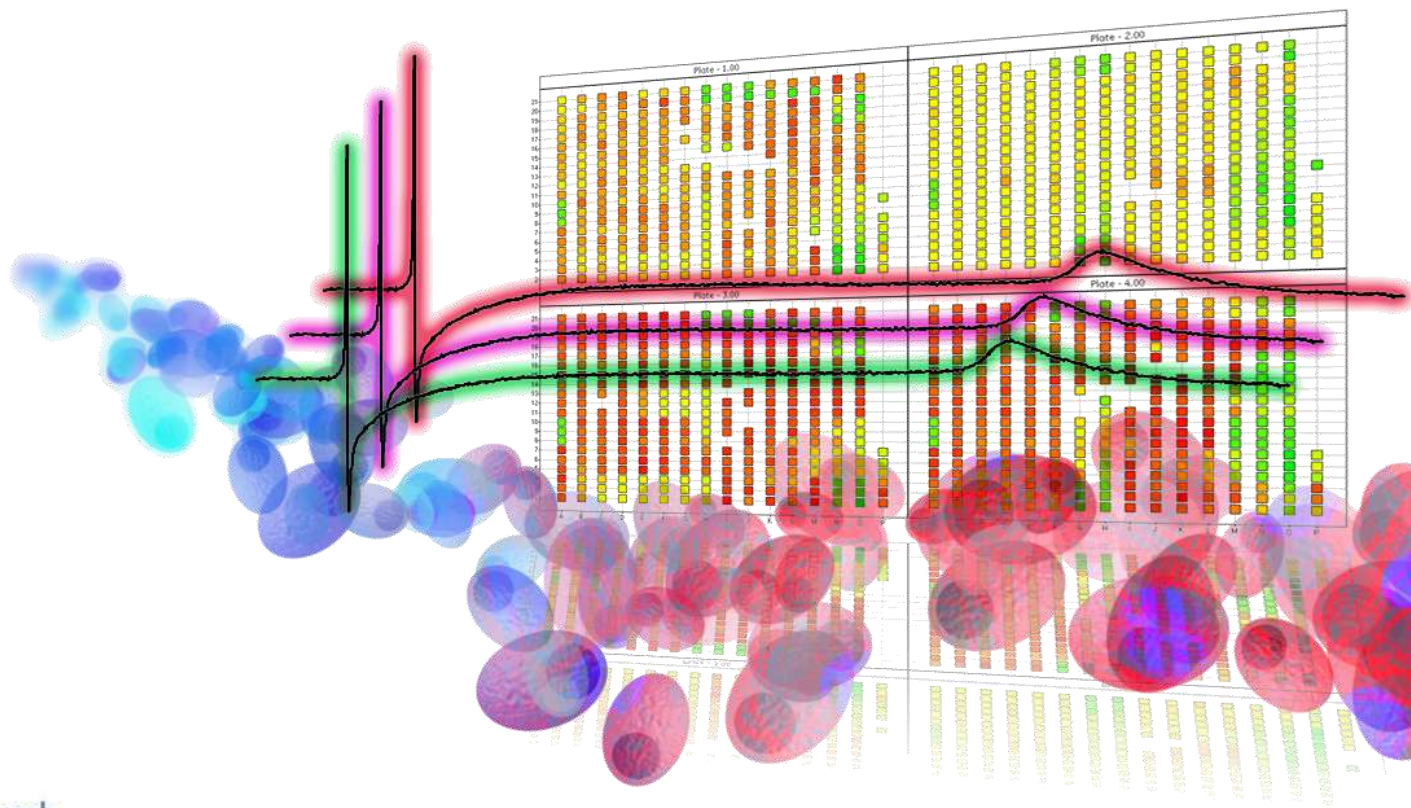


Innovating Pre-Clinical Drug Development: Towards an Integrated Approach to Investigative Toxicology in Human Models

Nick Thomas PhD
Principal Scientist
Cell Technologies
GE Healthcare

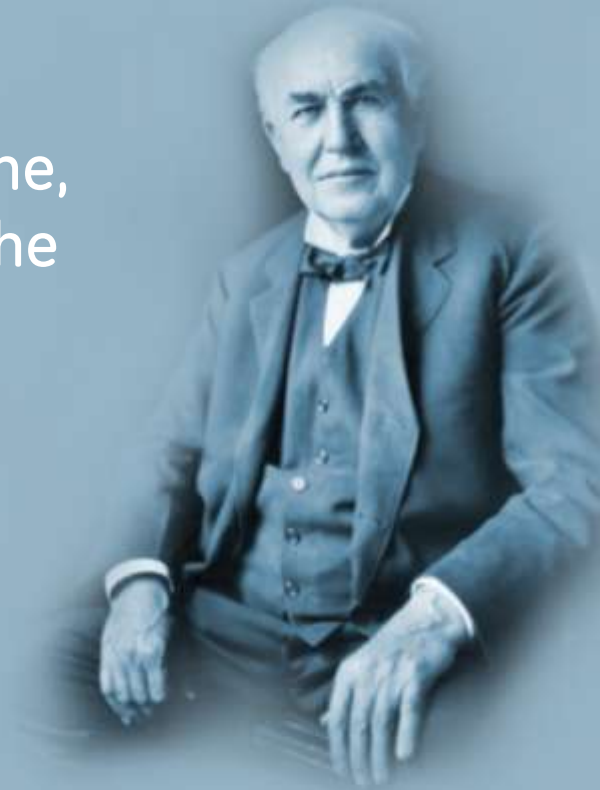


imagination at work

AIMBE/NIH Summit on Validation and Qualification of New
In Vitro Tools for the Pre-Clinical Drug Discovery Process
19th March 2012, NIH

The doctor of the future will give no medicine, but will interest his patients in the care of the human body, in diet, and in the cause and prevention of disease.

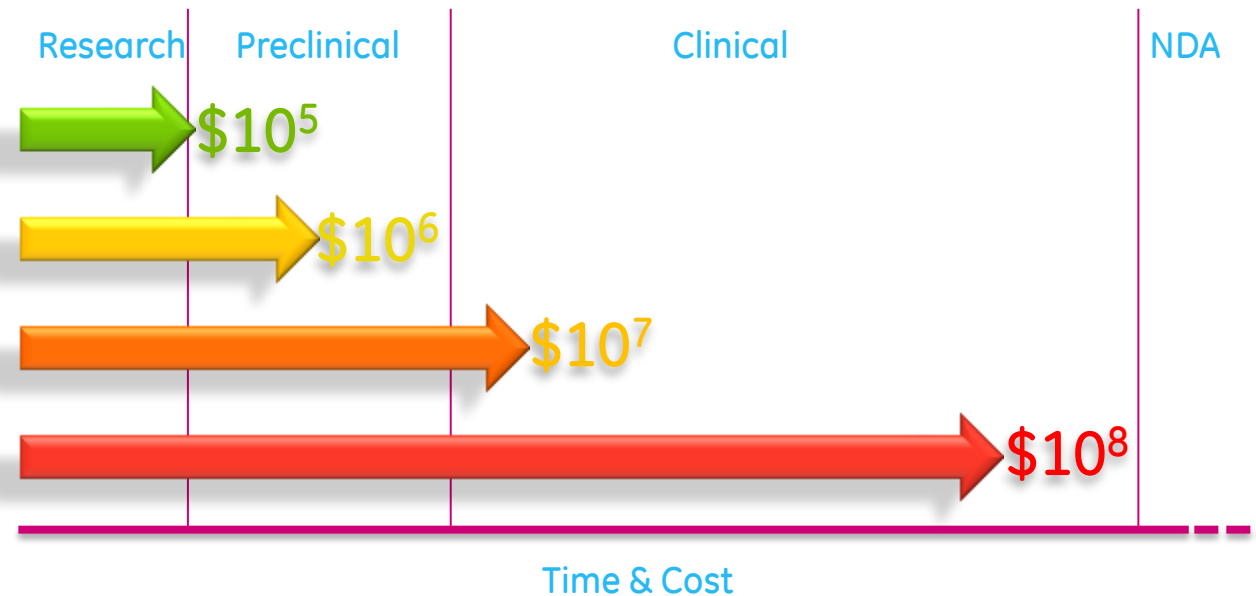
Thomas Alva Edison 1847– 1931



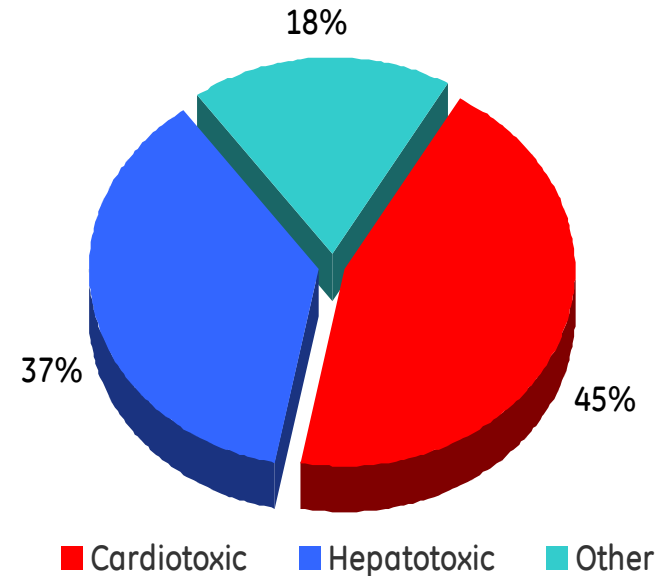
imagination at work

Drug Discovery & Development

Need for earlier and improved assessments



Drug Recalls 1994-2006



Stem Cells in Drug Development

Efficacy and safety assessment

High Throughput Screening

- stem cells economically viable ?
- assays 'dumbed down'
- applications in some disease areas ?

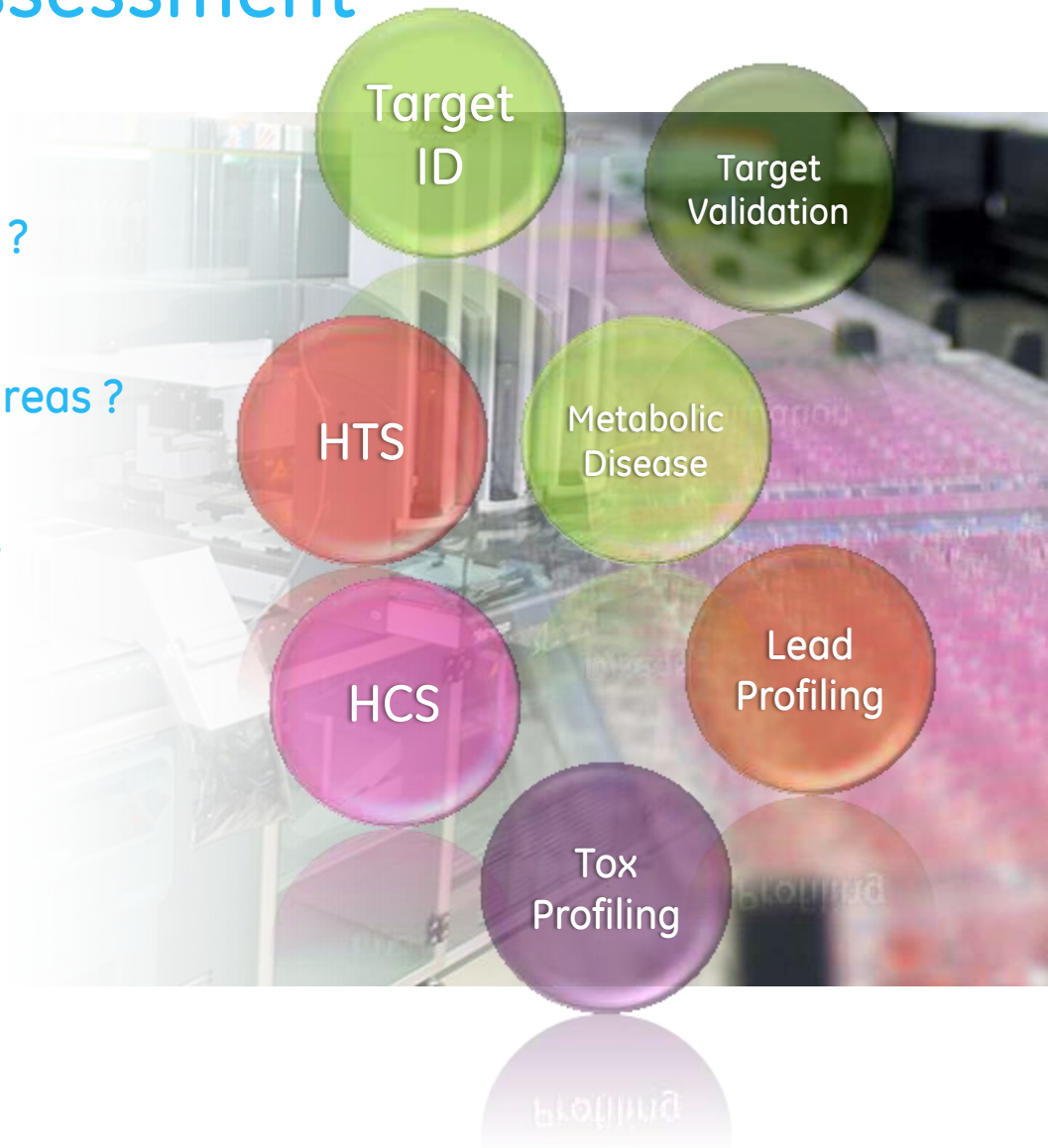
Secondary screening & profiling

- integrating efficacy and safety

Investigative Toxicology

- early stage attrition
- improved clinical predictivity
- integrated human models
- reduction in animal use

Adoption

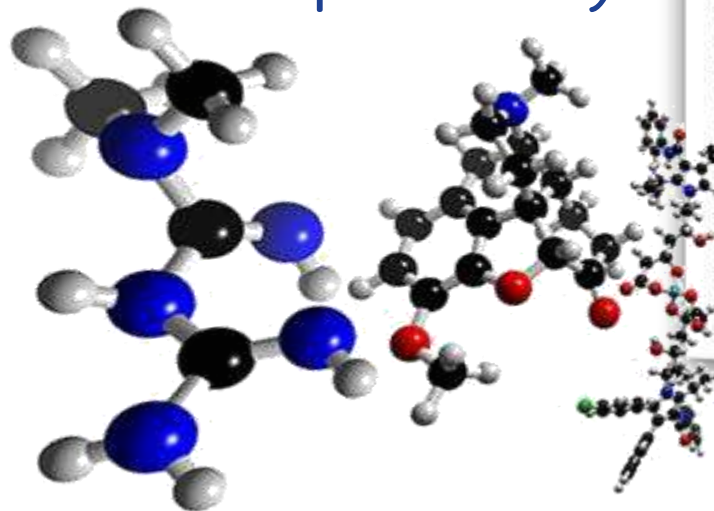


21st Century Toxicology

Toxicology at tipping point

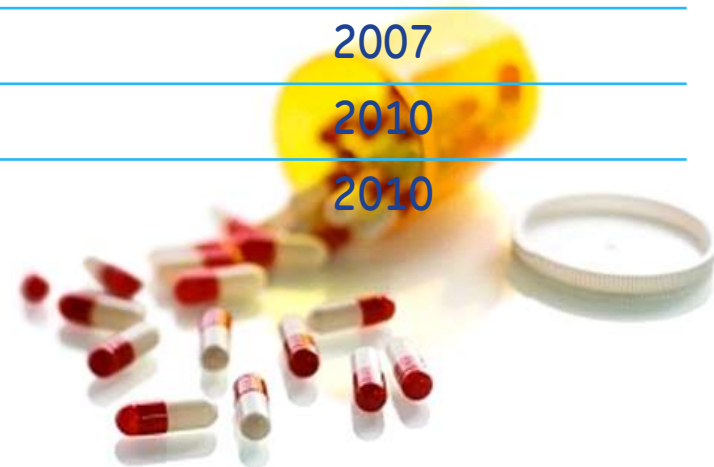
Stakeholders pushing for radical changes and adoption of new technologies

Pharma seeking in vitro models with improved clinical predictivity



Drug Cardiotoxicity

Drug	Class	Withdrawn
Terfenadine	Antihistamine	1998
Sertindole	Antipsychotic	1998
Astemizole	Antihistamine	1999
Grepafloxacin	Antibiotic	1999
Cisapride	Prokinetic	2000
Droperidol	Tranquilizer	2001
Levomethadyl	Opiate Dependence	2003
Rofecoxib	NSAID	2004
Tegaserod	Prokinetic	2007
Sibutramine	Appetite Suppressant	2010
Rosiglitazone	Antidiabetic	2010



Drug Toxicology

Current issues – problems & solutions

Using animal models to reflect human responses

- animals \neq humans
- animal \neq animal
- cross species testing may increase sensitivity but decrease specificity
- metabolism & MOA ?



