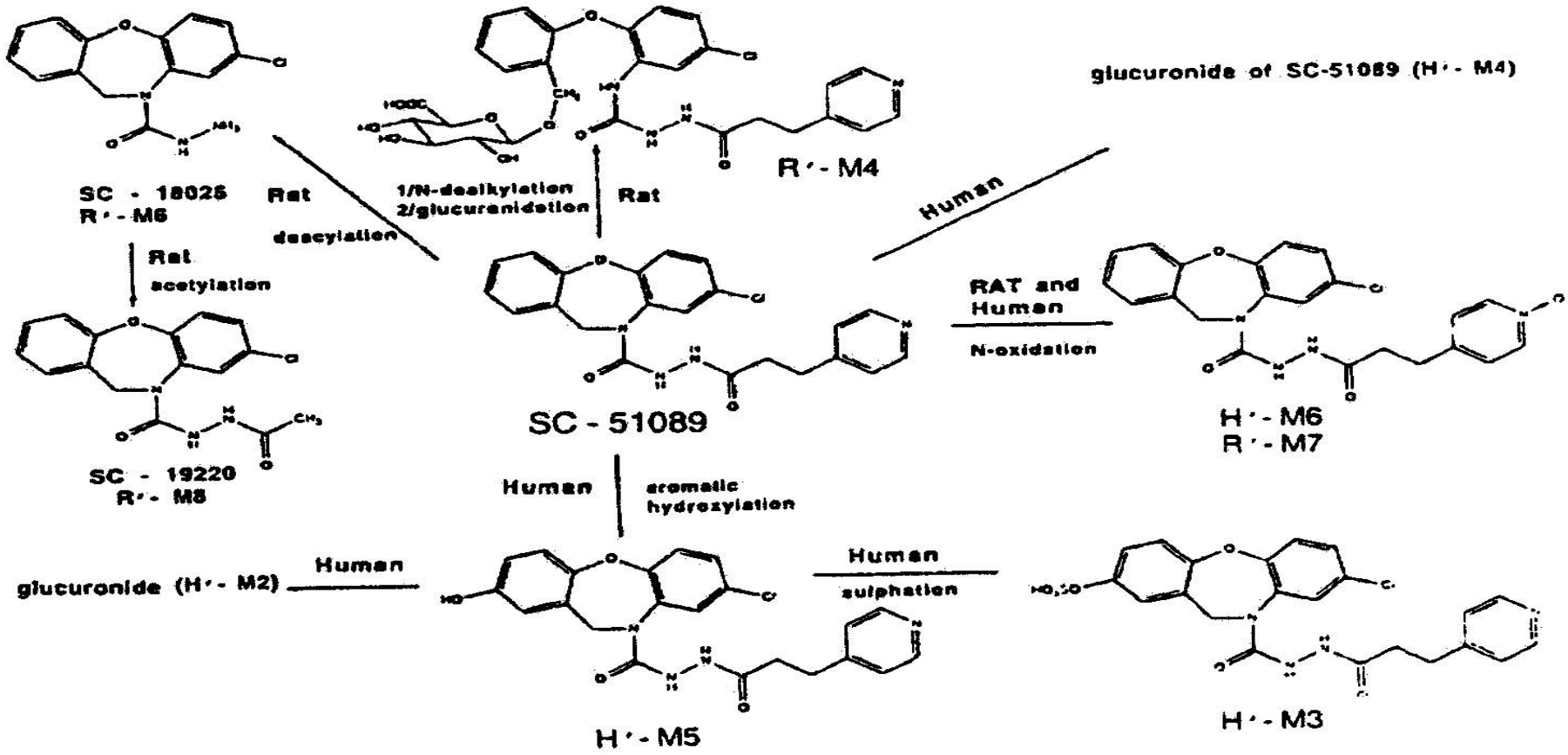


Coumarin 7-Hydroxylation in Rat and Human Liver Microsomes

(Easterbrook et al., CBI, 2001)



Metabolite Profiles in Rat and Human Hepatocytes



Lee et al. (1994), Xenobiotica 24: 25-36

Drug metabolism as a key determinant of species-species differences in drug toxicity

- An animal species which does not metabolize a parent drug to form metabolites found in human may under or over estimate drug toxicity in human
  - Underestimation of toxicity if the major toxic metabolites are human-specific
  - Overestimation of toxicity if the major toxic metabolites are animal-specific

Challenges in Today's Toxicology

- Urgent need for approaches to accurately predict human drug toxicity
  - Realization that classical toxicology is important but not adequate
- Challenge
  - Development and implementation of experimental systems for a more accurate prediction of human drug toxicity

Human-based In Vitro Experimental Systems for the Prediction of Human Drug Toxicity

Advances of Human Cell Culture Technologies

- Culture conditions established for the maintenance of differentiated functions for primary human cell cultures
- Increased practical applications in drug discovery and development
- Well-accepted commercial sources

Examples of Established Human Cell Systems

- Hepatocytes
- Endothelial cells
- Kidney tubule cells
- Osteoblasts/osteoclasts
- Astrocytes
- Airway epithelial cells
- Bone marrow cells/lymphocytes
- Various cell lines (e.g. Caco-2; MCF-7)

Key Components of an Alternative Experimental System for the Evaluation of Human Toxicants

- Human-specific metabolism
- Human target cells
- Predictive endpoints

Differentiated cells from key organs are critical to the success of an in vitro system for the evaluaton of human toxicity.

The use of dedifferentiated cell lines would not provide human-specific information, therefore would not improve upon current approaches

Hepatocytes as a Relevant Model for Human Hepatotoxicity Evaluation

- Relevant species: Human
- Relevant metabolism: Human hepatic metabolism
- Relevant cell type: parenchymal cells are known targets for hepatotoxicants

HepG-2 Cells

- Relevant species: Human
- Relevant organ: Liver
- Irrelevant target cells: Adenocarcinoma
- Irrelevant human metabolism: low (<1%) human liver drug metabolizing enzyme levels; embryonic rather than adult P450 isoforms

Successes in 3R

- *Refinement:* Metabolite profiling (hepatocytes from multiple species) for species selection for preclinical pharm/pk/tox studies
- *Reduction/replacement:* In vitro human liver system for Drug-drug Interactions for NDA – in vivo animal data considered inappropriate

