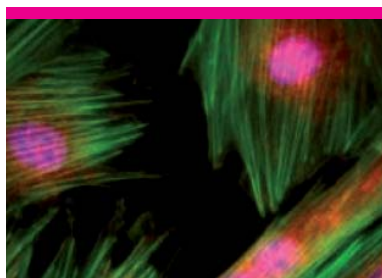


High Content Toxicology: CellCiphr™ Cardiotoxicity Profiling



in vitro TOXICOLOGY

Background Information



'The multiplexed image-based data captured by the CellCiphr™ tools offers advantages over single end point well-based assays by providing deeper insight into potential mechanisms of action.'

⁴Vernetti 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

- hERG Safety
- CellCiphr™ Cytotoxicity Profiling
- CellCiphr™ Hepatotoxicity Profiling

- Cardiotoxicity is one of the main reasons for drug withdrawals, accounting for 45% of all drugs withdrawn between 1994 and 2006.¹

- 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.^{2,3}

- The CellCiphr™ cardiotoxicity profiling assay assesses a panel of 8 key toxicity markers in H9c2 cardiomyocytes:

- Cell Loss
- Nuclear Size
- Apoptosis
- DNA Damage Response
- Mitochondrial Function
- ROS Generation
- Hypertrophy
- Steatosis

- 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

H9c2 cardiomyocytes

Toxicity Markers

8 key toxicity markers (cell loss, nuclear size, apoptosis, DNA damage response, mitochondrial function, ROS generation, hypertrophy, steatosis)

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 can be used from late in primary screening to early in preclinical phases for reliably identifying toxic compounds before entering expensive pre-clinical regulatory *in vivo* testing.

Figure 1

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

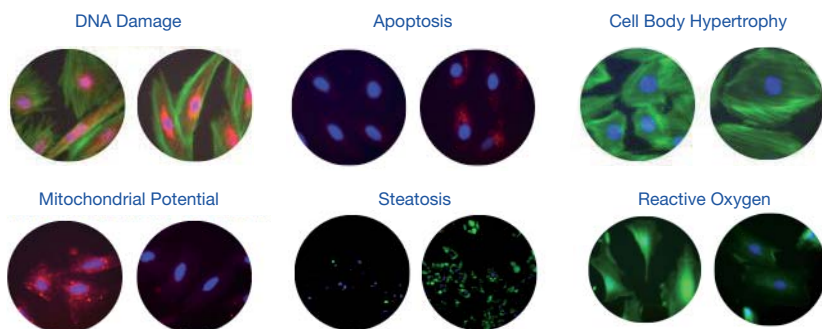


Figure 2

Example of a CellCiphr™ Toxicity Profiling Report.

Table 1

Data deliverables within the CellCiphr™ Cardiotoxicity 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	1 hr	24 hr
Earliest Toxic Indicator			Nuclear Size	148.9E-6	1.6E-3
Most Sensitive Toxic Indicator			Steatosis	23.7E-6	
General Indicators of Toxicity					
1		1	Cell Loss	69.5E-6	306.3E-6
2		2	Nuclear Size	23.7E-6	22.9E-6
3		3	DNA Damage Response	*	*
4		4	Apoptosis	31.0E-6	37.7E-6
5		5	Lysosomal Mass		
6		6	DNA Fragmentation	24.9E-6	34.3E-6
7		7	Mitochondrial Potential	59.7E-6	164.9E-6
8		8	Steatosis	39.8E-6	13.2E-6
<p>← AC50's for mechanistic indicators</p>					
Mechanistic Indicators					
Legend					
			*	- Excluded	
				- No Activity	
				- Not Measured	
Compound Correlations					
Within Test Set					
Compounds with a threshold Pearson's correlation coefficient of 0.8 or higher					
CLMN-3		0.92			
CLMN-18		0.81			
CLMN-14		0.86			
With CellCiphr® Database					
				Simvastatin	0.84
<p>← Correlations within set & with reference database</p>					
Profile Similarity Plots					
<p>← Profile comparison plots</p>					
<p>Profile Similarity Within Test Set Report compound (pink) Compounds in this set (r, g & b). X-axis: Acute, Early and Chronic Features #1-8 as above. Y-axis: - Log(AC50) (3 = mM, 9 = nM)</p>					
<p>Profile Similarity With CellCiphr DB Report compound (pink) CellCiphr DB compounds (r, g & b). Gray background represents maximum response in the set (top) and DB (bottom)</p>					
<p>Observations on Compound Physical Properties</p>					
<p>© 2010 Cyprotex Rev.02-02-000</p>					

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

¹ Dykens JA and Will Y (2007) *Drug Discovery Today* **12**; 777-785
² Abraham VC et al., (2008) *J Biomol Screen* **13(6)**; 527-537
³ Xu JJ et al., (2008) *Toxicol Sci* **105(1)**; 97-105
⁴ Verneti L et al. (2009) In *Drug Efficacy, Safety and Biologics Discovery: Emerging Technologies and Tools* Ed. Ekins S and Xu JJ; 53-74