Integrate range of predictive human cell models

Quality and robustness of toxicity cell models

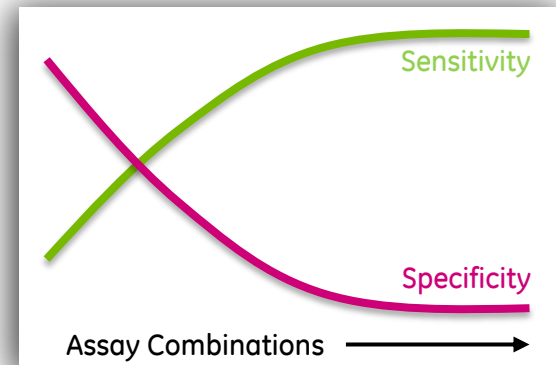
- scarcity of primary cells/tissues
- source variability
- more abundant models (immortalized/genetically engineered cells) may have reduced predictivity



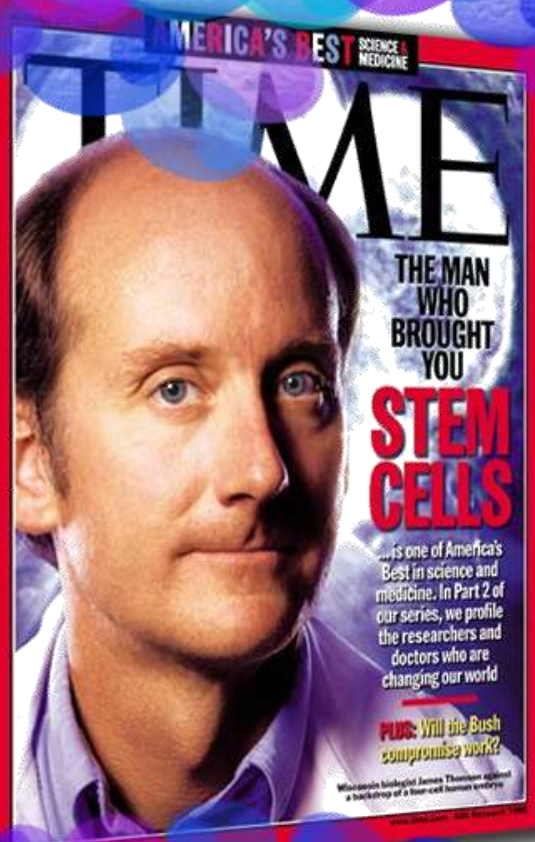
Integrate robust human stem cell derived models

Testing multiple endpoints leading to false-positives

- multiple testing increases sensitivity at cost of specificity
- different assay combinations yield varying predictivity
- testing multiple endpoints leads to false positives



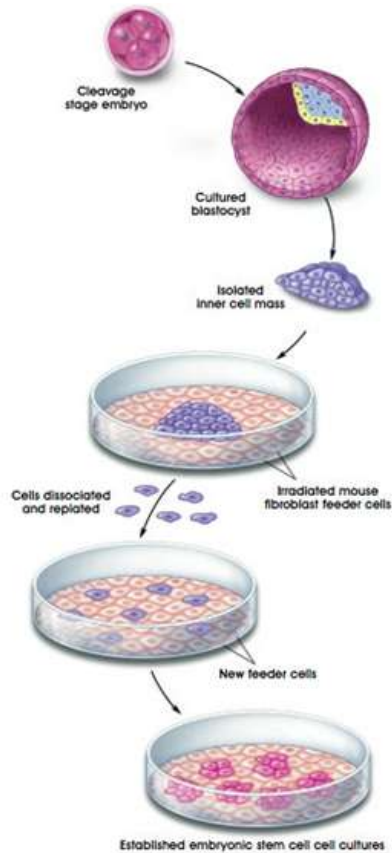
Integrate and standardize most predictive parameters



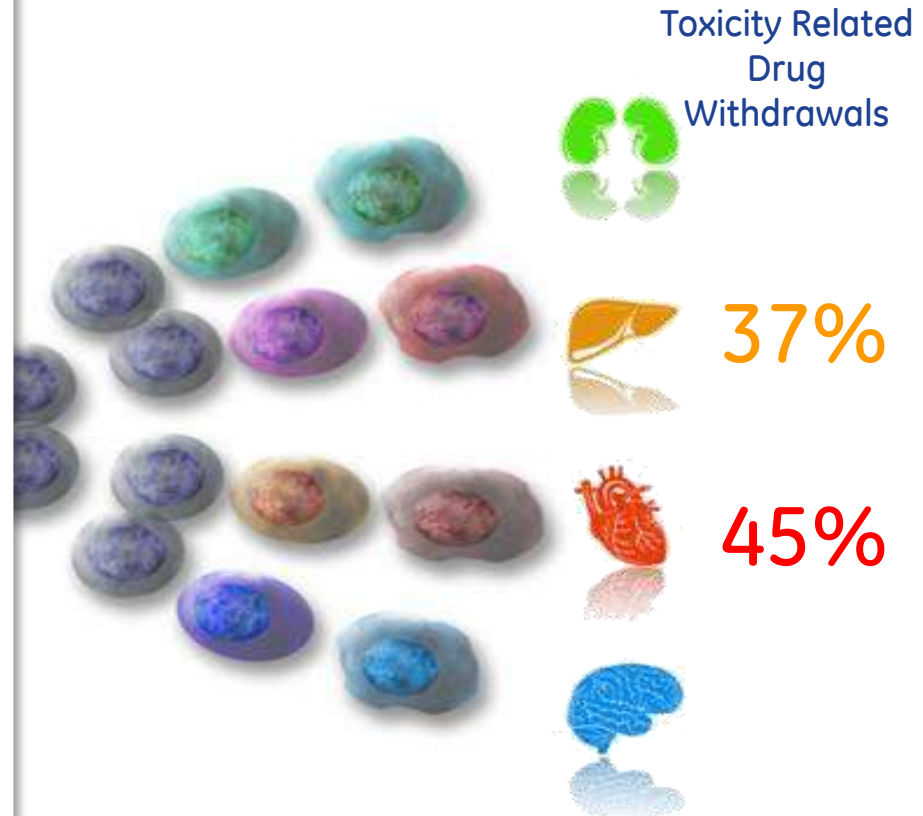
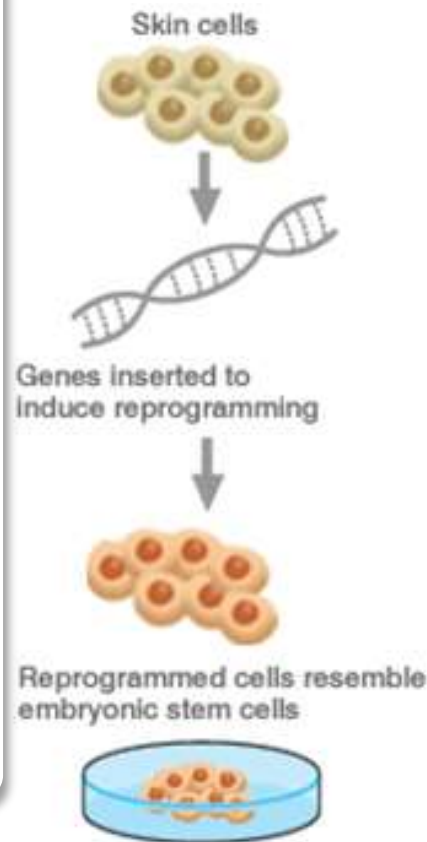
imagination at work

Stem Cells in Drug Toxicology

hESC

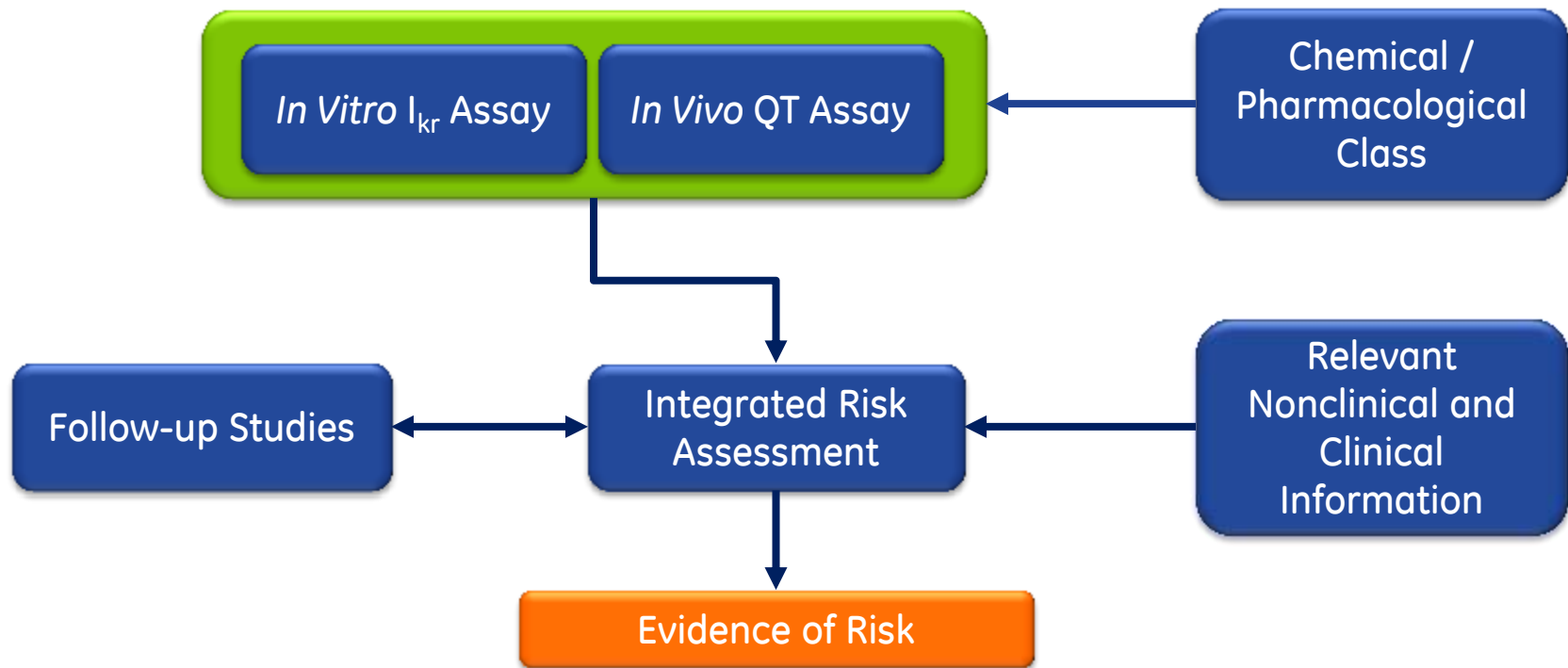


iPS



Cardiotoxicity

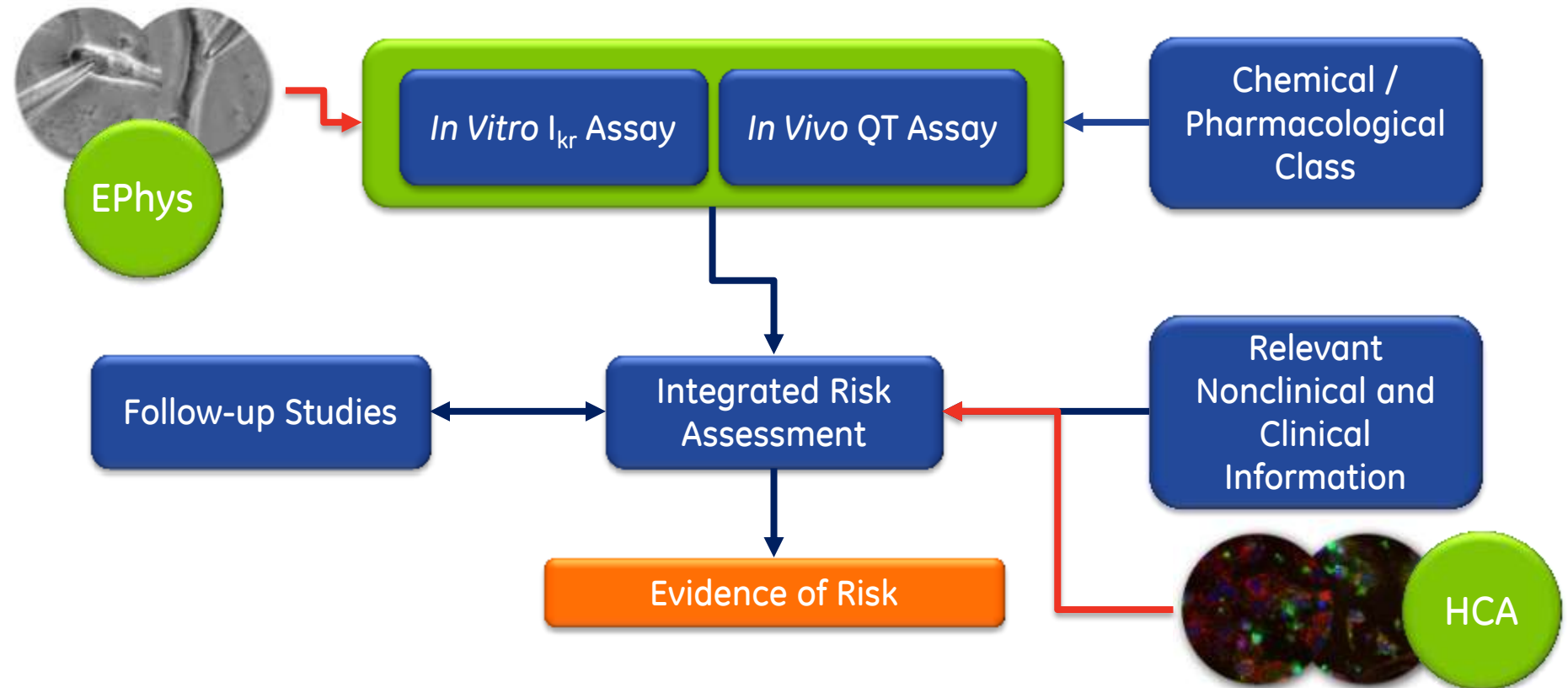
ICH S7B Nonclinical Testing Strategy



- Low level of integration - disparate engineered and ex-vivo model systems
- Focus on Ephys/QT assays (primarily hERG) - may miss non-EPhys liabilities