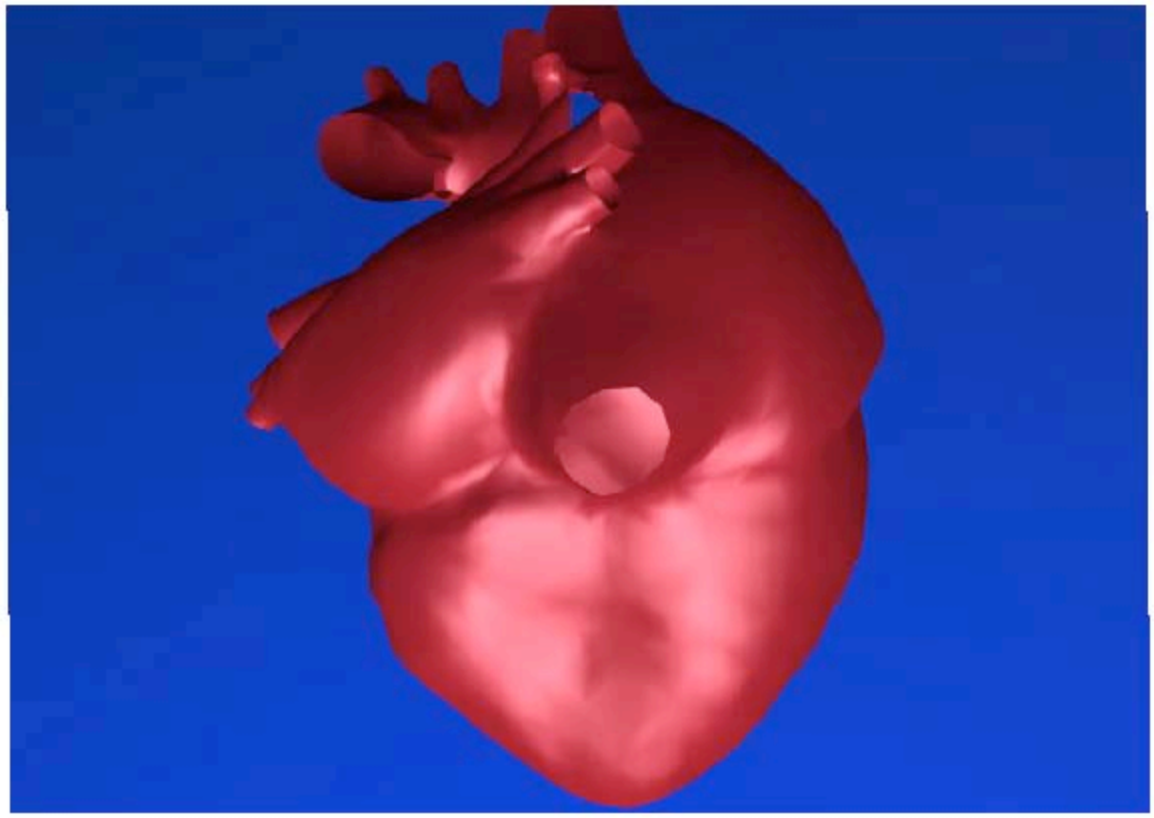
New Frontier: Human Toxicity Evaluation

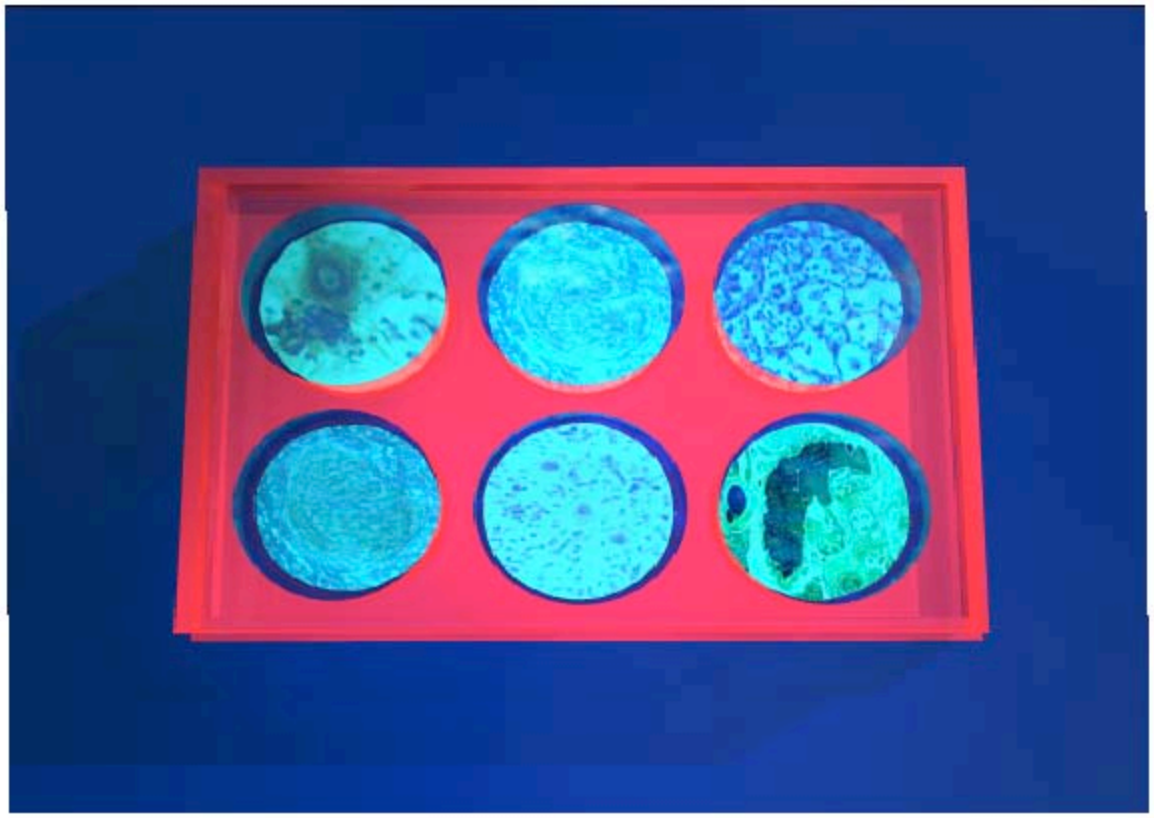
A major criticism of in vitro systems

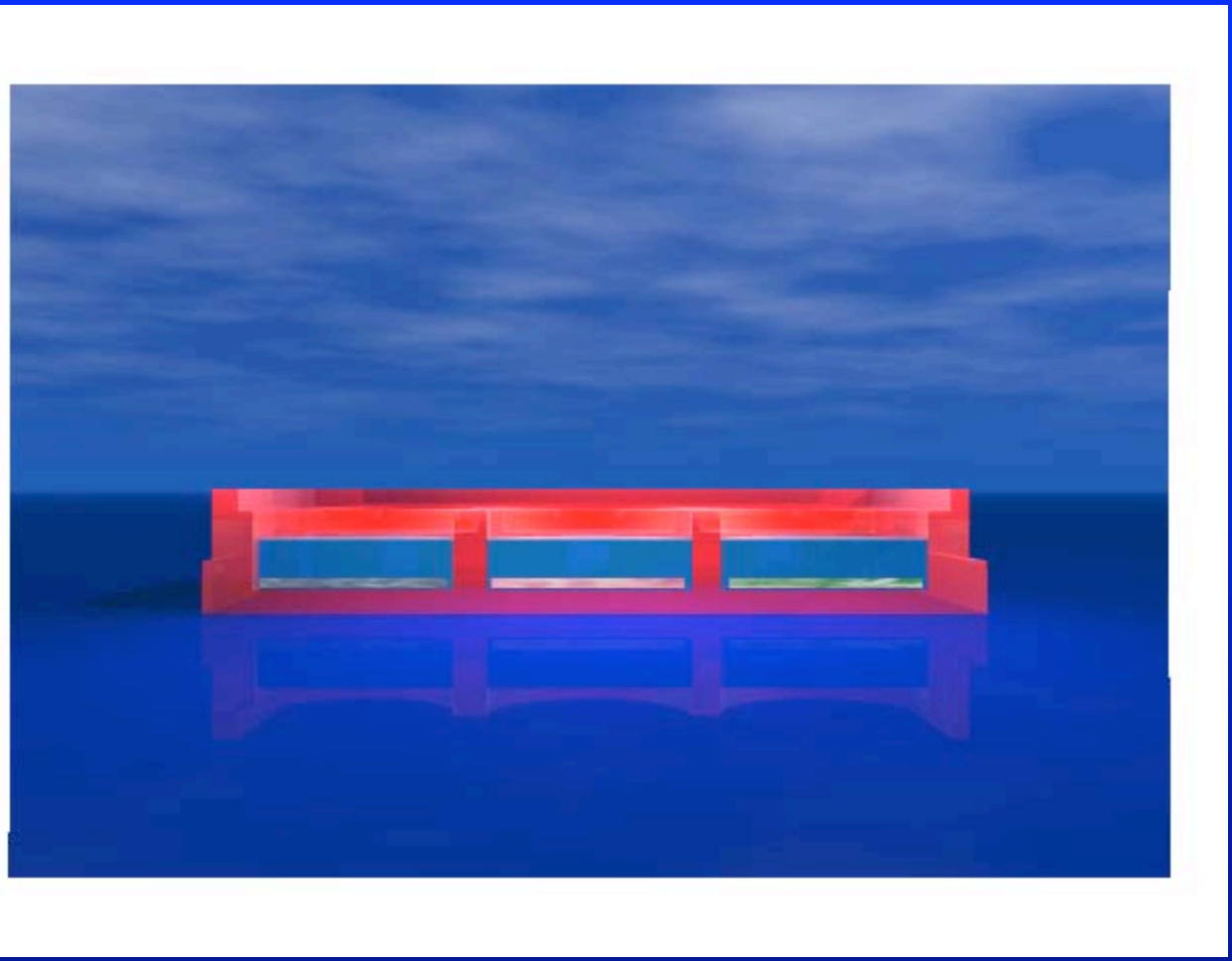
- Lack of multiple organ, systemic interactions
  - A toxicant and its metabolites may have multiple organ effects
  - A toxicant may be biotransformed by multiple organs, with metabolites from one organ may have effects on other organ(s)
  - Physiological changes (e.g. cytokine activation) in one organ may have effects on the toxicity of a toxicant on other organs

