

PROFILING COMPOUNDS WITH CARDIOTOXIC POTENTIAL USING HIGH CONTENT IMAGING IN RAT H9C2 CELLS AND HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES



Ann F. Hoffman³, Shannon M. Hamilton³, Karen L. Zipf³, Sei Kameoka¹, Joshua Babiarz¹, Brad Swanson², Wenbo Wang², Blake Anson², Kyle L. Kolaja¹

1. Nonclinical Safety, Roche, Nutley, NJ, USA.
2. Cellular Dynamics International, Madison, WI, USA.
3. Discovery Technology, Roche, Nutley, NJ, USA.

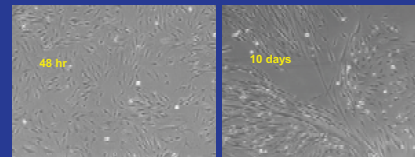
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Abstract

A high content imaging strategy was implemented to compare the toxicological properties of a set of compounds across two distinct cell systems, the rat H9C2 cell line and human induced pluripotent stem (hiPS) cell-derived cardiomyocytes. The use of a multiplexed approach includes parameters suitable for detecting DNA damage, nuclear morphology, mitochondrial membrane potential, oxidative stress, effects on lysosomal integrity, glutathione redox status, and apoptotic events. A test panel of compounds with known in vivo cardiotoxicological attributes including doxorubicin and many well characterized kinase inhibitors were used as reference set to assist in interpretation of the high content imaging assay results. The toxicity profile investigations revealed that cardiotoxics are more readily identified using hiPS cell-derived cardiomyocytes compared to H9C2 cells. In particular doxorubicin and other overt cardiotoxics induced a dose responsive change in cellular ATP, caspase 3/7 activity, and mitochondrial membrane potential in hiPS derived cardiomyocytes. This study supports the continued use of high content imaging and stem-cell derived models as a part of a safety evaluation strategy implemented during the early phase of drug discovery. As the availability of additional differentiated hiPS cell types are developed, high content imaging techniques can then be rapidly deployed to provide more complete insight into the early safety profile of a molecule.

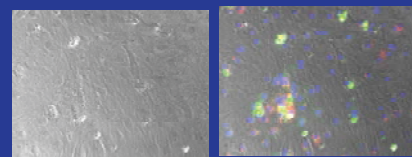
Rat H9C2 Characteristics

- To conserve myoblastic properties these cells should be maintained at < 70% confluency. Near confluency, the cells become striated.
- H9C2 cells show a lower proliferation rate such that a single plating density can be utilized over multiple analysis times
- Cell plating density is 7k/well for compound exposures of 24-72 hours
- Data exhibits great consistency between analysis time points and replicates



Characteristics: Cryopreserved human iCell Cardiomyocytes

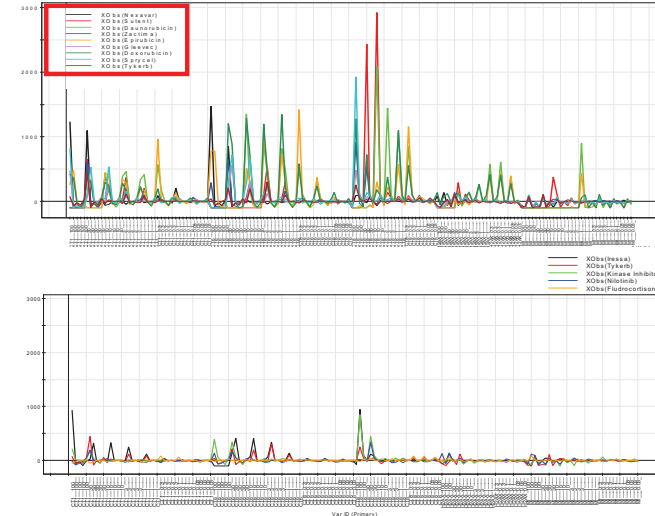
- Cells are maintained for up to 7 days to acquire HCS data
- iCell cardiomyocytes are allowed to equilibrate over 3 days to achieve beating monolayers
- Cell plating density is 20k/well for compound exposures of 24-48 hours
- Careful and gentle technical handling of the cryopreserved iCell cardiomyocytes are key to maximizing optimal pre-plating viability



HCS Assay Feature Results of iCell Cardiomyocytes: Cardiotoxics & Non-Cardiotoxics