Cardiotoxicity ICH S7B

Vision for integrating hESC-Cardiomyocytes

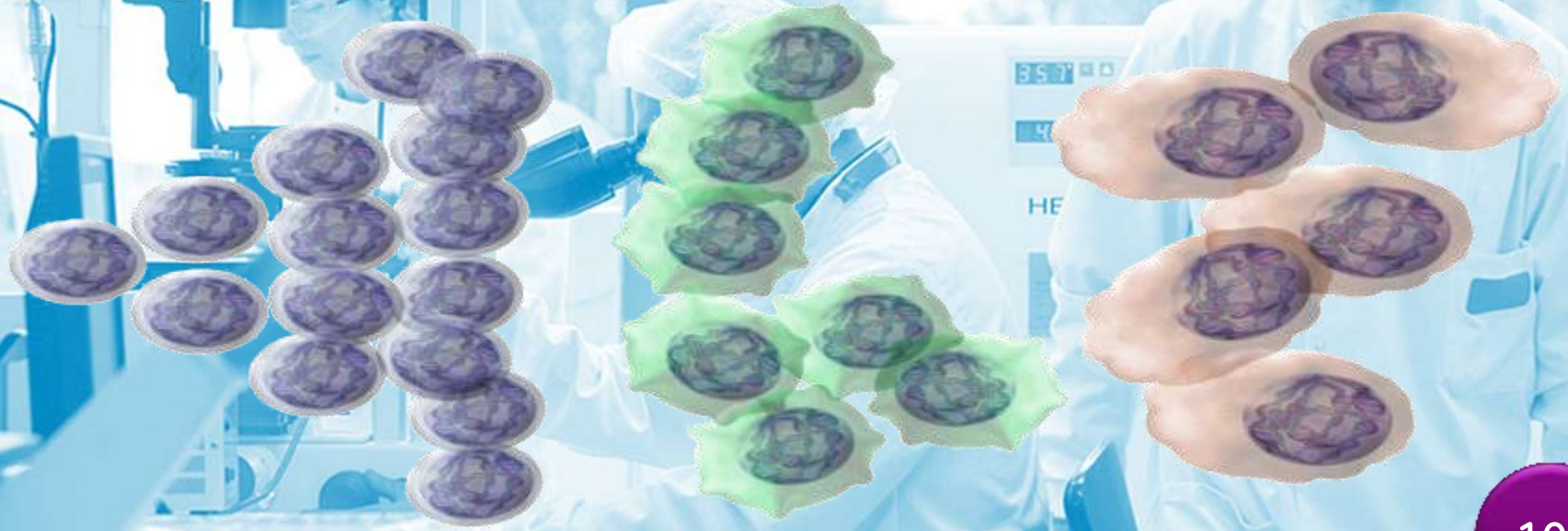


- hESC-CM complementing/replacing in-vitro hERG assay with global ion channel liability surveillance
- HCA assays complementing EPhys for functional cardiac liabilities