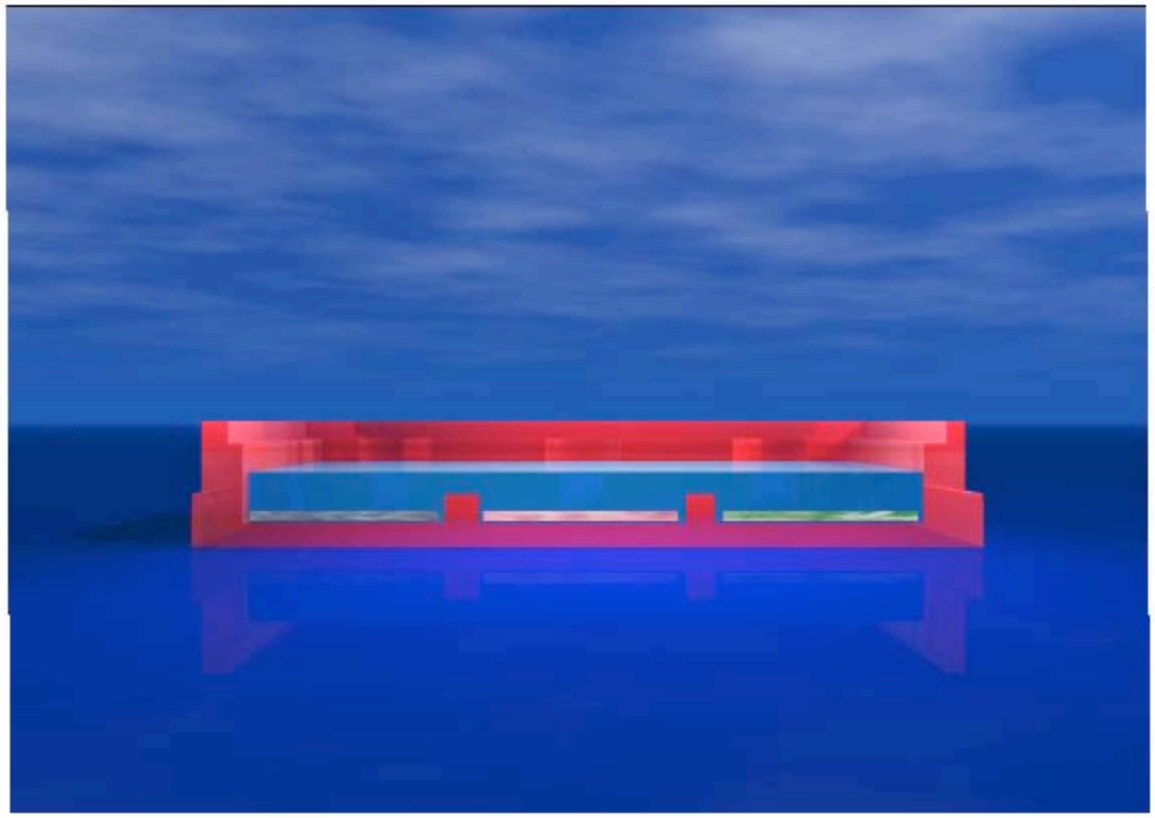
Integrated Discrete Multiple Organ Cell Culture (patent pending)