HCS Assay Feature Results in rat H9C2 cells: Cardiotoxics & Non-Cardiotoxics



Compound Analysis of ATP in iCell Cardiomyocytes

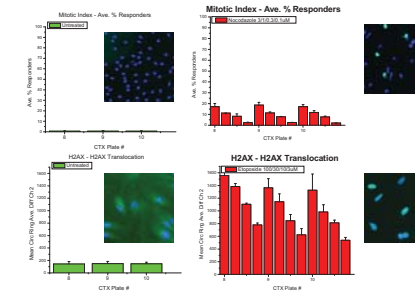
Name/Project	In Vivo Cardiotox?	IC50 ATP-24h	IC50 ATP-72h
Nexavar	Yes	13	12
Sutent	Yes	16	16
Daurorubicin	Yes	17	2
Zactima	Yes	23	15
Epirubicin	Yes	26	3
Gleevec	Yes	46	20
Doxorubicin	Yes	47	2
Sprycel	Yes	72	41
Iressa	No	47	33
Tykerb	No	65	50
Norepinephrine	in vivo	189	174
Isoprenaline	in vivo	>110	60
Kinase Inhibitor	No	>500	50
Nilotinib, Tasigna	No	>200	>200
Fludrocortisone	No	>500	>500

Protocols, Probes and Methods for HCS Assays

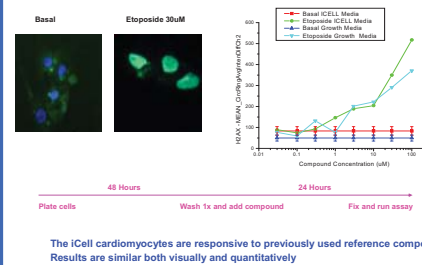
Time	Human IPS cells	Rat Transformed H9C2	Fluorescent Probes
24 hour	DNA Damage	Mitotic Arrest	Hoechst 33258 for Nucleus
	Oxidative Stress	DNA Damage	Ab to PhosphoHistone H3
48 hour	Live/Dead	Oxidative Stress	Dihydroethidium
	Lysosomal	Lysosomal	Carboxyfluorescein
	Mitochondrial	Mitochondrial	LysoTracker Green or Red
72 hour		Mitochondrial	MitoTracker Deep Red

1. Plate H9C2 at 7000 cells/well, iCell Cardiomyocytes cells at 20,000 attached cells/well
2. Incubate H9C2 cells at 37C/5% CO2 O/N proceed to #3.
3. For iCell Cardiomyocytes cells wash after 48 hours, then incubate another 72 hours at 37C/7% CO2
4. Remove conditioned media replacing with 50 ul diluted compound in H9C2 growth media or iCell Maintenance medium.
5. After incubation, add an equal volume (50ul) of staining cocktail. (DO NOT REMOVE MEDIA)
 - 1) Lysosomal Physiology CT1M - 30 minutes prefix at 37C
 - 2) Live/Dead CT3M - 60 minutes prefix at 37C
 - 3) Mitochondrial Physiology CT5M - 30 minutes prefix at 37C
 - 4) Lysosomal and Mitochondrial Physiology CT6 - 30 minutes prefix at 37C
6. Aspirate and add 100 ul fixative to each well, incubate 10 minutes RT.
7. Remove fixative, wash 1x with 100ul PBS.
8. Add 200ul PBS and seal plate, read on Cellomics VTIArrayScans

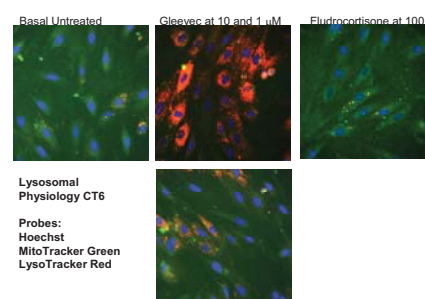
Mitotic Index & H2AX HCS



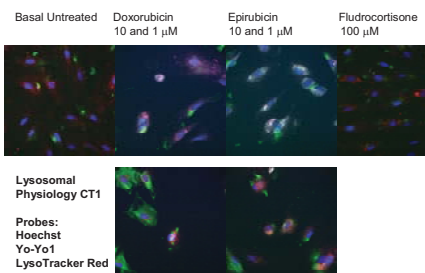
Assay Development Results for H2AX



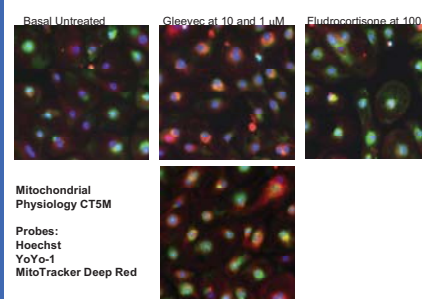
Representative Composite Images for rat H9C2 cells