Cytiva Cardiomyocytes

H7 hESC

Cardiomyocytes



Expansion

Differentiation

0 5 10 15 20 25 30

Media 1

Media 2

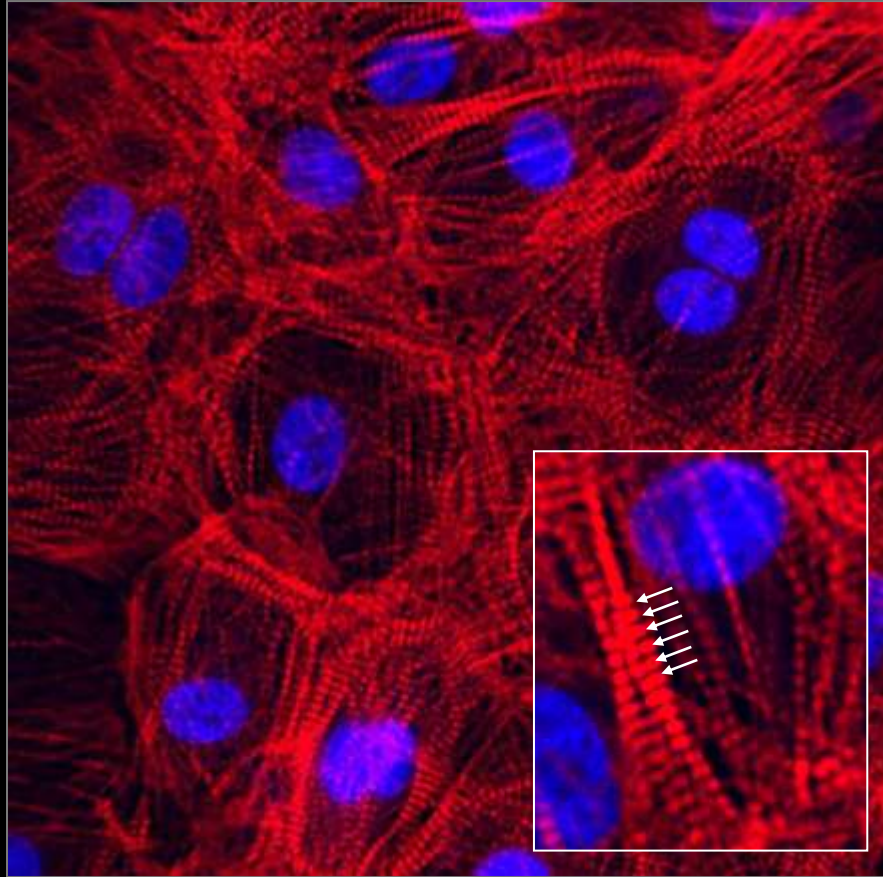
Growth Factors

Feed

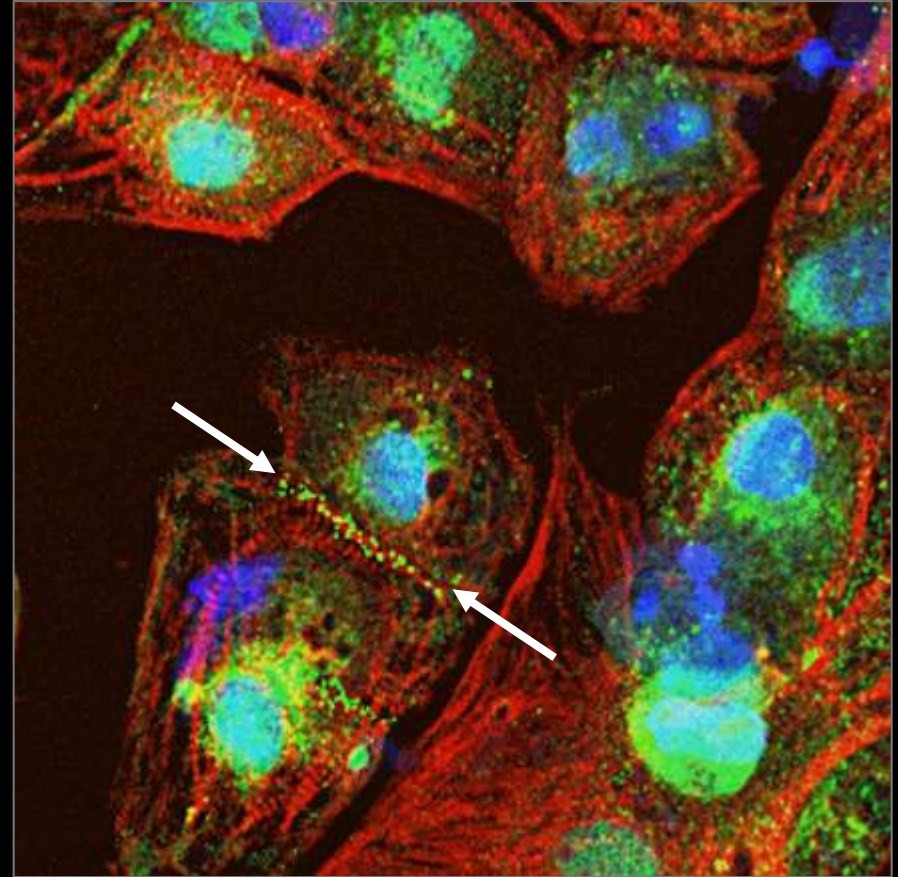
10^9



Cytiva Cardiomyocytes



DNA Troponin I



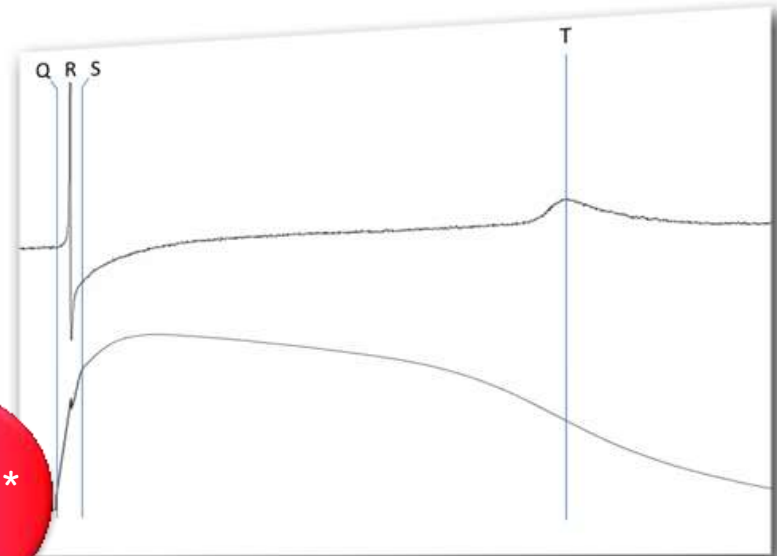
DNA Connexin 43 Troponin I

Cardiomyocytes in Drug Safety

Electrophysiology

HERG
Na⁺ & Ca²⁺ Channels
QT Prolongation

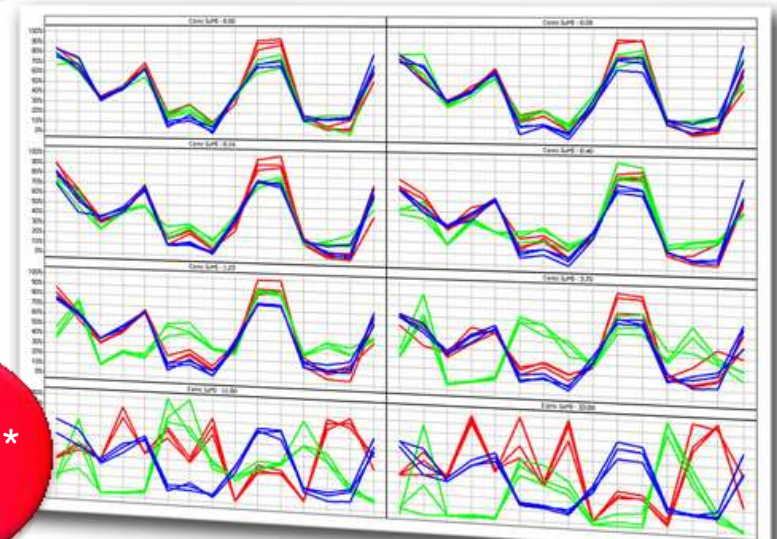
25%*



High Content Analysis

Mitochondria
Membrane Integrity
Ca²⁺ Homeostasis
Morphology

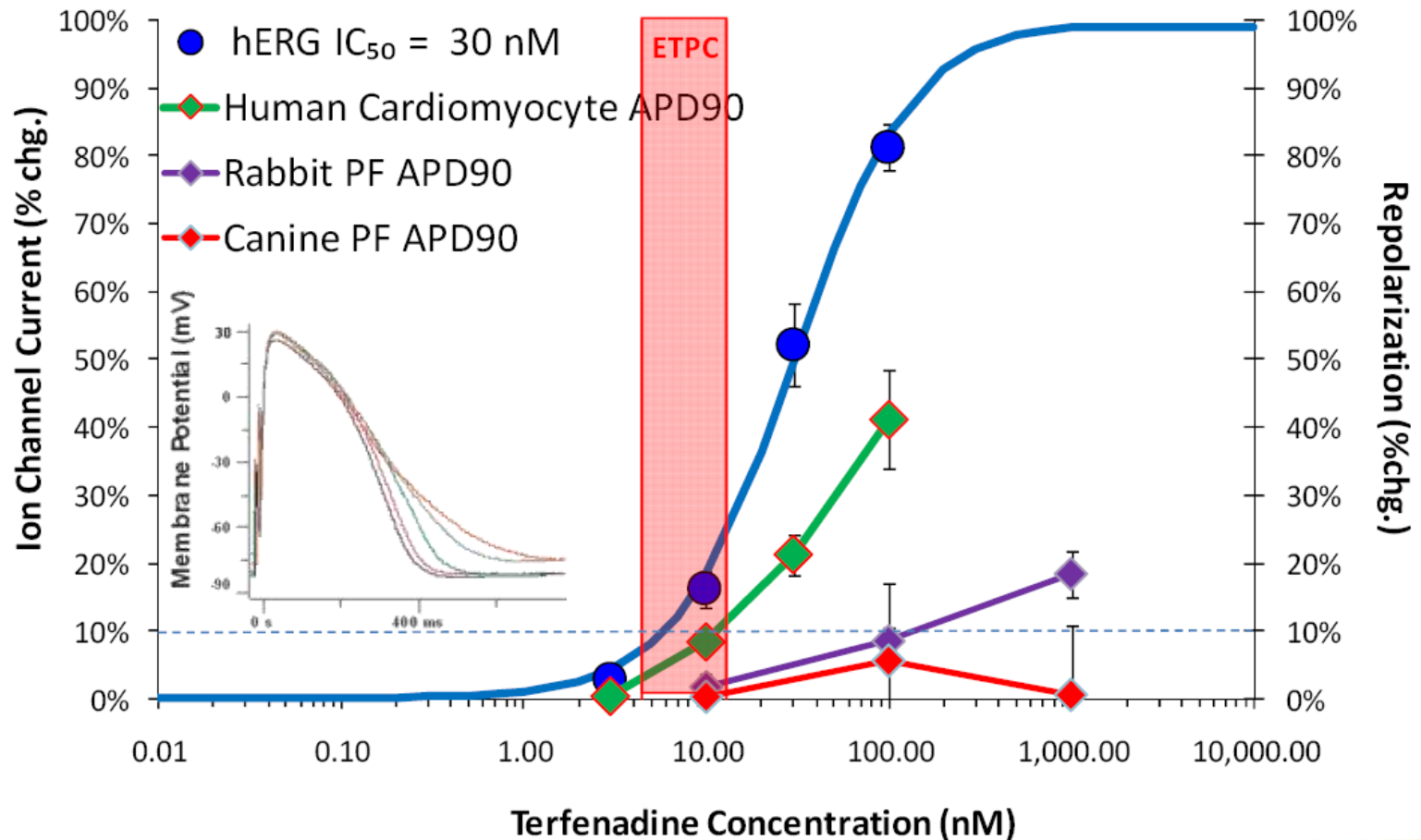
75%*



*AZ study of failed compounds

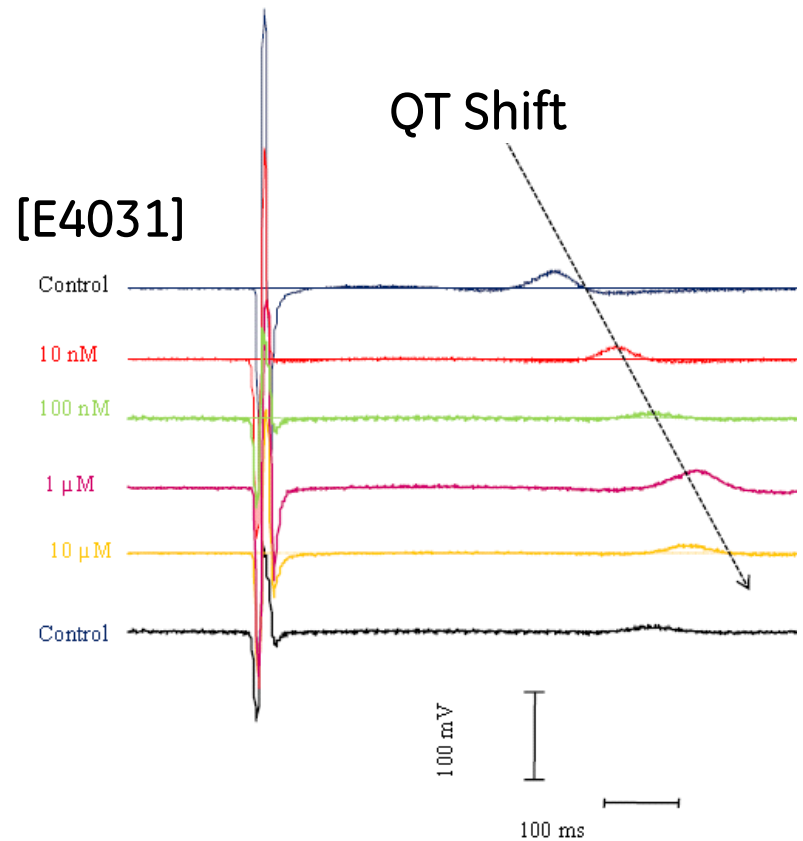
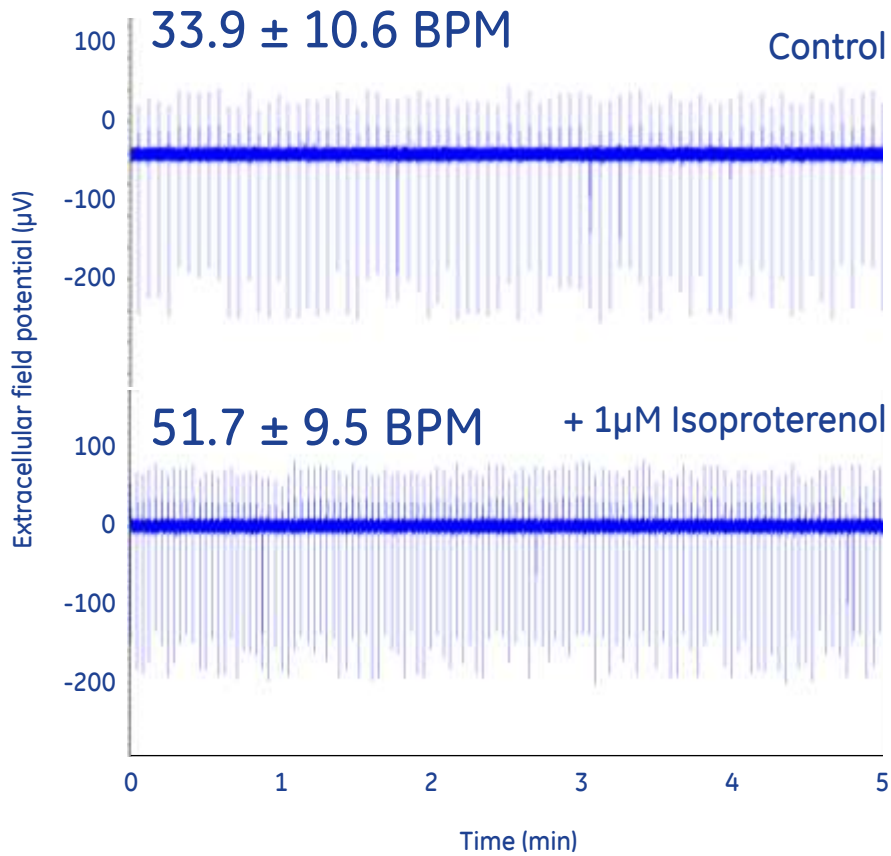
Manual Patch Clamp

Species variation in Terfenadine APD₉₀ prolongation



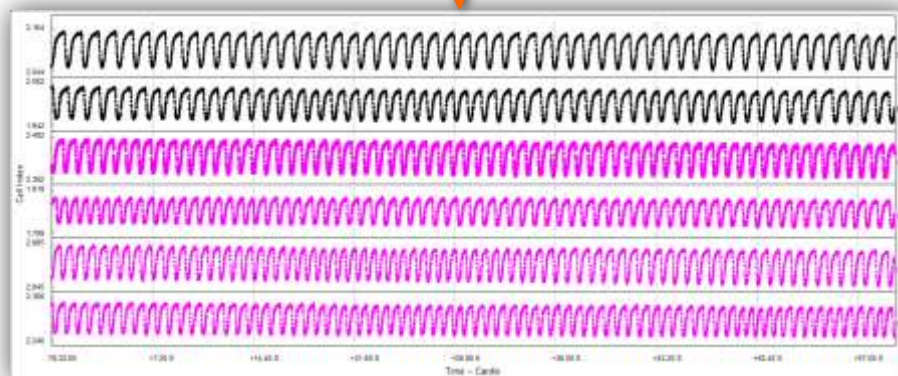
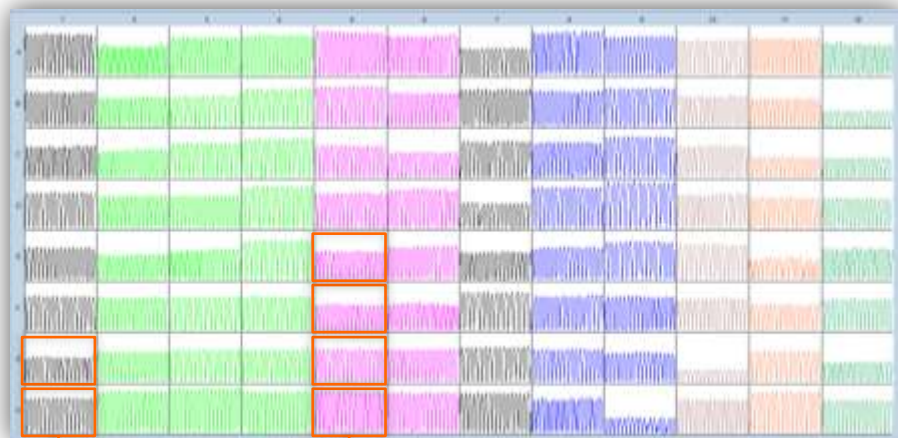
Multi-Electrode Array (MEA)

Integrated extracellular voltage measurement



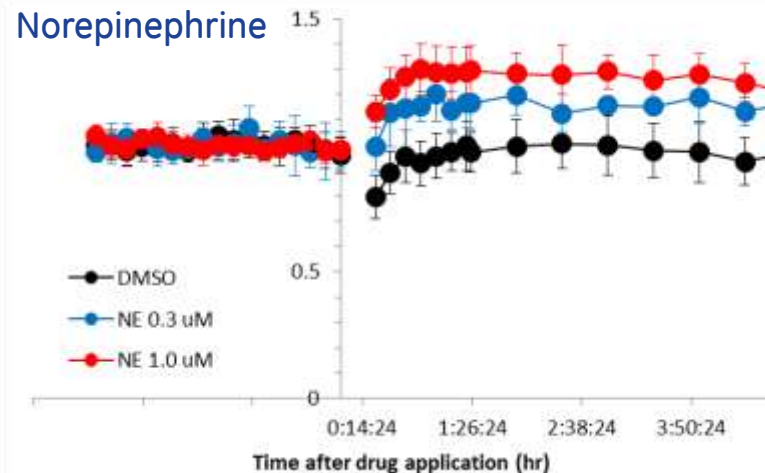
Label Free Beat Rate Measurement

xCELLigence RTCA Cardio Instrument

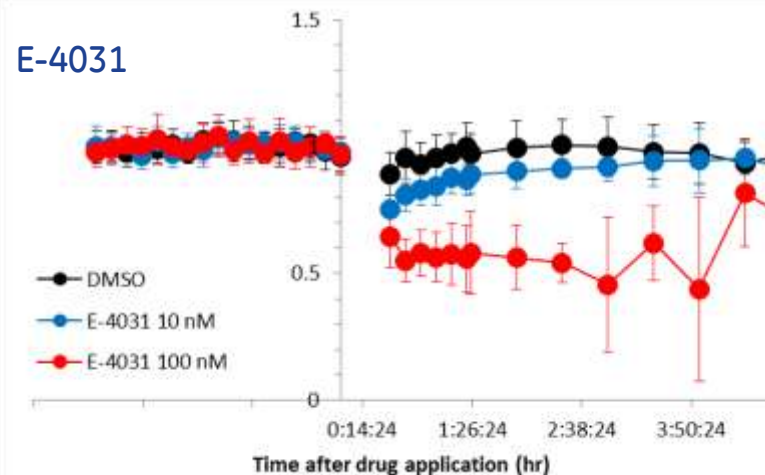


Data generated by Chantest using GEHC Cytiva™ cardiomyocytes on a Roche xCELLigence instrument as part of a GEHC/Chantest/Genentech collaboration.