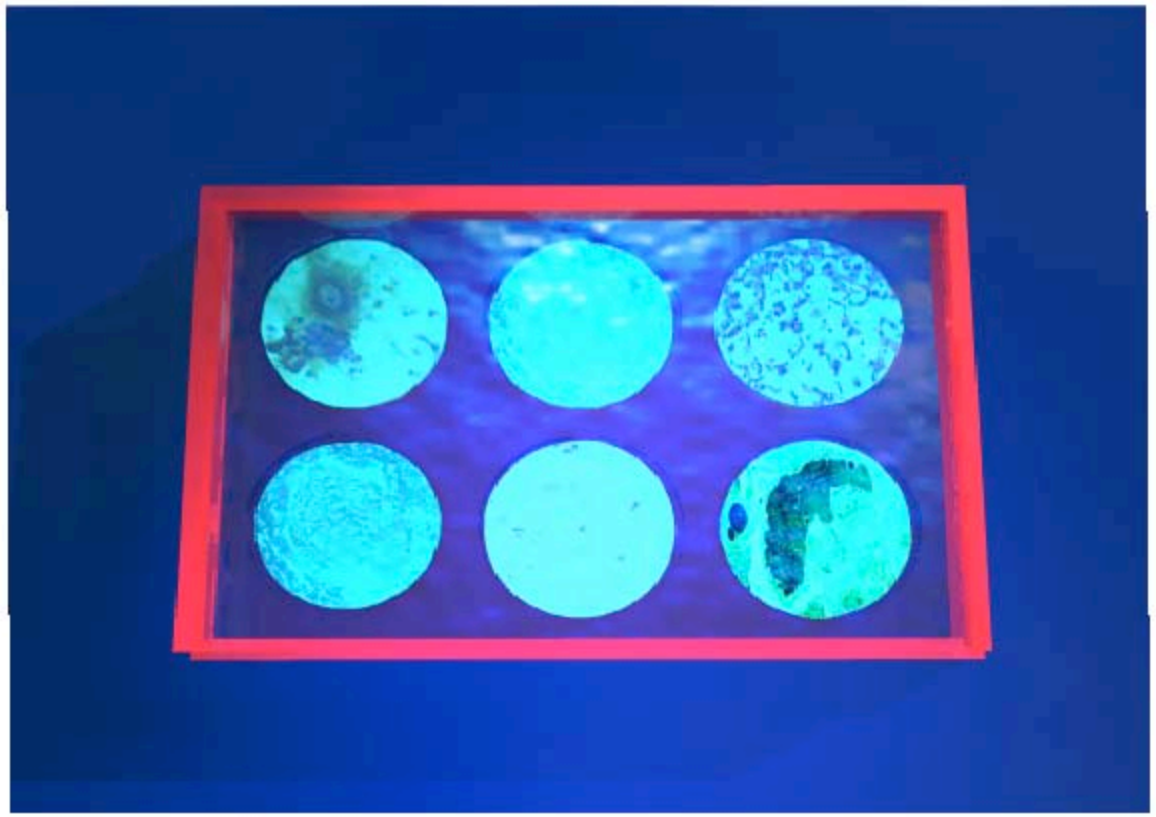
Li et al., Chem Biol Interact, 2004



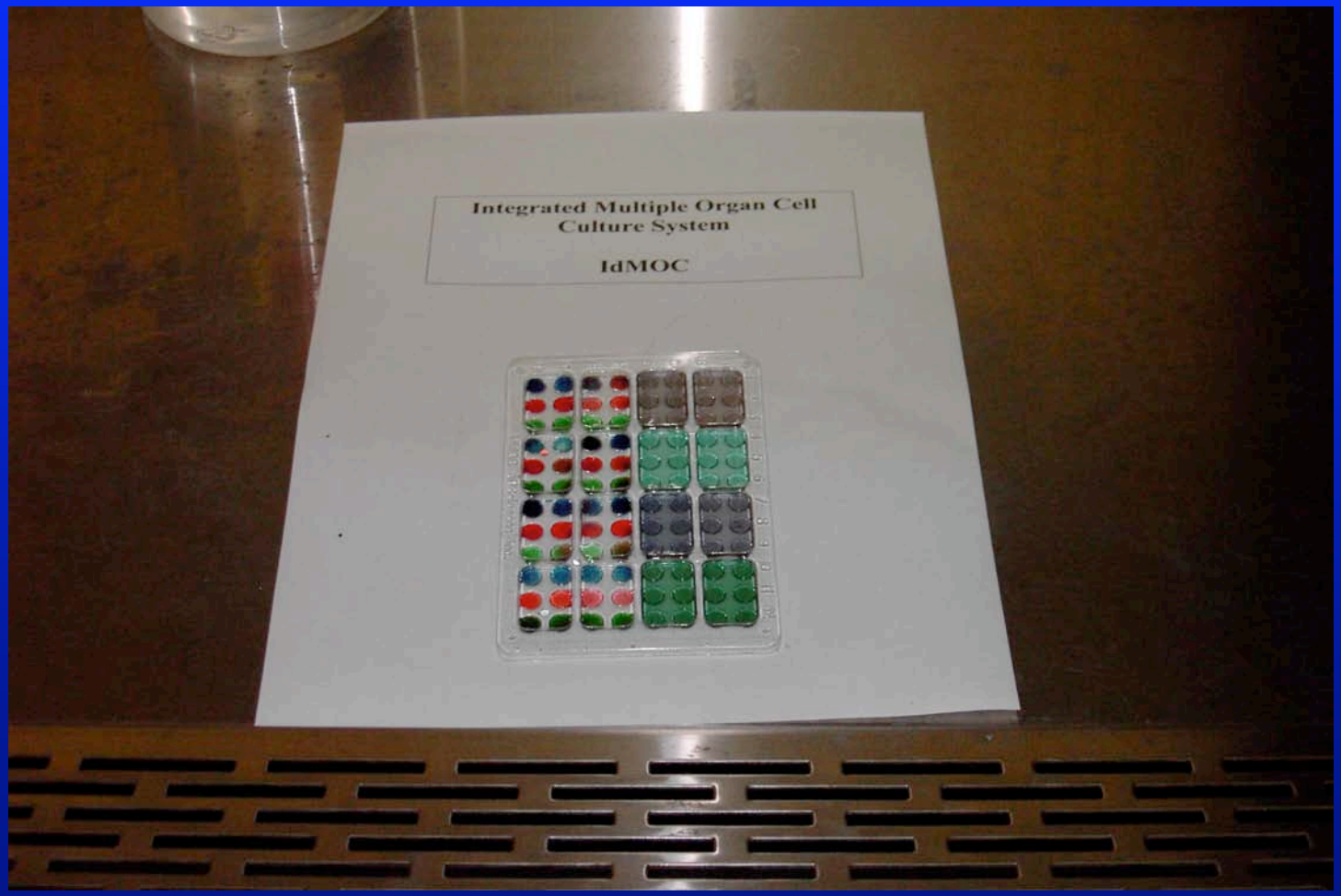








IdMOC 16x6



Cytotoxicity Endpoints (established)

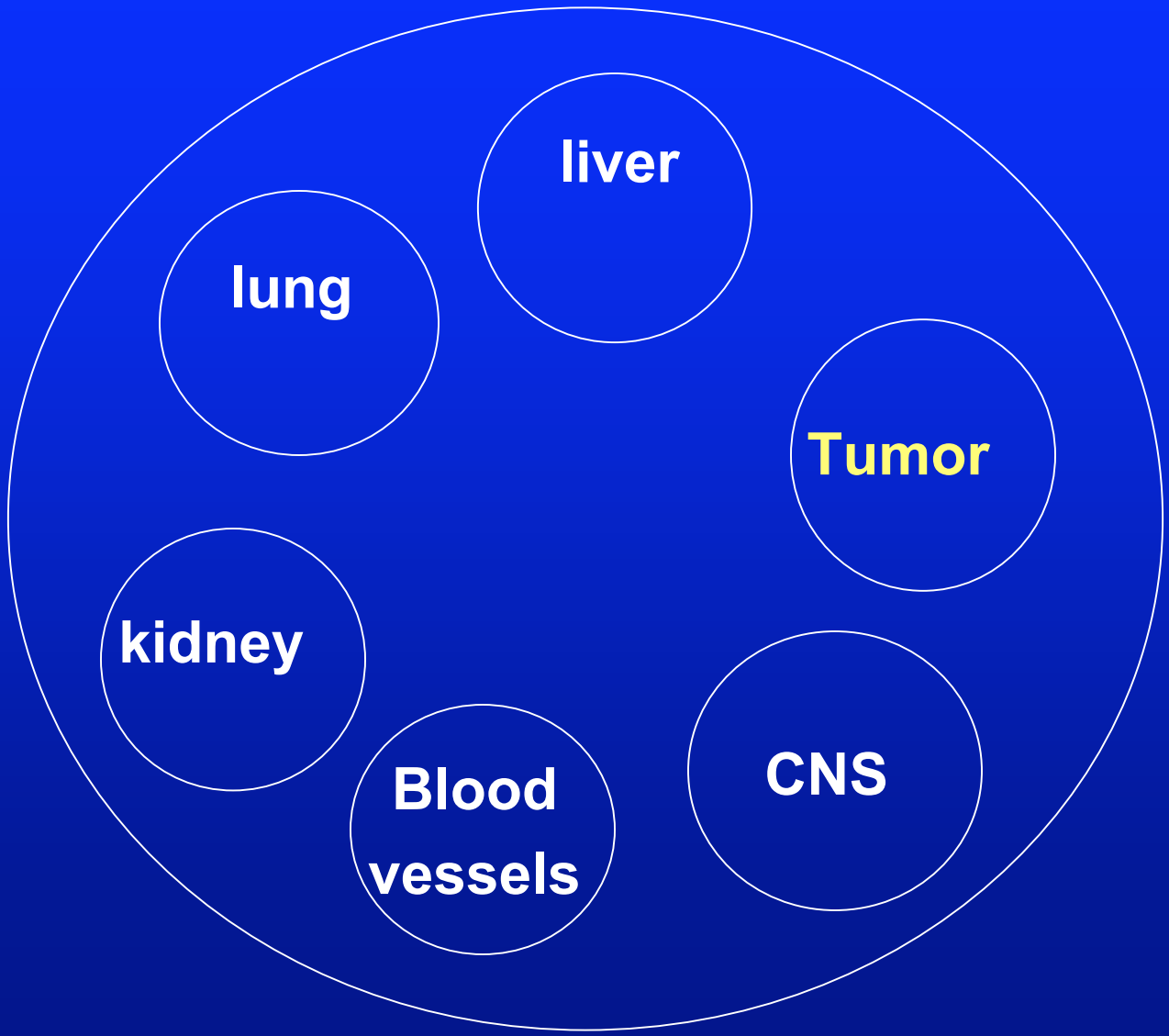
- ATP
- Cell number
- Protein
- Enzyme release
- MTT/MTS
- Alamar blue metabolism
- Neutral red uptake
- Caspase apoptosis

Predictive/Mechanistic Endpoints (in development)

- Toxicogenomics
- Metabolomics

IdMOC Evaluation of Anticancer Agents

idMOC model of a tumor-bearing man



Tamoxifen

- Estrogen antagonists for the treatment of estrogen-dependent breast cancer
- Known estrogen receptor independent toxicity towards multiple tissues

