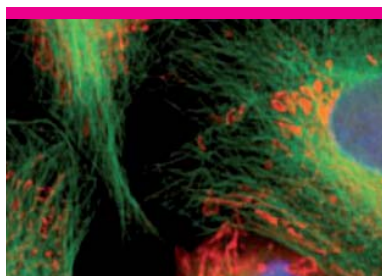


High Content Toxicology: CellCiphr™ Cytotoxicity Profiling in HepG2 Cells

cyprotex

in vitro TOXICOLOGY

Background Information



'The CellCiphr™ Cytotoxicity Profiling system offers a rapid, inexpensive, cell-based method capable of accurate and sensitive identification of compounds associated with severe *in vivo* toxicity with the additional benefit of no occurrence of false positives.'

³Vernetti 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™ Hepatotoxicity Profiling
- CellCiphr™ Cardiotoxicity Profiling

- 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 fluorescence imaging to simultaneously analyse multi-parametric indicators of cellular toxicity. It can detect cell death as well as mechanisms of cell death and can cover a wide spectrum of cytopathological changes.^{1,2}
- The CellCiphr™ cytotoxicity profiling assay assesses a panel of 10 key toxicity markers in HepG2:
 - Cell loss
 - Cell cycle arrest
 - Nuclear size
 - Oxidative stress
 - Stress kinase activation
 - DNA damage response
 - Mitochondrial function I
 - Mitochondrial function II
 - Mitosis marker
 - Cytoskeletal disruption
- Using Cyprotex's proprietary database of *in vitro* profiling and *in vivo* toxicity data, the CellCiphr™ Classifier system can rank and classify unknown compounds against known toxic effects improving the accuracy of toxicity prediction.
- 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

Instrument

Cellomics ArrayScan® VTI (Thermo Scientific)

Analysis Method

High Content Screening with CellCiphr™ Classifier System

Cell Type

HepG2

Toxicity Markers

10 key toxicity markers (cell loss, cell cycle arrest, nuclear size, oxidative stress, stress kinase activation, DNA damage response, mitochondrial function I, mitochondrial function II, mitosis marker and cytoskeletal disruption)

Test Compound Concentration

10 point dose response curve in duplicate at 1 hr, 24 hr and 72 hr exposure

Data Delivery

CellCiphr™ toxicity report (see table 1)

CellCiphr™ Toxicity Profiles are analysed with proprietary visual and quantitative data mining tools including CellCiphr™ Classifiers, correlation analysis, and cluster analysis.

Figure 1

Representative high content screening images for untreated (image on left) and treated (image on right) HepG2 cells illustrating key toxicity markers.

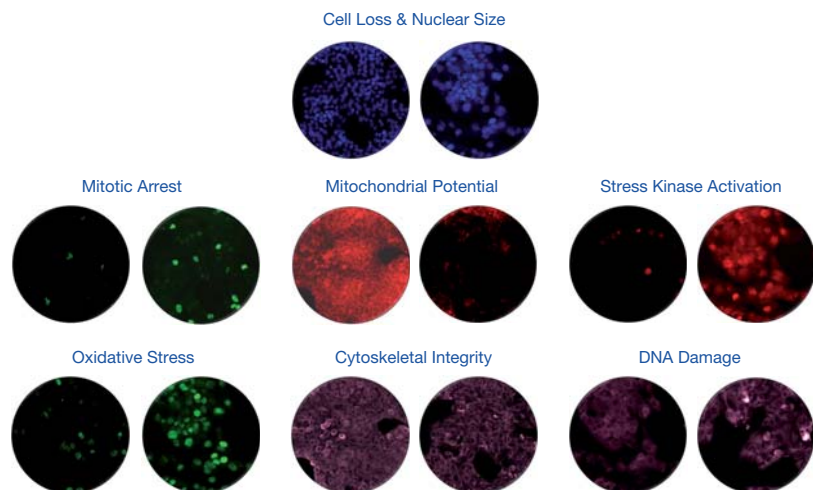


Figure 2

Example of a CellCiphr™ Toxicity Profiling Report.

Table 1

Data deliverables within the CellCiphr™ Cytotoxicity 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

Classification		Safety Rank	6/20	Safety Risk Index	Mod Risk
<p>← Safety Alert & Rank</p>					
Indicators		#	Measured Effects	AC ₅₀ (M)	
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)		Activity (-log ₁₀ M)			
Compounds in this set (r, g & b).		Acute Early Chronic			
X-axis: Acute, Early and Chronic Features #1-8 as above.		1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8			
Y-axis: -Log(AC ₅₀) (3 = mM, 9 = nM)		← Profile comparison plots			
Profile Similarity With CellCiphr DB		Report compound (pink)			
CellCiphr DB compounds (r, g & b).		Activity (-log ₁₀ M)			
Gray background represents maximum response in the set (top) and DB (bottom)		1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8			
Observations on Compound Physical Properties					
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The CellCiphr™ Classifier profiles unknown 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

- 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 Biologicals Discovery: Emerging Technologies and Tools* Ed. Ekins S and Xu JJ; 53-74