Norepinephrine

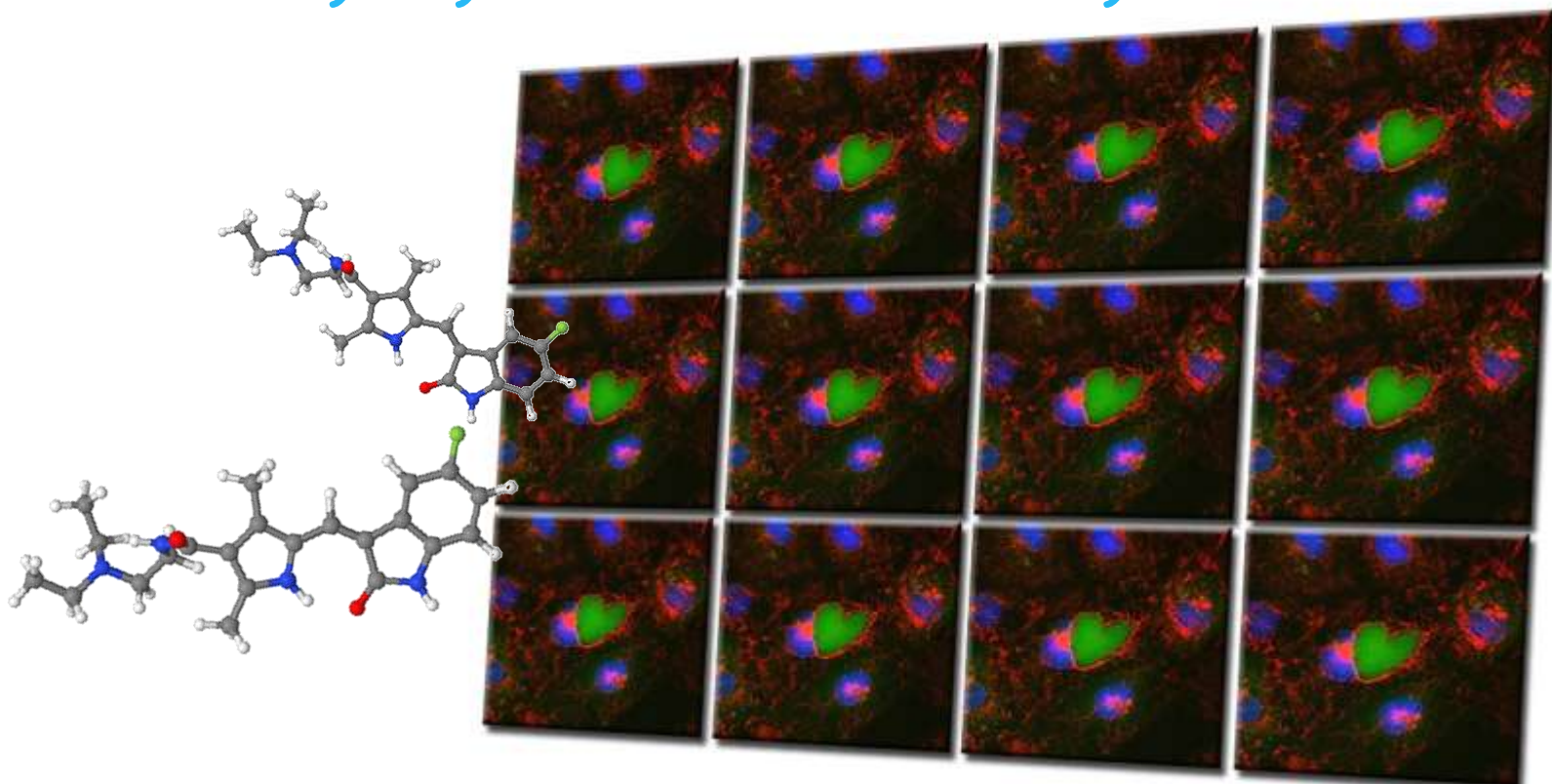


E-4031



Cardiotoxicity Profiling of Anticancer Drugs

Cytiva Cardiomyocytes & IN Cell Analyzer

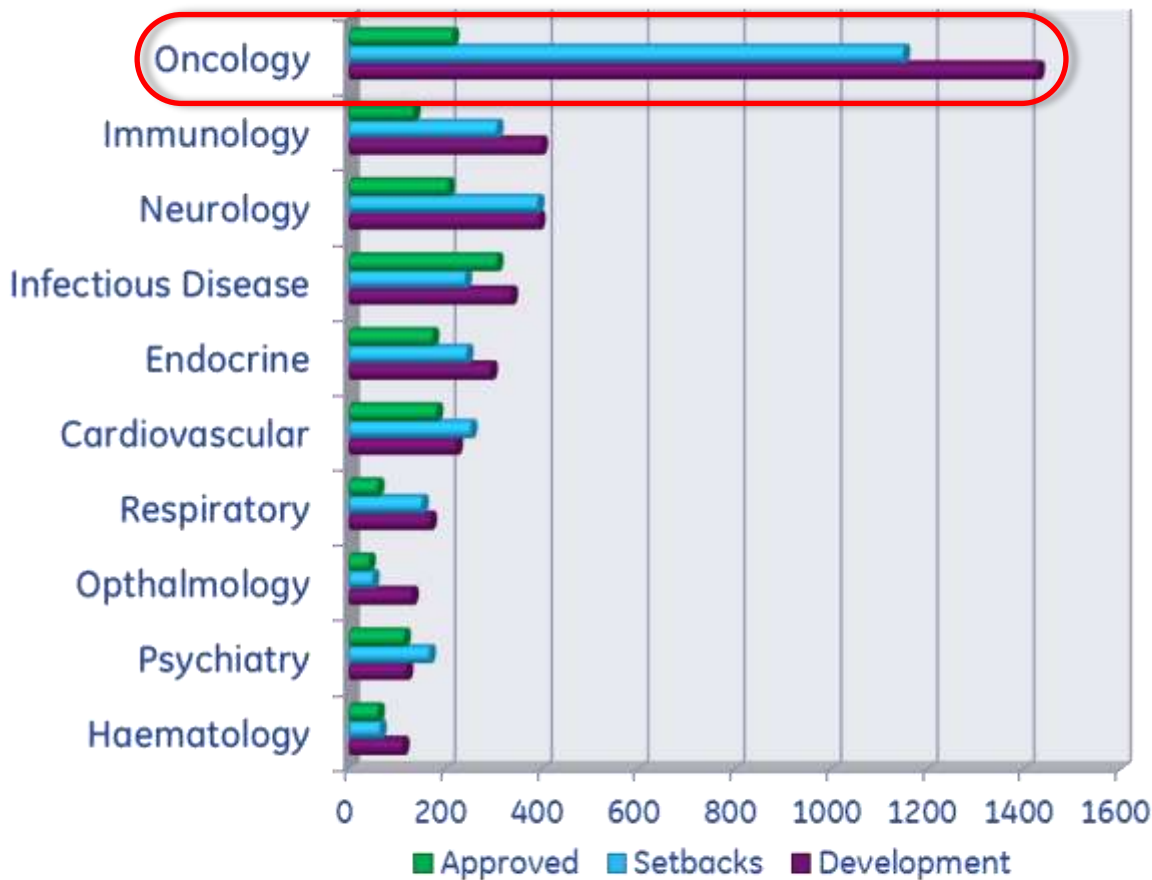


imagination at work

Genentech
A Member of the Roche Group

Cardiotoxicity & Anticancer Drugs

Drug Pipelines



Data from: Drug pipeline: Q411. Mak H.C. 2012
Nature Biotechnol. 30,15

Toxicity in Drug Development

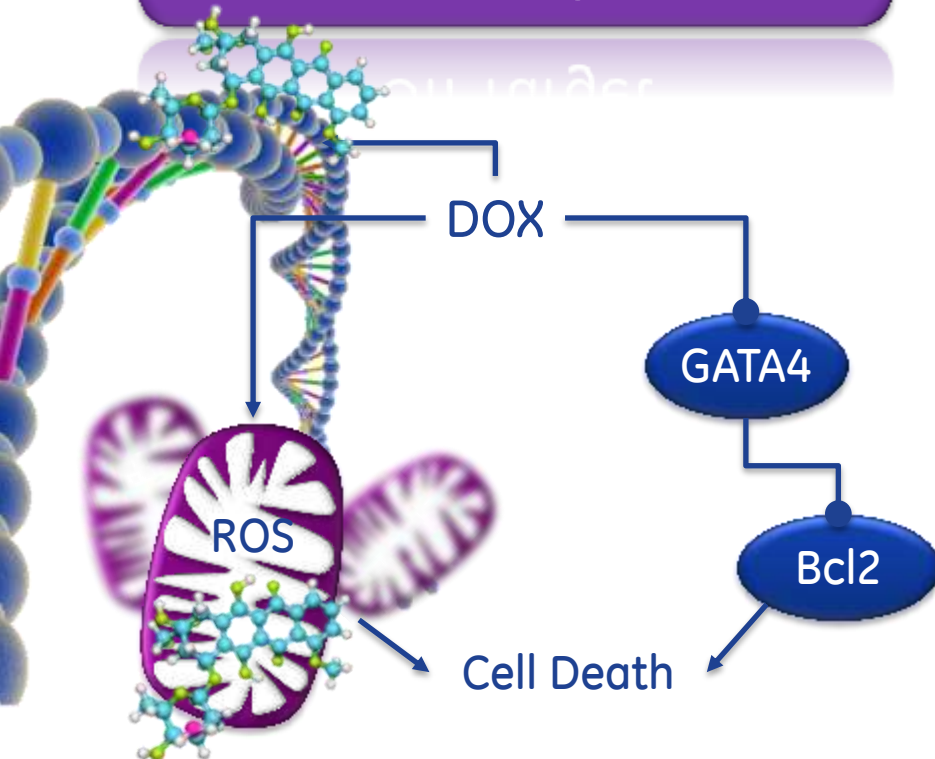


- Hepatotoxicity
- Nephrotoxicity
- Cardiotoxicity
- Rhabdomyolysis
- Other

Data from; Wilke RA et al. Nature Reviews Drug Discovery
2007 6, 904-916

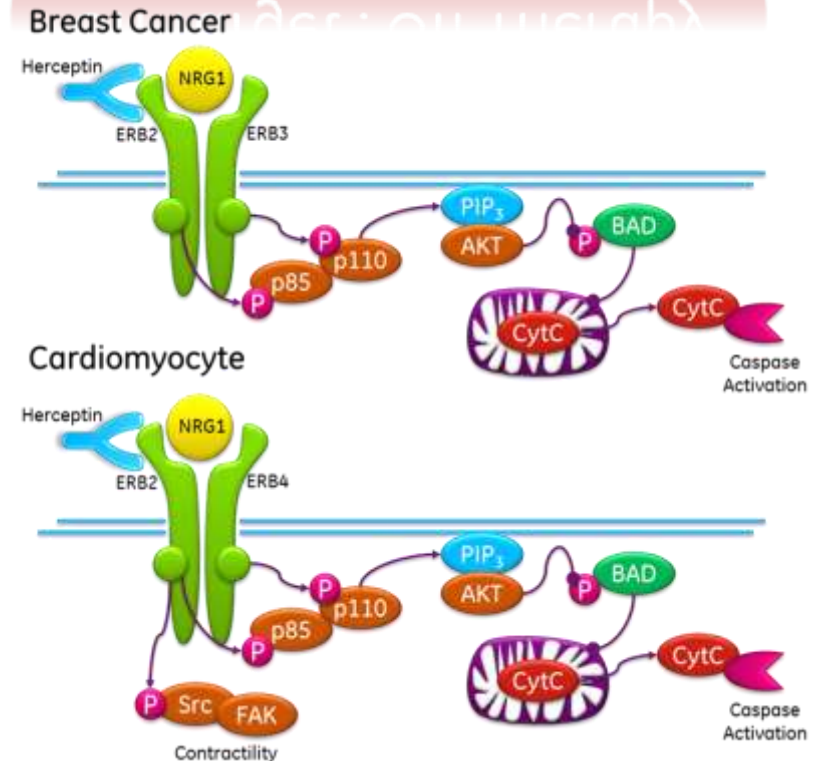
Cardiotoxicity of Anticancer Drugs

Off Target



Adapted from; Kobayashi S. et.al. FASEB Journal. 2006;20:800-802

On Target : Off Therapy



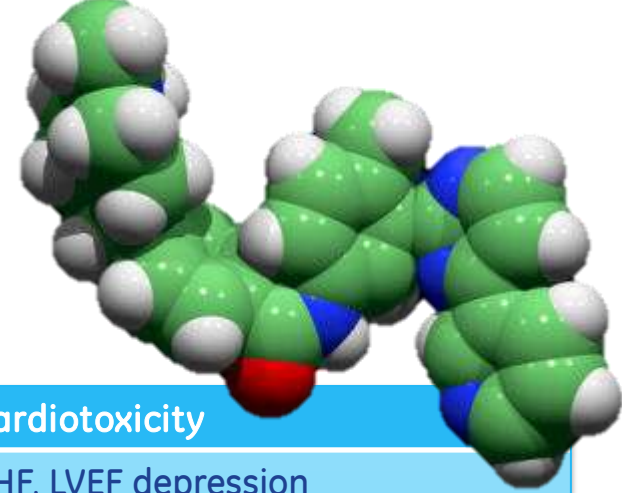
Adapted from; Hansel et.al. Nature Reviews Drug Discovery 2010 ;9 325

Small molecules

Biologics

Cancer & Cardiotoxicity

Tyrosine kinase inhibitors

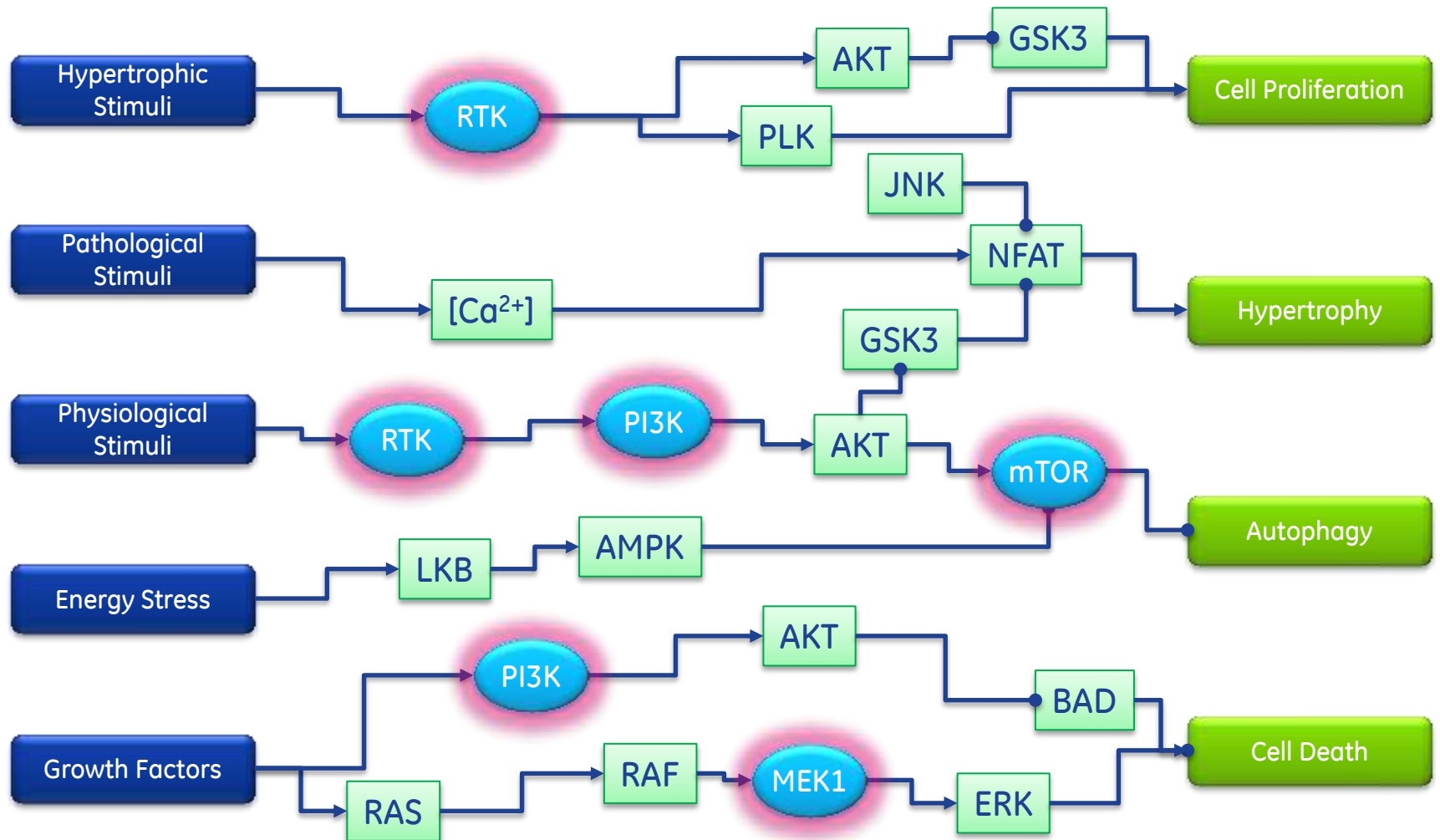


Drug	TK Target	Indications	Cardiotoxicity
Imatinib	Bcr-Abl, c-kit, PDGFR	CML, PhALL, GIST, CMML, CEL, DFSP	CHF, LVEF depression
Dasatinib	Bcr-Abl, c-kit, PDGFR, Src	CML	QT prolongation, Peripheral oedema
Nilotinib	Bcr-Abl, c-kit, PDGFR	CML	QT prolongation
Sunitinib	VEGFR, RET, PDGFR, c-kit	RCC, GIST	Hypertension, LVEF depression, CHF, MI
Sorafenib	VEGFR, c-kit, PDGFR, FLT3, RAF1	RCC, HCC	Acute coronary syndrome, MI, Hypertension
Lapatinib	EGFR, ERBB2	Breast Ca	Asymptomatic LVEF depression
Gefitinib	EGFR	NSCLC	Not reported
Erlotinib	EGFR	NSCLC, Ca pancreas	Not reported

Data from: Orphanos G.S. et.al. Cardiotoxicity induced by tyrosine kinase inhibitors 2009; Acta Oncologica, 48: 964-970

Cancer & Cardiotoxicity

Overlapping Cardiac Signalling & Oncology Targets



Adapted from; Force T & Kolaja K.L. Cardiotoxicity of kinase inhibitors: the prediction and translation of preclinical models to clinical outcomes. 2011; Nature Reviews Drug Discovery 10, 111-126

Cardiotoxicity assay compounds

Oncology focus

Compound	Target	Compound	Target
Amiodarone (+ve control)	K ⁺ Channel/Adrenergic	Nifedipine (-ve control)	Ca ²⁺ Channel
Dasatinib (Sprycel)	Tyrosine Kinase	NVP-BEZ235	PI3K
Tasocitinib	Tyrosine Kinase	PIK 90	PI3K
Pazopanib (Votrient)	Tyrosine Kinase	LY 294002	PI3K
Axitinib	Tyrosine Kinase	SB 202190	p38
Mubritinib	Tyrosine Kinase	VX 702	p38
Tyrphostin AG1478	Tyrosine Kinase	Rapamycin	mTOR
Vandetanib (Zactima)	Tyrosine Kinase	Temsirolimus	mTOR
Vatalanib	Tyrosine Kinase	PD 98059	MEK1
Sorafenib (Nexavar)	Tyrosine Kinase	PD 325901	MEK1
Sunitinib (Sutent)	Tyrosine Kinase	PD 184352	MEK1
Lapatinib (Tyverb)	Tyrosine Kinase	U 0126	MEK1
Imatinib (Gleevec)	Tyrosine Kinase	Pimecrolimus	FKBP-12
GECT-Y	Tyrosine Kinase	Entinostat	HDAC