Cell Types Co-cultured in IdMOC

- Human Hepatocytes (Liver)
- Human Aortic Endothelial Cells (Blood Vessel)
- Human Astrocytes (CNS)
- Human Renal Proximal Tubule Cells (Kidney)
- Human Small Airway Epithelial Cells (Lung)
- MCF-7 (Human Breast Adenocarcinoma)

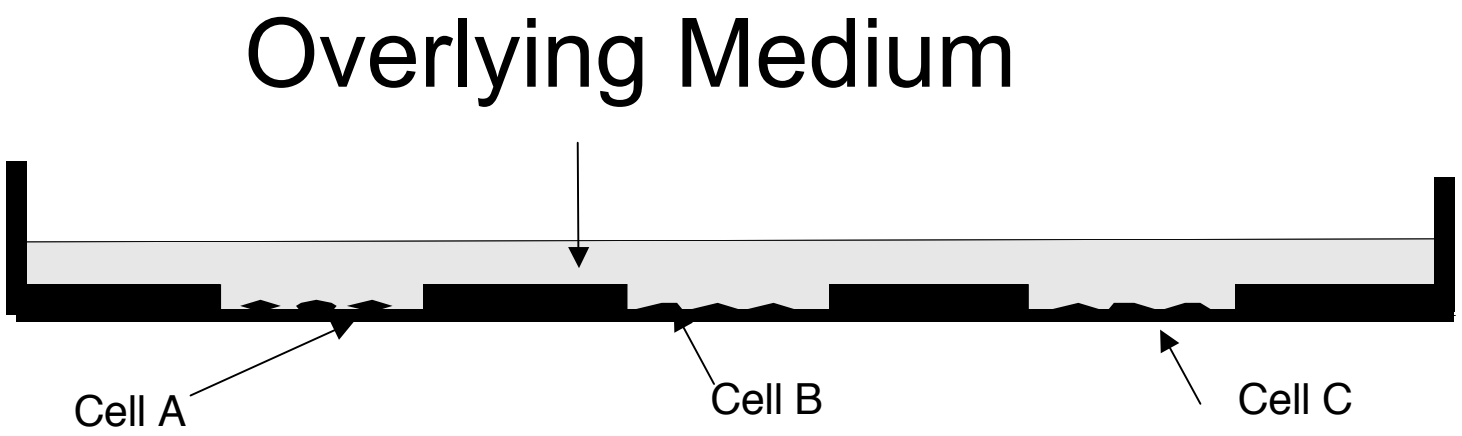
Evaluation of Anticancer Agent: Tamoxifen

- Can quantitative results on cytotoxicity be generated?
- What are the effective concentrations for 50% cell killing (EC50), 90% cell killing (EC90), and 99% cell killing (EC99)?
- What are the therapeutic index values for each cell type?

Procedures

- Day 1: Plating of each cell type in its respective medium into individual wells of the IdMOC
- Day 2: Removal of media, replace with MEM containing various concentrations of tamoxifen (0 – 200 uM)
- Day 3: Removal of treatment medium, addition of ATP reagent into individual wells to quantify ATP content in each cell type after treatment

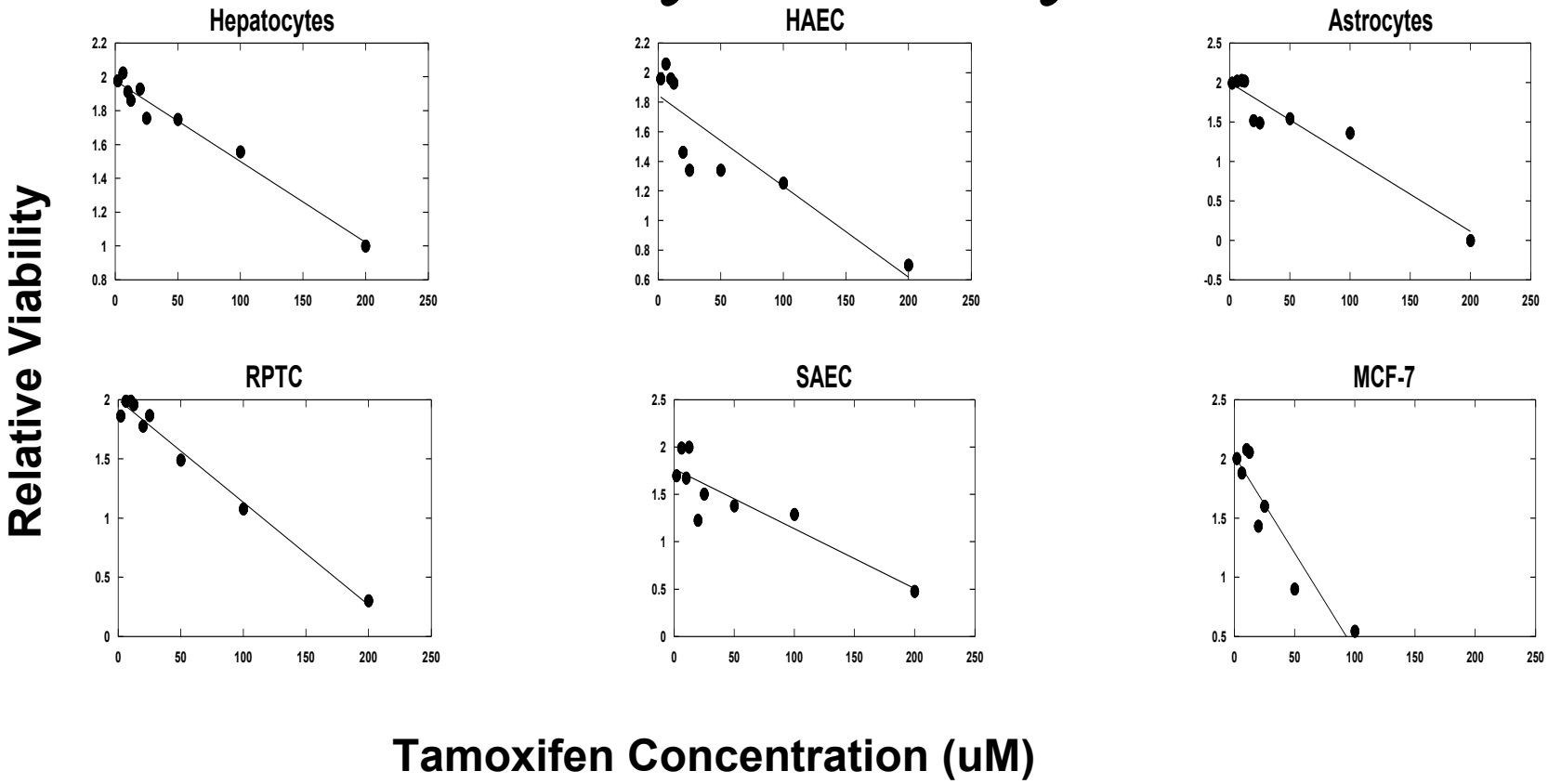
Schematic Diagram for IdMOC



Relative Viability

$$\text{Relative Viability (\%)} = \frac{\text{ATP (Treatment)}}{\text{ATP (Solvent Control)}}$$

