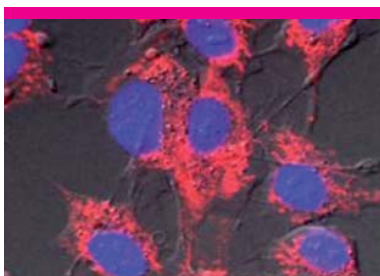


# High Content Toxicology: CellCiphr™ Hepatotoxicity Profiling



*in vitro* TOXICOLOGY

## Background Information



'Multiple cells types or panels are required to cover the broad range of *in vivo* toxicity mechanisms'

<sup>4</sup>Vernetti L, Irwin W, Giuliano KA, Gough A, Johnson K and Taylor DL (2009)

*In Drug Efficacy, Safety and Biologics Discovery: Emerging Technologies and Tools* (Ed. Ekins S and Xu JJ) John Wiley & Sons, New Jersey; 53-74

### Related Services

- CellCiphr™ Cytotoxicity Profiling
- CellCiphr™ Cardiotoxicity Profiling

- Hepatotoxicity is one of the main reasons for drug withdrawals, accounting for 37% of all drugs withdrawn between 1994 and 2006.<sup>1</sup>
- Drug toxicity is typically a combination of multiple mechanisms. A single experimental approach is unlikely to be predictive of the complexity involved in cellular toxicity.
- High Content Screening uses automated fluorescence imaging to simultaneously analyse multi-parametric indicators of cellular toxicity. It can detect general cell death and/or mechanisms of cell death and can cover a wide spectrum of cytopathological changes.<sup>2,3</sup>
- The CellCiphr™ hepatotoxicity profiling assay assesses a panel of 8 key toxicity markers in primary rat hepatocytes:
  - Cell Loss
  - DNA Fragmentation
  - Nuclear Size
  - Apoptosis
  - Steatosis
  - Phospholipidosis
  - Mitochondrial Function
  - DNA Damage Response
- Using Cyprotex's extensive database of *in vitro* profiling and *in vivo* toxicity data, the CellCiphr™ Classifier system can rank and classify unknown compounds against known toxic effects, allowing the prediction of organ toxicity.
- For drug-discovery programs using CellCiphr™ toxicity profiling to filter out toxic compounds at the start of the hit to lead stage, Cyprotex projects a saving in direct costs of over \$91 million. For programs using CellCiphr™ to selectively advance compounds with reduced risk of attrition due to toxicity, Cyprotex projects a \$35 million increase in value for the typical clinical pipeline.

### Protocol

#### Instruments

Cellomics ArrayScan® VTI (Thermo Scientific)

#### Analysis Method

High Content Screening with CellCiphr™ Classifier System

#### Cell Type

Primary rat hepatocytes

#### Toxicity Markers

8 key toxicity markers (cell loss, DNA fragmentation, nuclear size, apoptosis, steatosis, phospholipidosis, mitochondrial function, DNA damage response)

#### Test Compound Concentration

10 point dose response curve in duplicate at 1 hr, 24 hr and 48 hr exposure

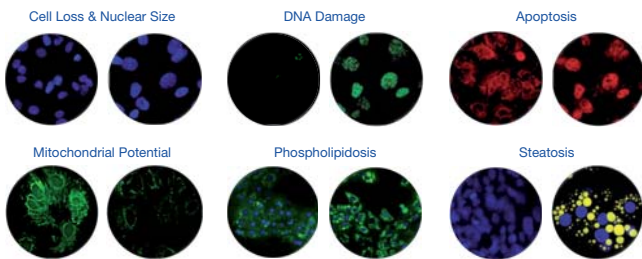
#### Data Delivery

CellCiphr™ toxicity report (see table 1)

# CellCiphr™ Toxicity Profiling investigates exposure at 3 different time points to assess early and late stage toxic response markers.