Functional Cardiotoxicity

Disruption of cellular integrity

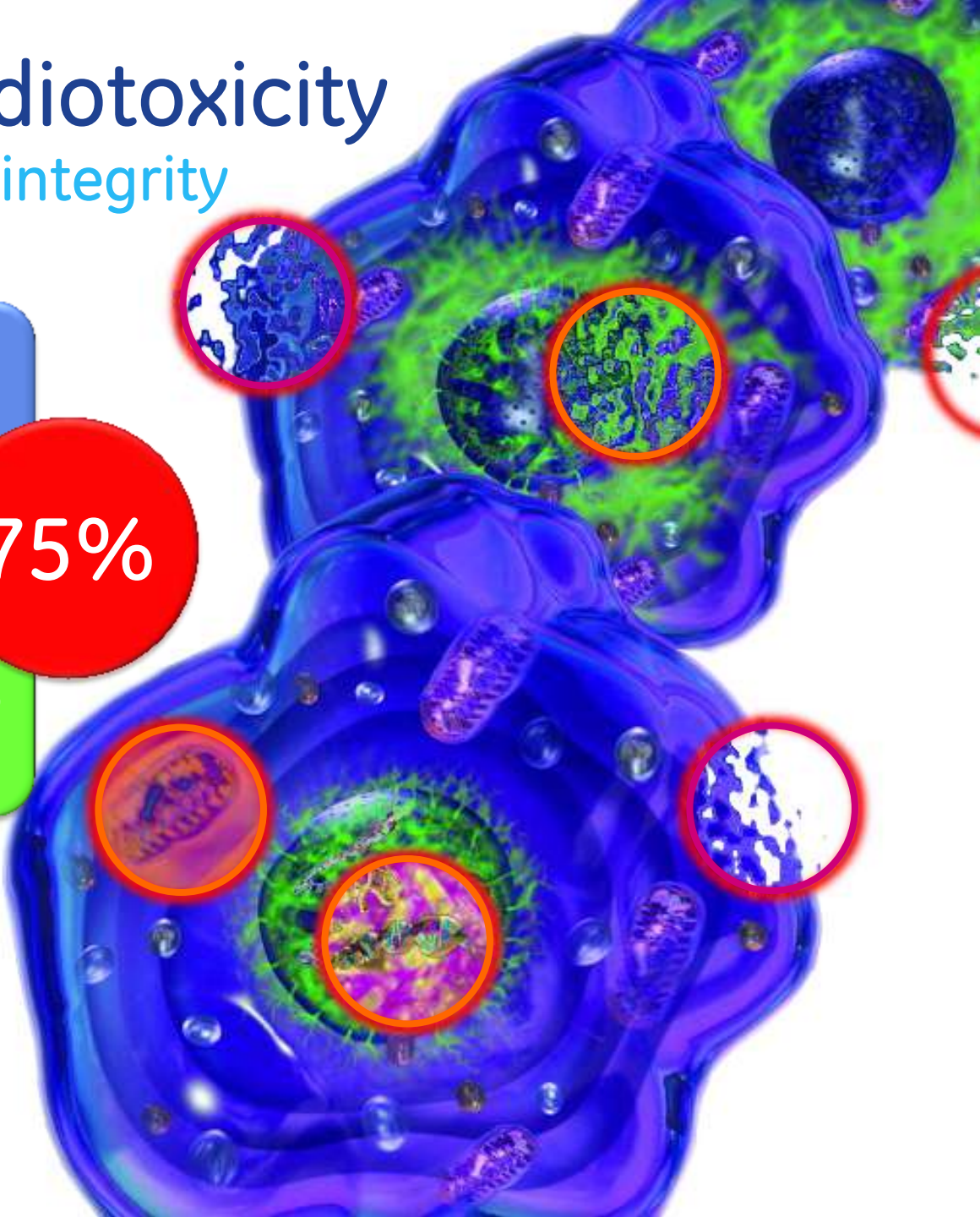
Membrane Integrity

Dissipation of gradients,
organelle disruption, loss of
homeostasis.....

Biochemical Integrity

Disruption of signal transduction,
synthesis, metabolism,
cytoskeletal machinery.....

75%



imagination at work

High Content Analysis (HCA)

Cardiomyocyte Toxicity Assay

Fluorescent Dyes

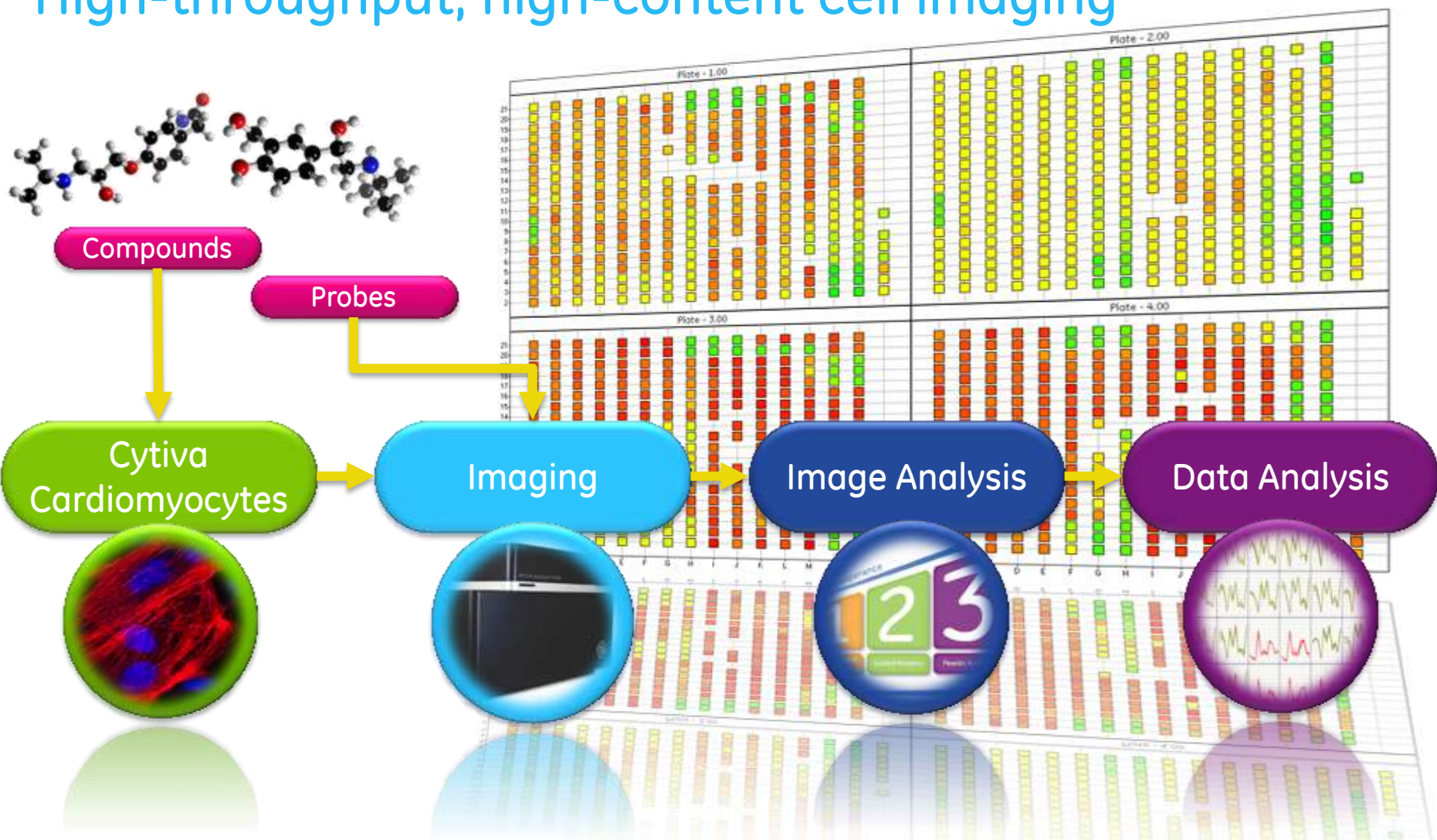
Cytiva Cardiomyocytes



Cell Biology In-Situ & In-Context

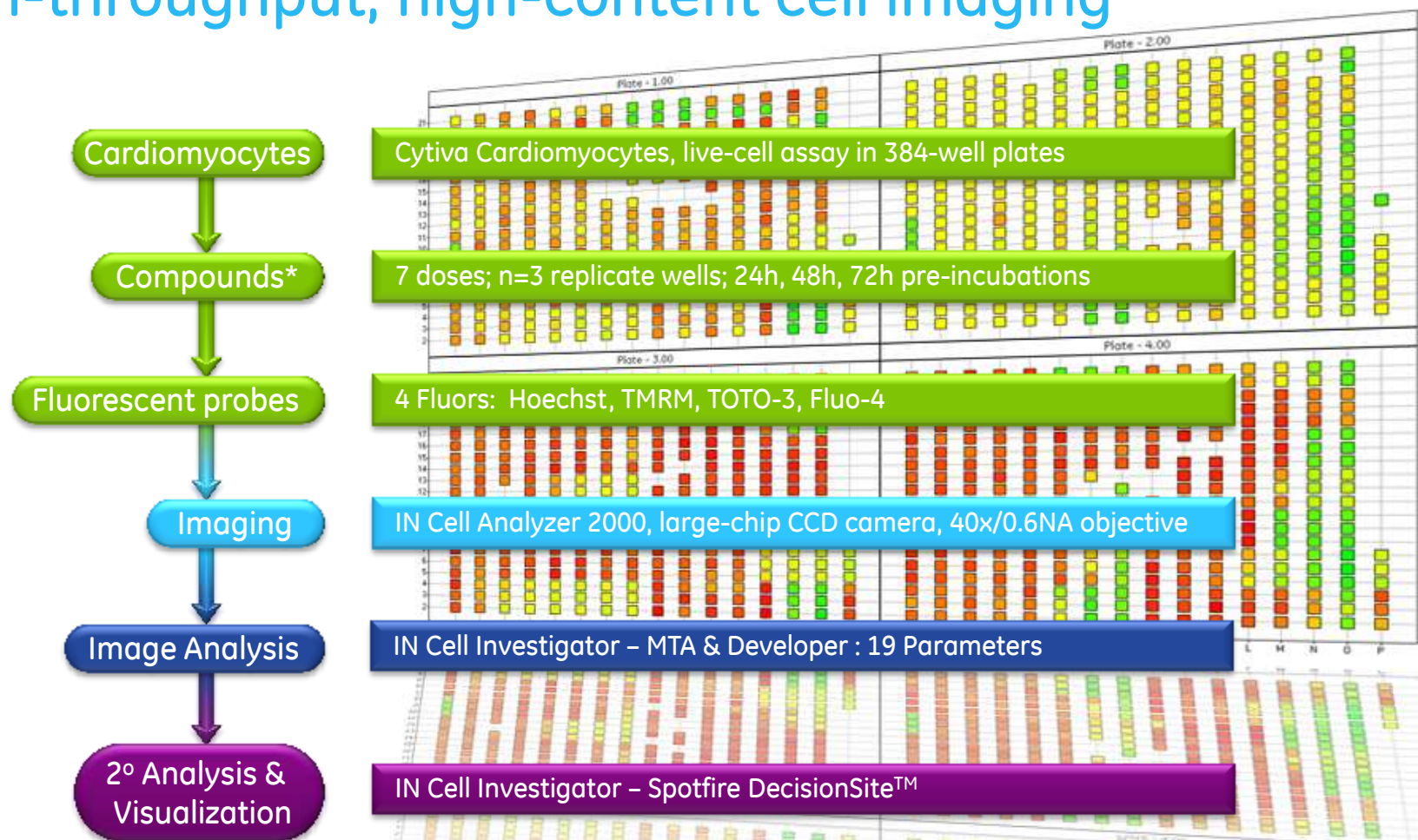
Cardiotoxicity Assay Workflow

High-throughput, high-content cell imaging



Cardiotoxicity Assay Workflow

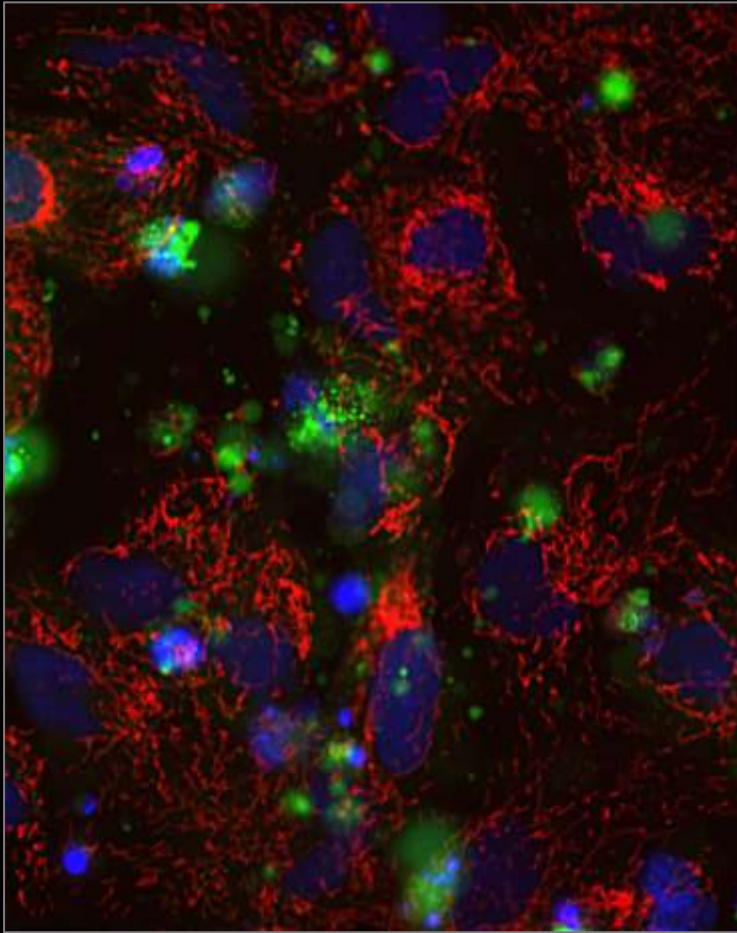
High-throughput, high-content cell imaging



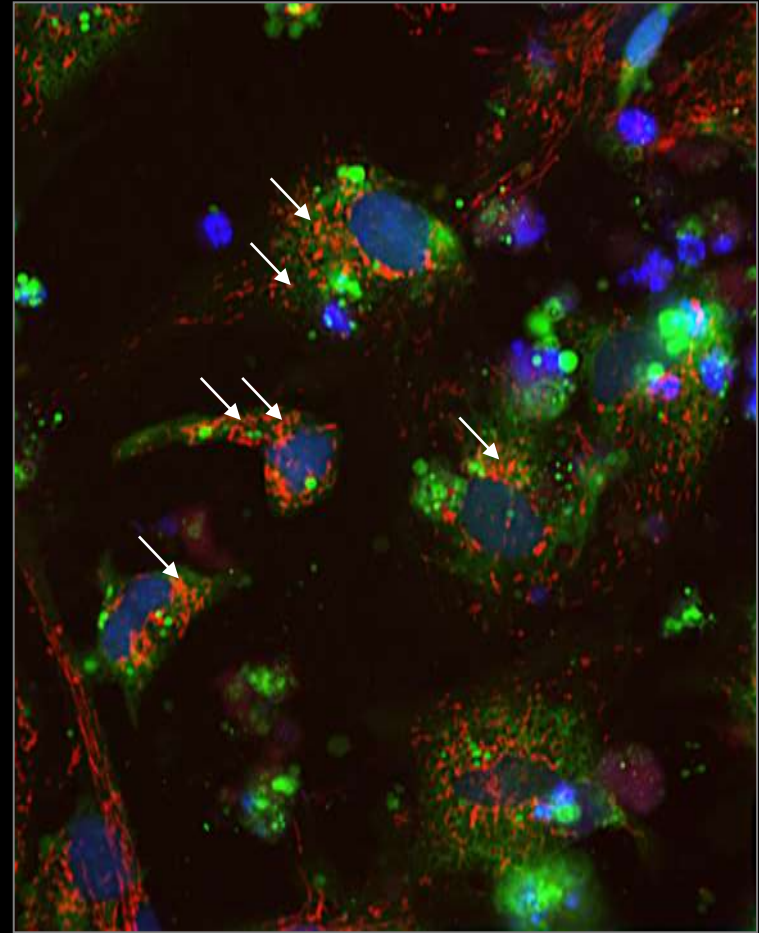
*Compounds blinded until data analysis completed