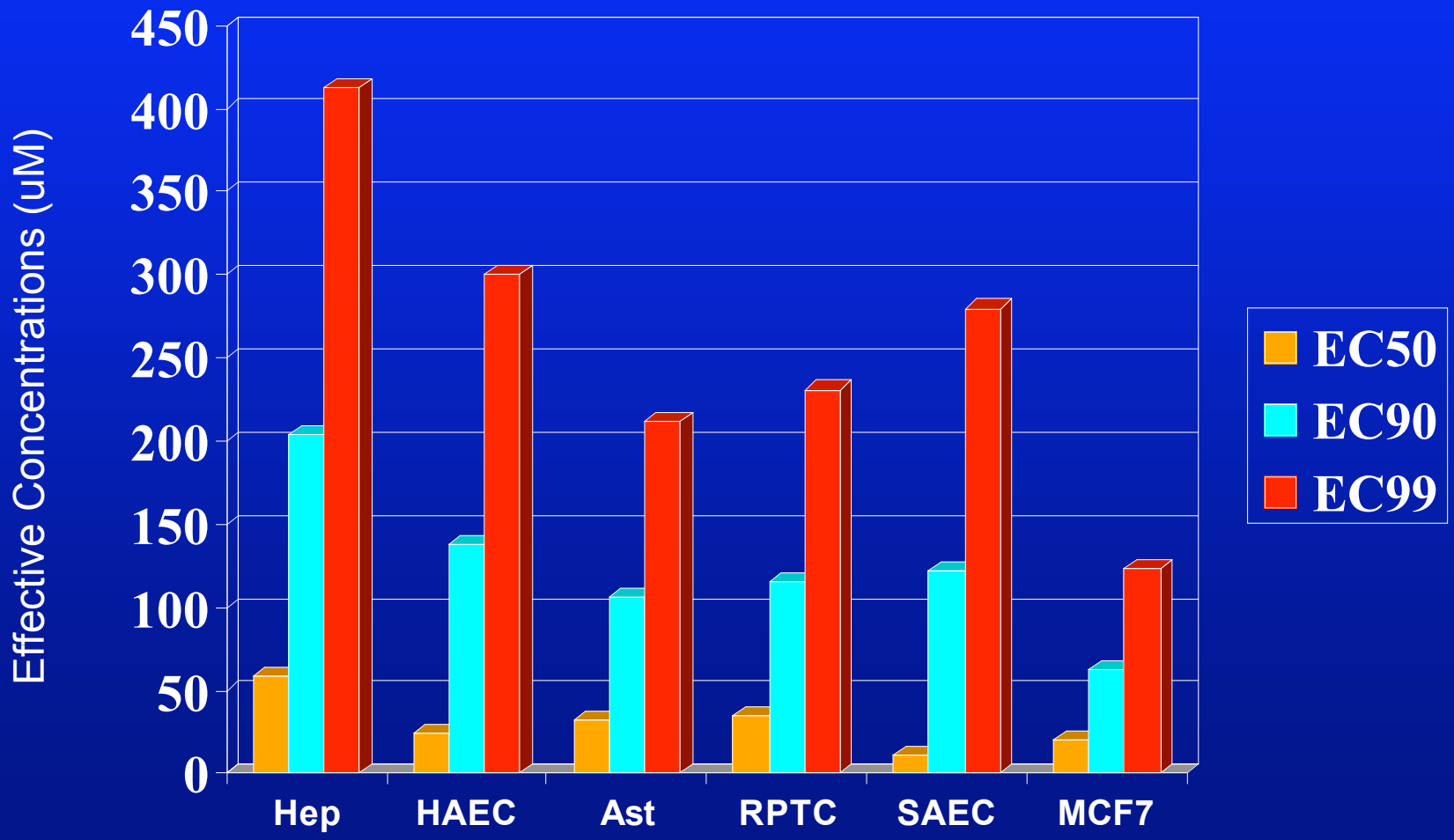
IdMOC Evaluation of Tamoxifen Cytotoxicity



Effective Concentrations (EC)

- Equation: Log (RS) = a + b (uM drug)
- EC50: Drug concentration for 50% RS
- EC90: Drug concentration for 90% RS
- EC99: Drug concentration for 99% RS

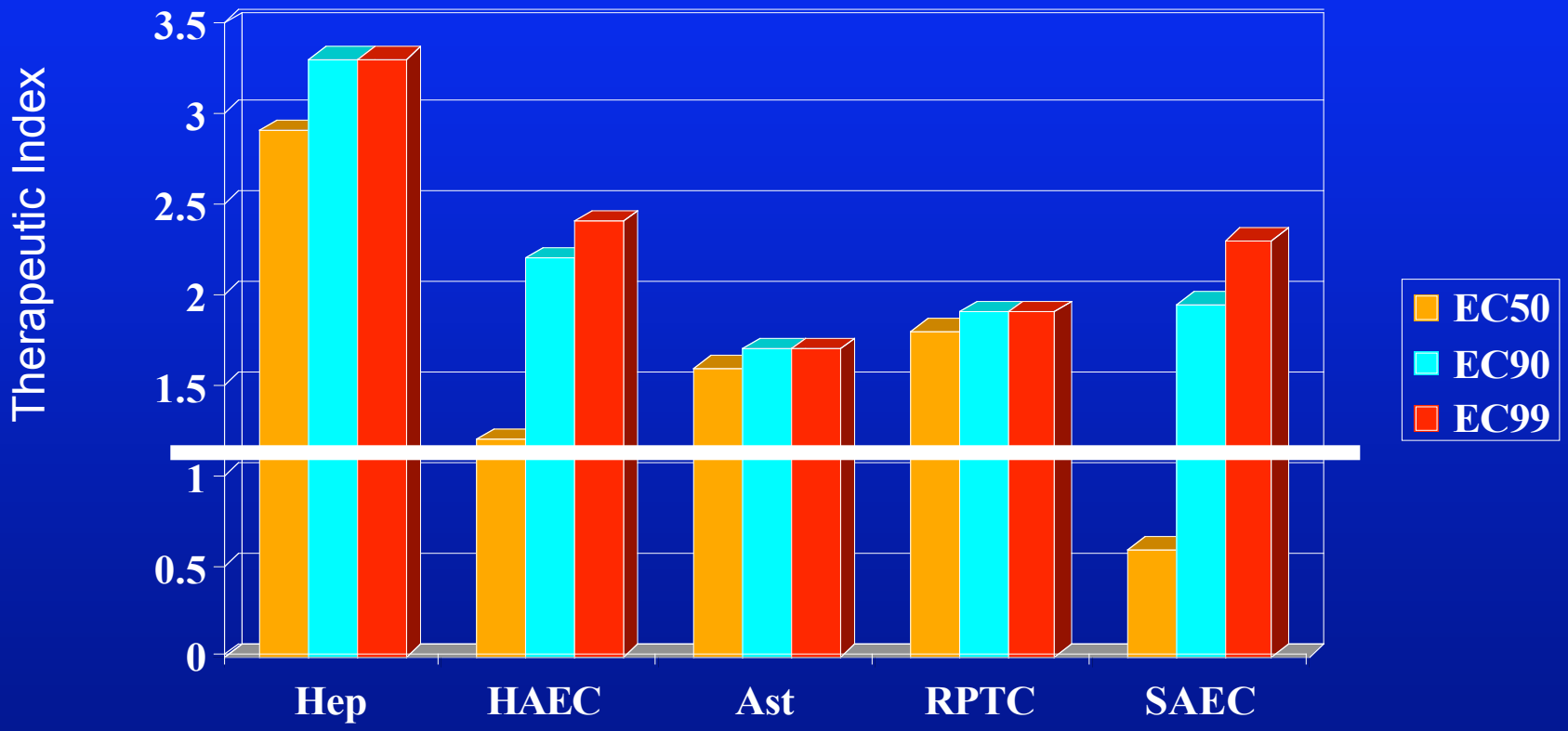
EC Values for the Multiple Cell Types



Therapuetic Index

- A common measure of safety – a ratio of toxic dose over therapeutic dose
- TI = EC (normal tissue)/EC (cancer)

In Vitro Therapeutic Index Values for the Multiple Cell Types



Relative Sensitivity of Multiple Cell Types towards Tamoxifen Cytotoxicity

EC50:

SAEC > MCF-7 > HAEC >Astrocytes >RPTC > Hepatocytes

EC90:

MCF-7> Astrocytes > RPTC > SAEC > HAEC > Hepatocytes

EC99:

MCF7>Astrocytes > RPTC > SAEC > HAEC > Hepatocytes

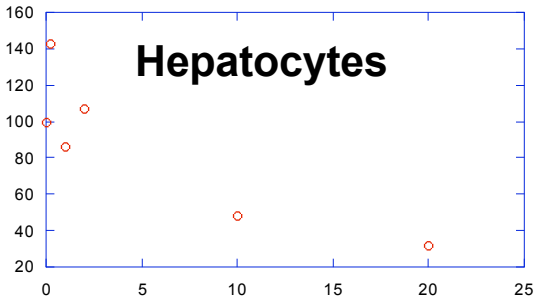
Summary: Tamoxifen

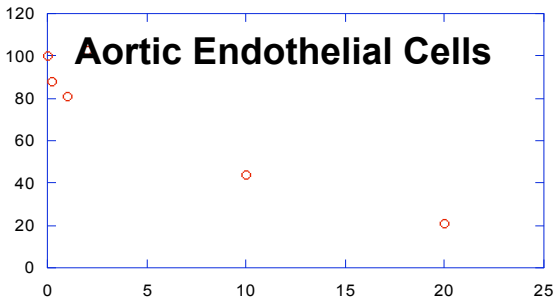
- Quantitative result obtained for the multiple cell types co-cultured in IdMOC
- EC values can be calculated for the calculation of TI
- MCF-7 as the most sensitive towards tamoxifen cytotoxicity
- Hepatocytes as the most resistant towards tamoxifen cytotoxicity

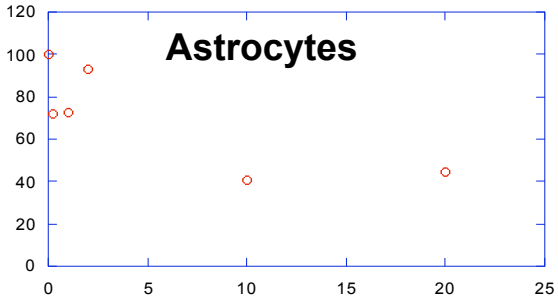
Cyclophosphamide

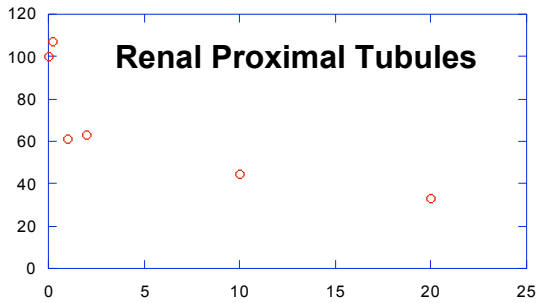
- Anticancer agent with known multiple organ toxicity
- Requires metabolic activation to be toxic

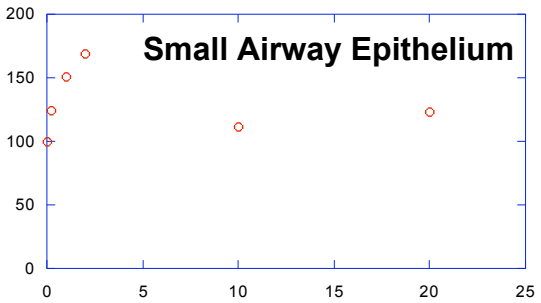
Cyclophosphamide Cytoxicity in IdMOC





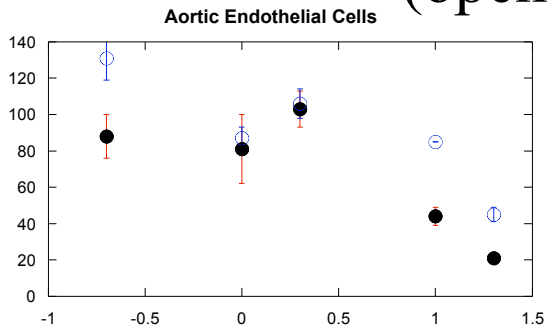


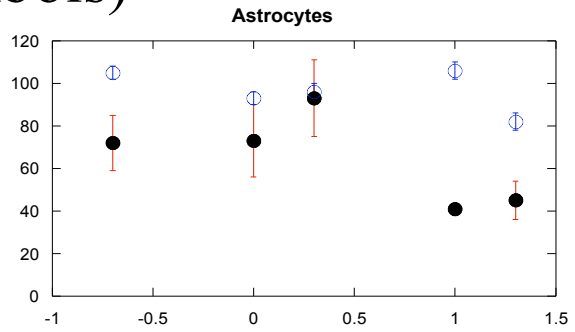


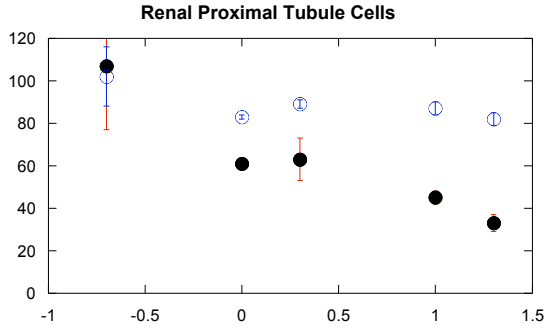


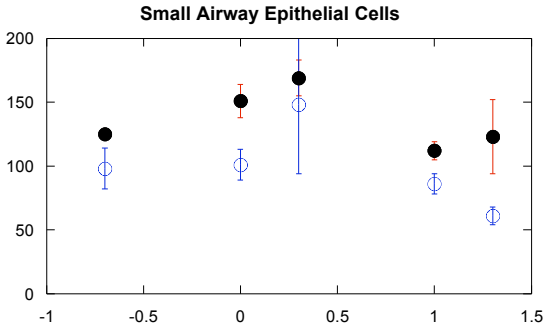
Cyclophosphamide Cytotoxicity:

IdMOC (filled symbols) vs Single Cell Type Cultures (opened symbols)









Summary: Cyclophosphamide

- Requires high doses to be cytotoxic (consistent with literature values for in vitro cytotoxicity with hepatocytes)
- Cytotoxicity in HAEC, astrocytes, and RPTEC higher in IdMOC than SCTC, illustrating multiple organ interactions in IdMOC

Her TS with IdMOC

- Station 1: IdMOC Production
  - 20 uL per well into IdMOC-96
- Station 2: Treatment
  - Addition of 720 uL of treatment medium
  - Incubation for required period
  - Addition of 10 uL of MTT reagent
  - Incubation for 3 hrs followed by medium removal
- Station 3: Endpoint Quantification
  - Addition of solvent for dissolution of MTT crystals
  - Color quantification