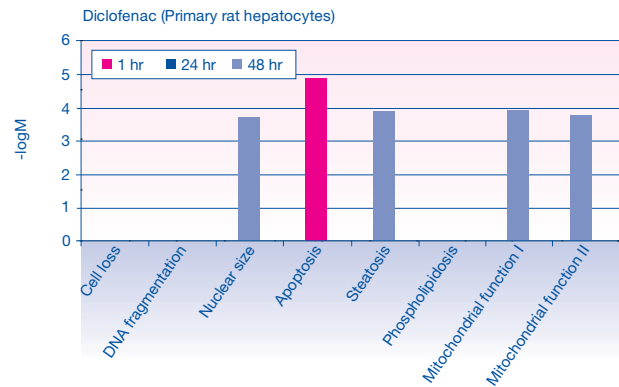
**Figure 1**

Representative high content screening images for untreated (image on left) and treated (image on right) rat hepatocytes illustrating key toxicity markers.



**Figure 2**

CellCiphr™ hepatotoxicity profile for diclofenac in fresh rat hepatocytes.<sup>4</sup>



**Figure 3**

Example of a CellCiphr™ Toxicity Profiling Report.

Classification		Safety Rank	6/20	Safety Risk Index	Mod Risk
<b>Indicators</b> # <b>Measured Effects</b> <b>AC<sub>50</sub> (M)</b>					
Maximum Tolerated Dose		>70% Cell Loss		148.9E-6	1.8E-3
Earliest Toxic Indicator		Nuclear Size		23.7E-6	
Most Sensitive Toxic Indicator		Steatosis		13.2E-6	
General Indicators of Toxicity		1	Cell Loss	69.5E-6	306.3E-6
		2	Nuclear Size	23.7E-6	22.9E-6
Mechanistic Indicators		3	DNA Damage Response		*
		4	Apoptosis	31.0E-6	37.7E-6
		5	Lysosomal Mass		
		6	DNA Fragmentation	24.9E-6	34.3E-6
		7	Mitochondrial Potential	59.7E-6	164.9E-6
		8	Steatosis	39.8E-6	13.2E-6
			Legend	* - Excluded - No Activity - Not Measured	
Compound Correlations		Within Test Set		With CellCiphr® Database	
Compounds with a threshold Pearson's correlation coefficient of 0.8 or higher		CLMN-3	0.92	Simvastatin	0.84
		CLMN-18	0.81		
		CLMN-14	0.86		
Profile Similarity Within Test Set		Profile Similarity Plots			
Report compound (pink)		Acute Early Chronic			
Compounds in this set (r, g & b).		Activity (-logM)			
X-axis: Acute, Early and Chronic		Max Within CLMN-25 CLMN-3 CLMN-18 CLMN-14			
Features #1-8 as above.					
Y-axis: - Log(AC50) (3 = mM, 9 = nM)					
Profile Similarity With CellCiphr DB		Profile comparison plots			
Report compound (pink)		Activity (-logM)			
CellCiphr DB compounds (r, g & b).		Max DB CLMN-25 Simvastatin N/A N/A			
Gray background represents maximum response in the set (top) and DB (bottom)					
Observations on Compound Physical Properties					
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**Table 1**

Data deliverables within the CellCiphr™ Hepatotoxicity Profiling Report.

## CellCiphr™ Toxicity Profiling Report

CellCiphr™ Safety Risk Index

Maximum Tolerated Dose

Earliest Toxic Indicator

Most Sensitive Toxic Indicator

General Indicators of Toxicity

Mechanistic Indicators of Toxicity

CellCiphr™ correlation analysis, including comparison with other compounds in the project, compounds in the reference database, and CellCiphr™ ToxProfile Similarity plots

The CellCiphr™ Classifier profiles known compounds against an extensive set of reference compounds for which safety data are available. The profiles for the reference compounds are used to create a proprietary classification algorithm that provides a rank order of risk of failure in safety studies (Safety Risk Index).

## References

- Dykens JA and Will Y (2007) *Drug Discovery Today* **12**; 777-785
- Abraham VC et al., (2008) *J Biomol Screen* **13(6)**; 527-537
- Xu JJ et al., (2008) *Toxicol Sci* **105(1)**; 97-105
- Vernetti L et al. (2009) In *Drug Efficacy, Safety and Biologics Discovery: Emerging Technologies and Tools Ed.* Ekins S and Xu JJ; 53-74