Cytiva Cardiotoxicity Assay

Control



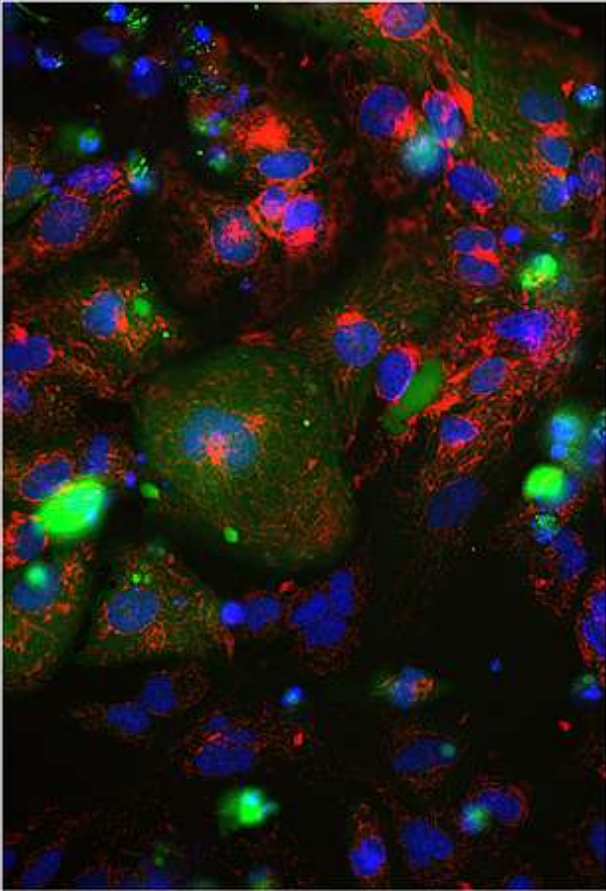
Amiodarone



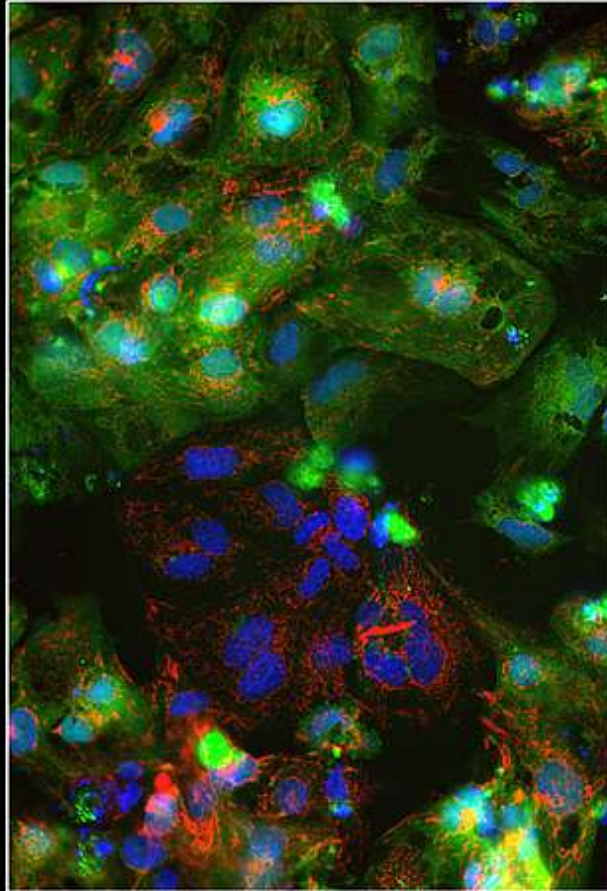
DNA Mitochondria Ca²⁺

Vatalanib

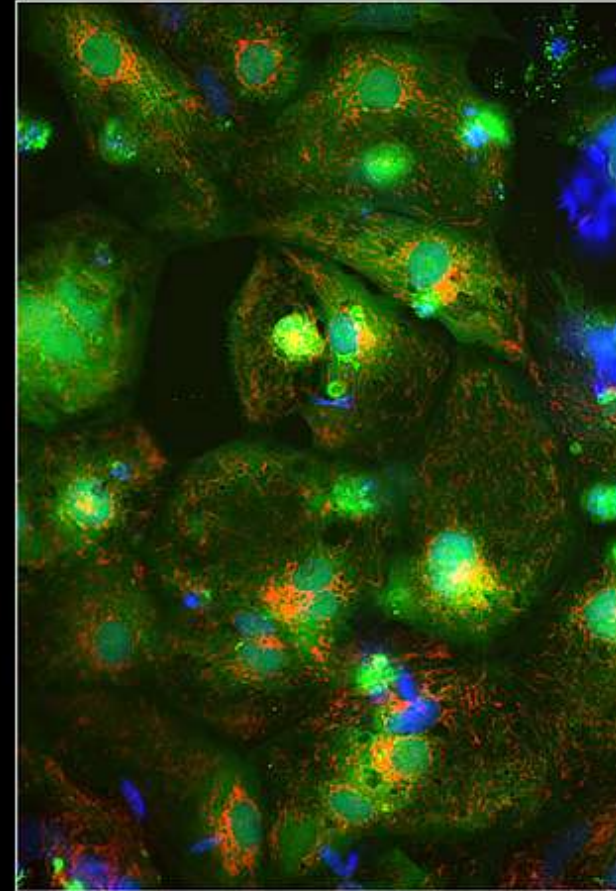
1.24 μ M



11.0 μ M



33.0 μ M



72 hours DNA Mitochondria Ca²⁺



imagination at work

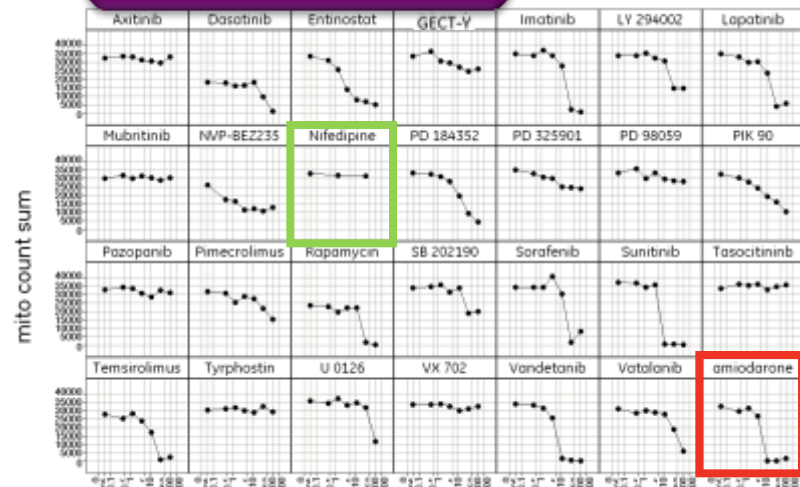
Dose response data

4/19 parameters 72 hours

 Nifedipine (-ve control)

 Amiodarone (+ve control)

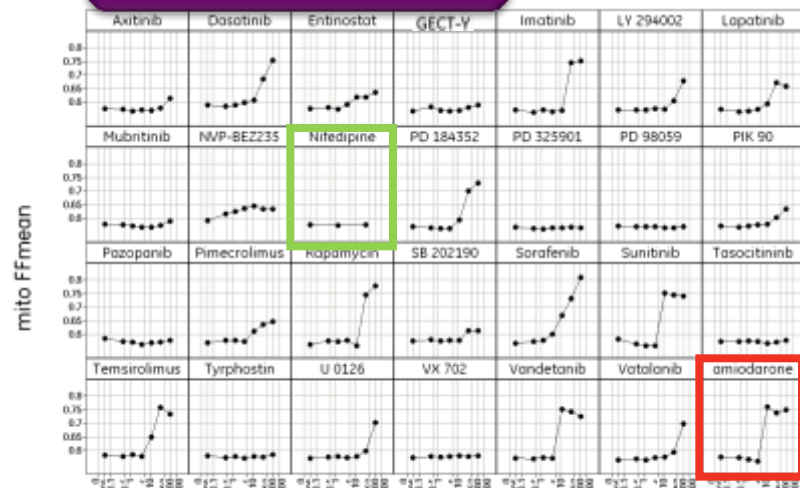
Mitochondrial Count



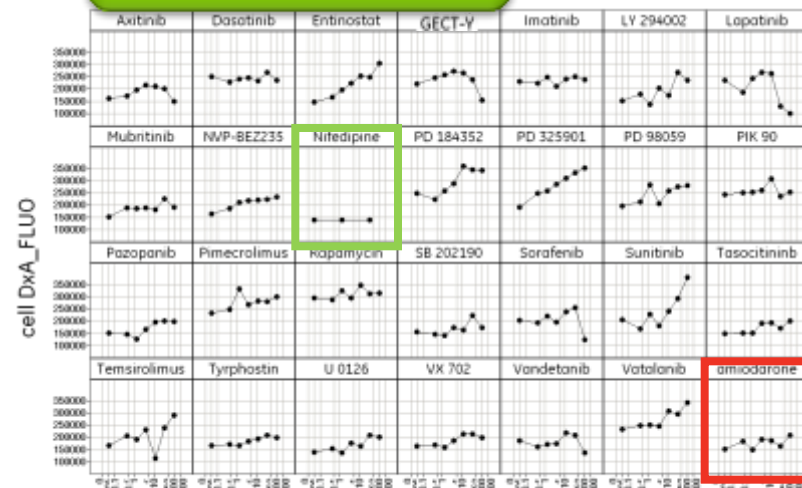
Viability



Mitochondrial Form Factor



Cell Calcium

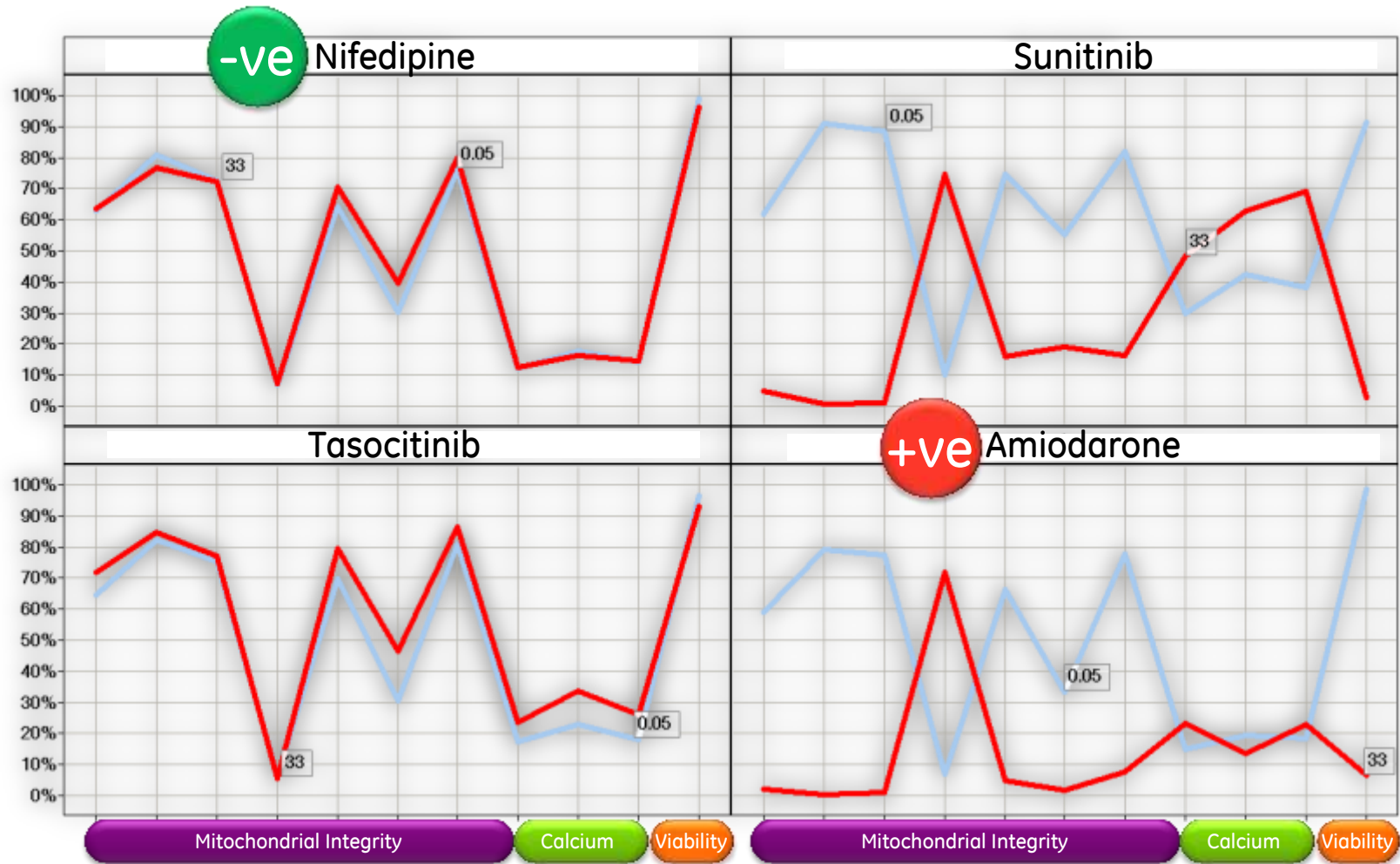


Conc (uM)

Conc (uM)