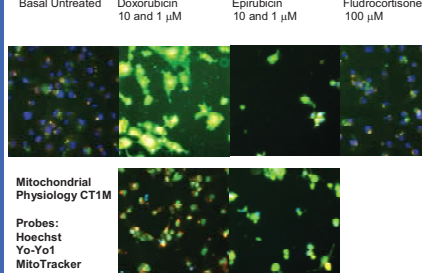
Representative Composite Images for rat H9C2 cells



Representative Composite Images for iCell Cardiomyocytes



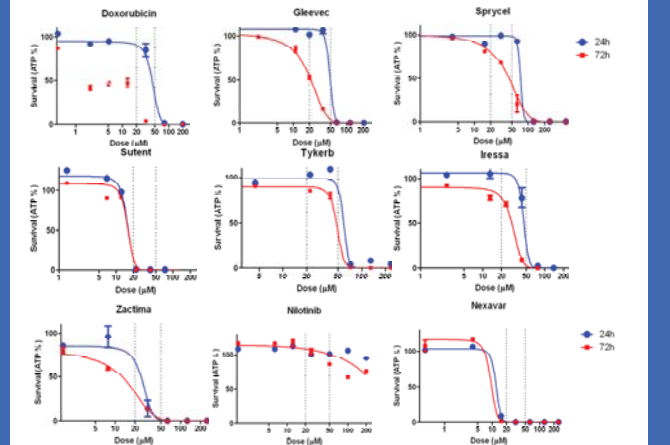
Representative Composite Images for iCell Cardiomyocytes



One HCS Assay One File Multiple feature parameters collected

H2AX	UniquePLATEID
1 Unique Plate Descriptor	MEAN_H2AX_TRANS_NORM
2 MEAN_CircRingAngleDFDC2	MEAN_NUC_AREA_FCT
3 MEAN_NucleusCh1	CTC_CELL_CNT
4 SelectedCellCount	MEAN_NucAngleCh1
5 MEAN_NucAngleCh1	MEAN_NucShapeWRC1
6 MEAN_NucShapeWRC1	MEAN_NucShapeP2ACH1
7 MEAN_NucShapeP2ACH1	MEAN_NucTotAreaCh1
8 MEAN_NucTotAreaCh1	VALID_FIELDS
9 ValidFieldCount	ValidCellCount
10 ValidCellCount	MEAN_CircTotAreaCh2
11 MEAN_CircTotAreaCh2	MEAN_CircAngleCh2
12 MEAN_CircAngleCh2	MEAN_RingAreaCh2
13 MEAN_RingAreaCh2	MEAN_RingTotAreaCh2
14 MEAN_RingTotAreaCh2	MEAN_CircRingAngleRatioCh2
15 MEAN_CircRingAngleRatioCh2	

Cells=iCell D32



Summary

- Human iCell cardiomyocytes have been successfully applied to multiple High Content Imaging assays as well as biochemical assays. The responses to standard reference compounds: valinomycin, etoposide, and rotenone, show data that is similar to data acquired using the "cardiomyocyte-like" cell line, rat H9C2.
- Results of the HCS data suggest that feature measurements quantifying mitochondrial and lysosomal physiology, DNA damage and oxidative stress are key elements affected by many of these known in vivo cardiotoxics.
- The data from the biochemical ATP assay appears to correctly classify the limited set of compounds as it relates to cardiotoxicity in vivo data.
- Further effort will be required to determine correlations between the HCS multi-feature data and the in vivo cardiotoxicity classifications of these compounds. Other known cardiotoxics and non-cardiotoxics will need to be evaluated in these and additional HCS and biochemical assays.
- The resultant data supports the potential that human iCell cardiomyocytes maybe a useful screening strategy in detecting early safety liabilities of preclinical candidates.