Summary: IdMOC

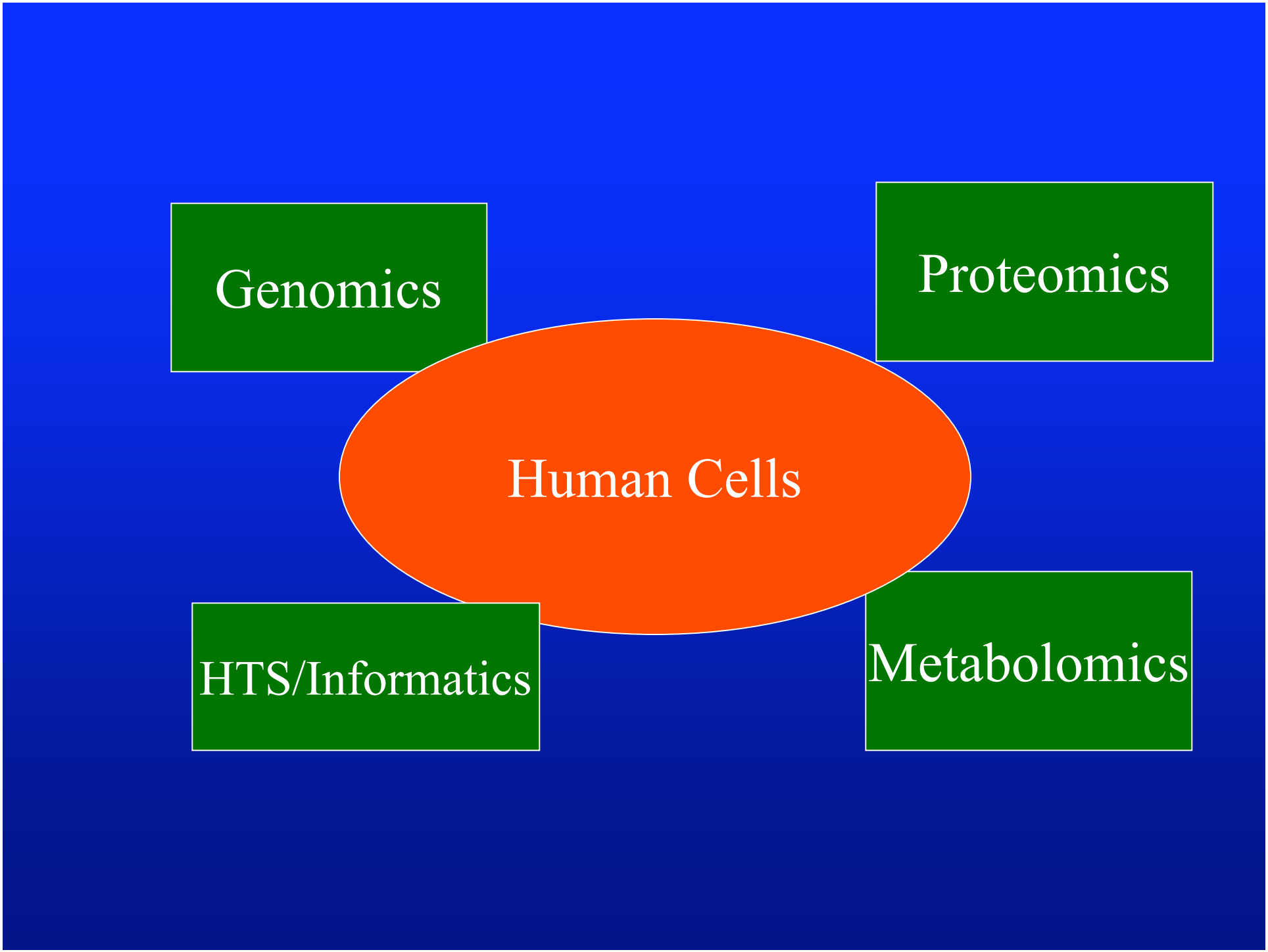
- Quantitative data on the effects of a toxicant on cells from multiple organs
  - Treatment of multiple cell types under "identical" experimental conditions
  - Multiple organ interaction
- Differential cytotoxicity illustrated by results with tamoxifen (selective cytotoxicity towards breast cancer cells)
- Evaluation of metabolism-related toxicity as illustrated by results with cyclophosphamide
- Higher Throughput Screening technologies developed

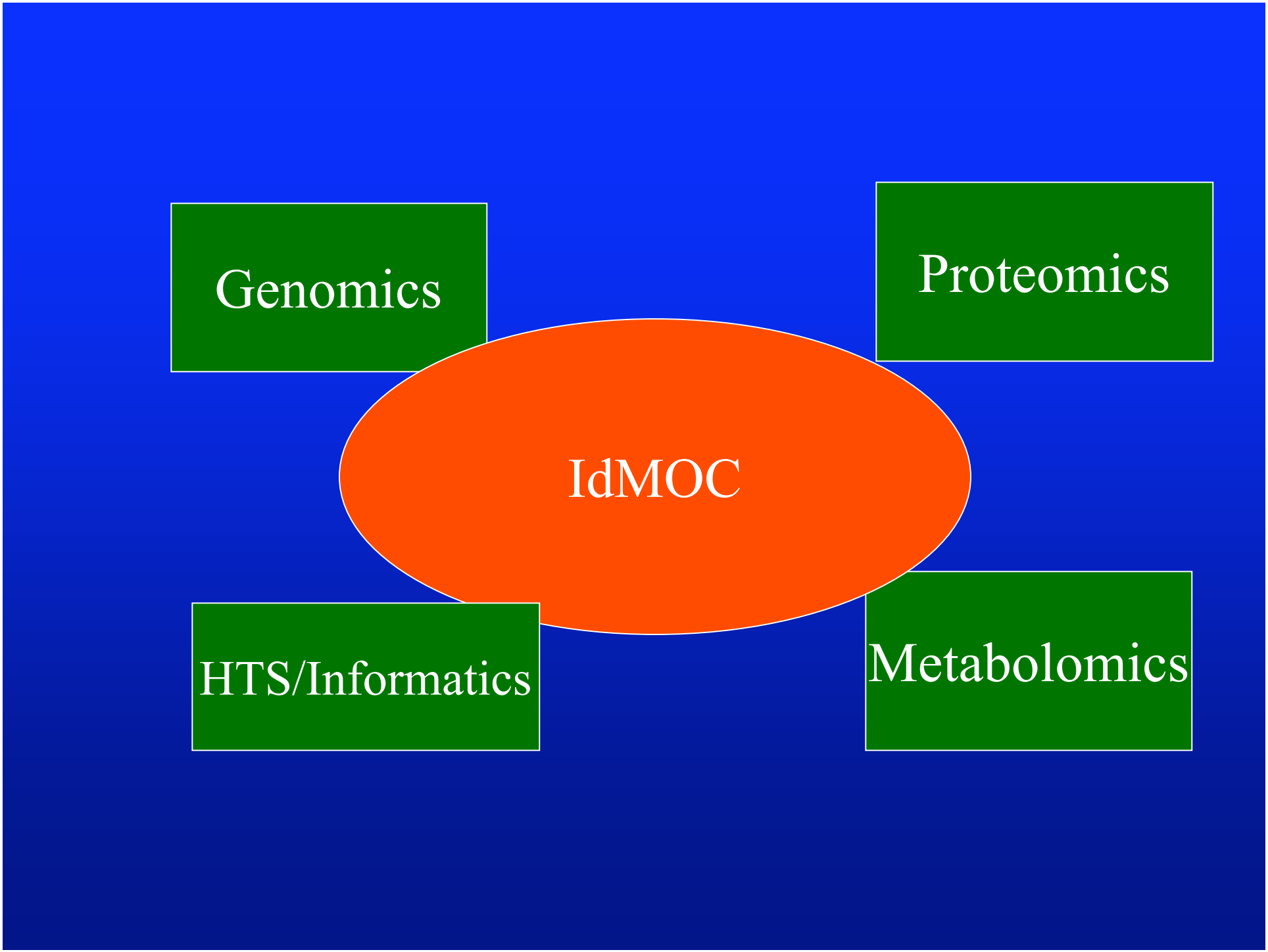
IdMOC: An Universal Tool for Drug Discovery and Development

- Co-culturing of primary cells from multiple organs with a common overlying medium, thereby modeling an organism (e.g. human) with multiple organs sharing a common body fluid
  - Discrete cultures allowing the evaluation of organ-specific effects
  - Interconnected culture allowing multiple organ metabolism
- Can be applied towards most disciplines of drug development, including metabolism, distribution, toxicity, and efficacy
- *IdMOC as a tumor-bearing man: an effective tool for the discovery of anticancer drugs*

Major technological advancements in the new millennium

- Information technology
- Genomic/proteomic/metabolomic technologies
- HTS technologies
- Human cell technology


Genomics
Proteomics
Human Cells
HTS/Informatics
Metabolomics


Genomics
Proteomics
IdMOC
HTS/Informatics
Metabolomics

I’m right, I use the most relevant experimental system

Acknowledgment

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