Cardiotoxicity Assay

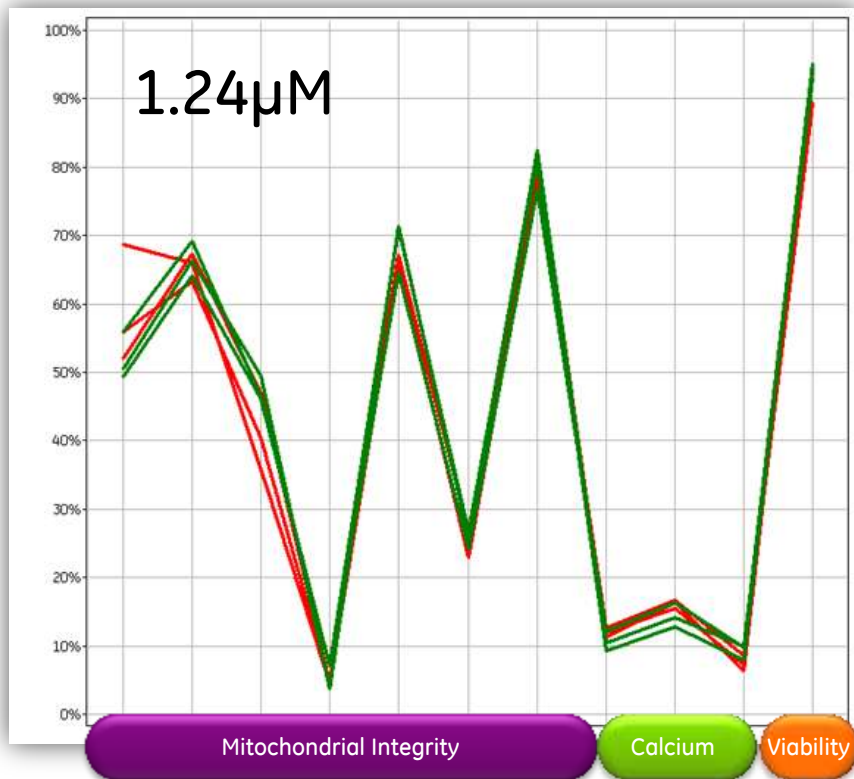
Multi-parameter Phenotypic Profiling



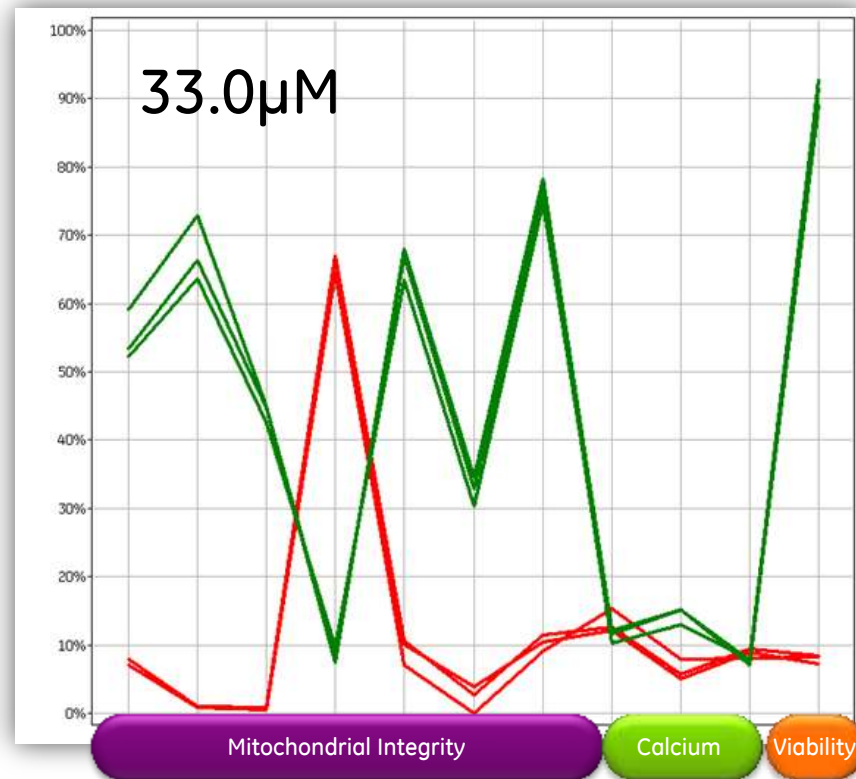
Data Profiling

Assay reproducibility

Nifedipine (n=3) Amiodarone (n=3)

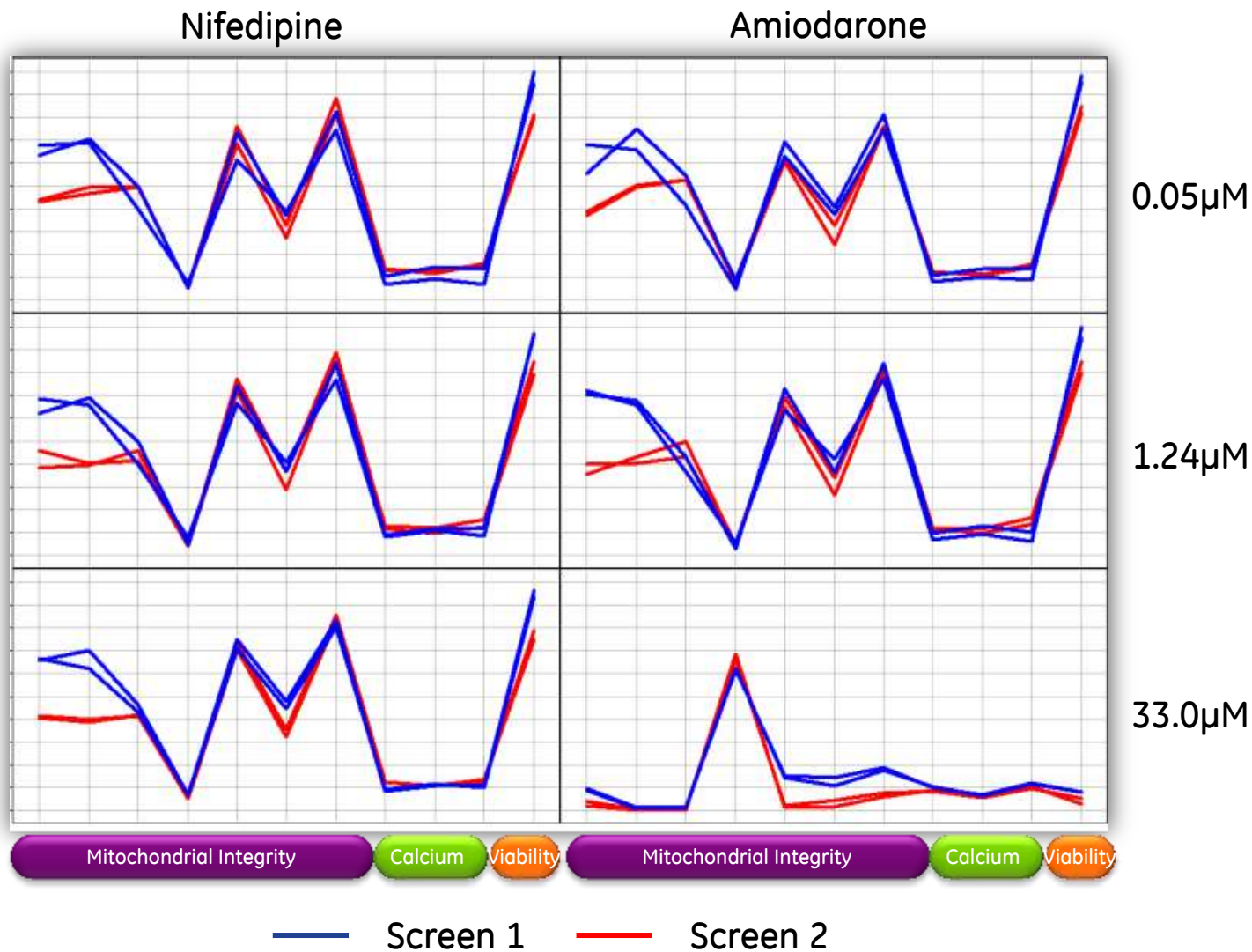


Nifedipine (n=3) Amiodarone (n=3)



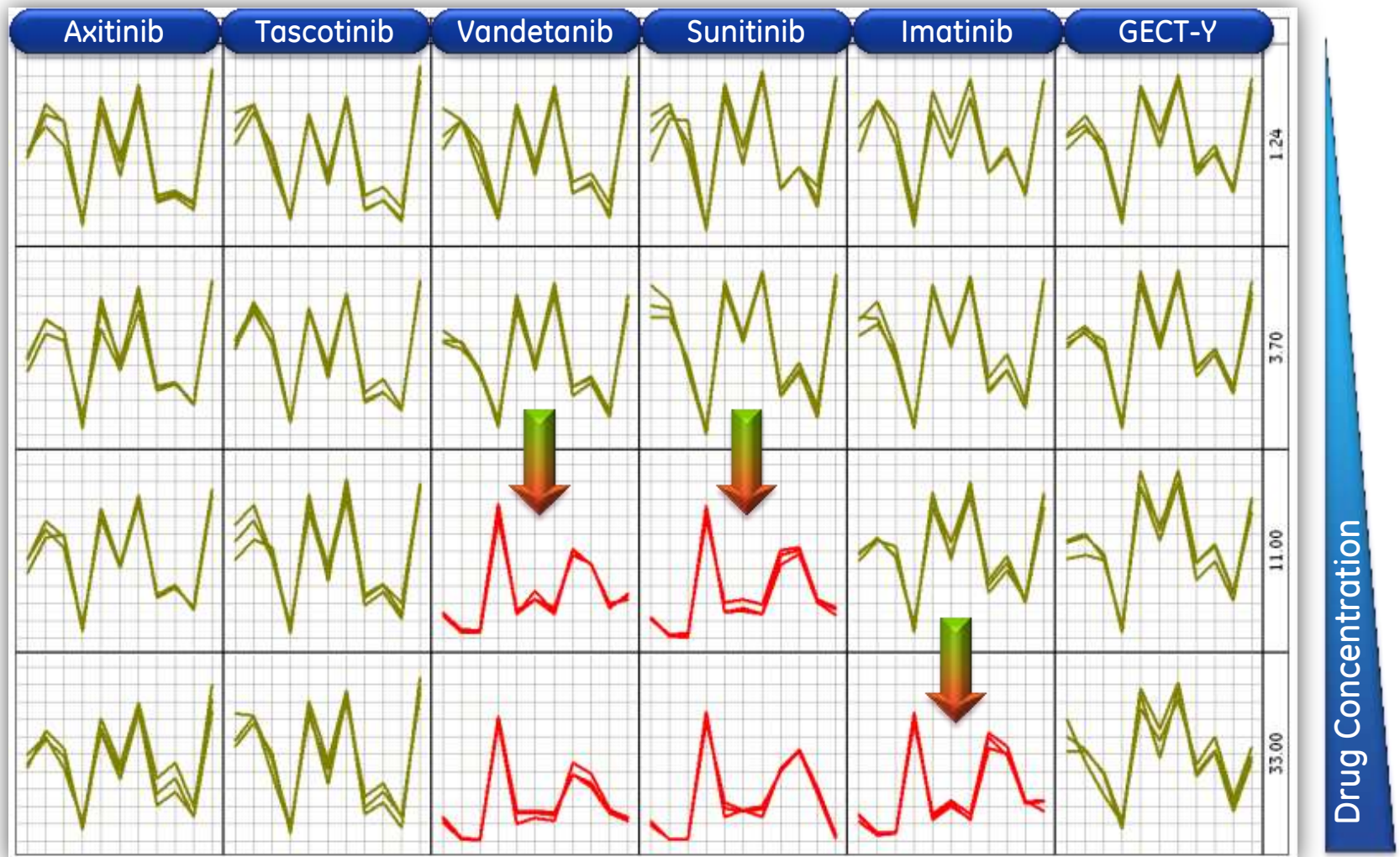
Data Profiling

Screen reproducibility



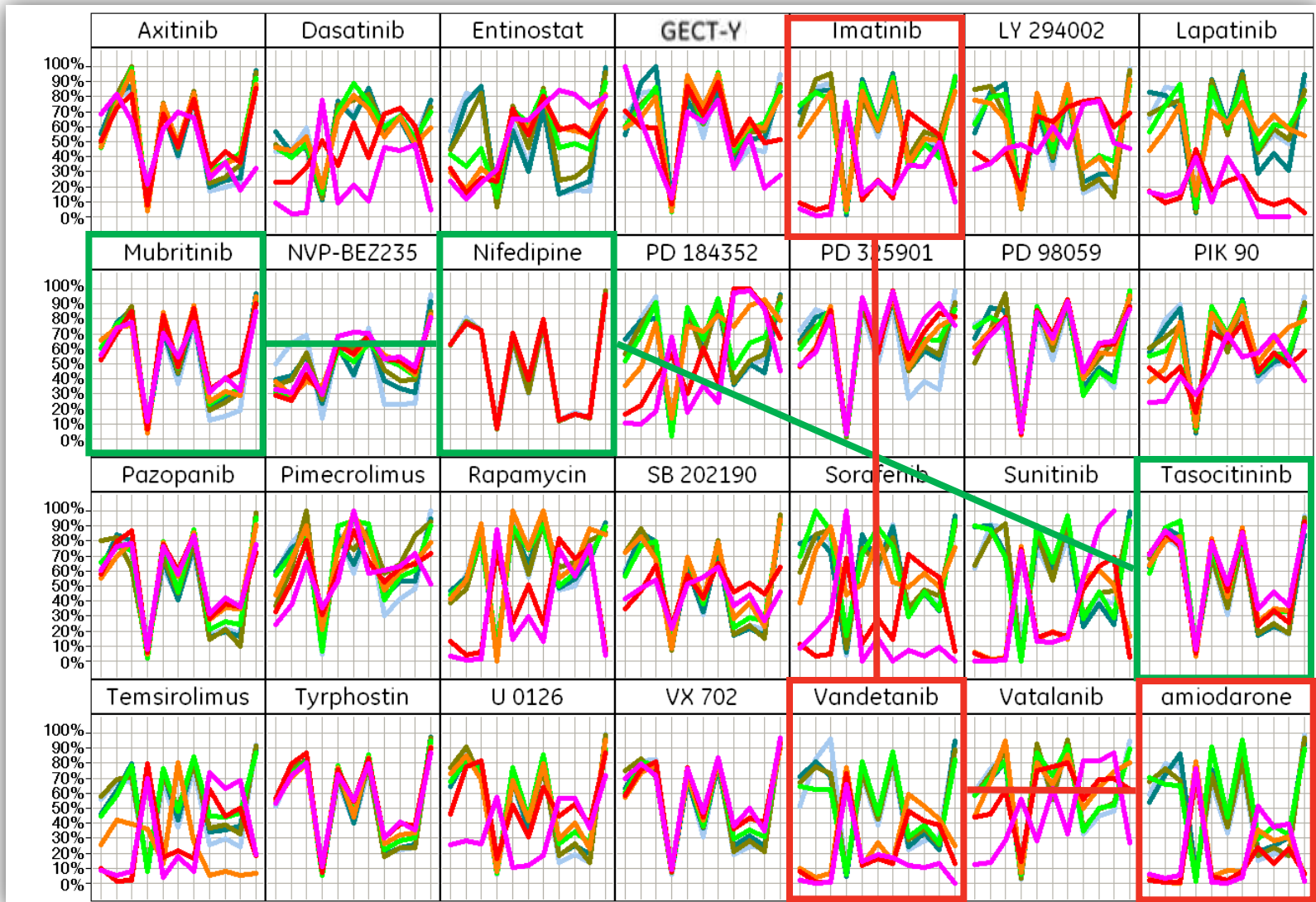
Profiling Anti-Cancer Drugs

Tyrosine kinase inhibitors

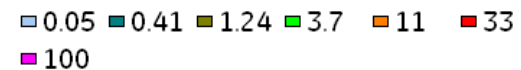


Drug Concentration

Data Profiling

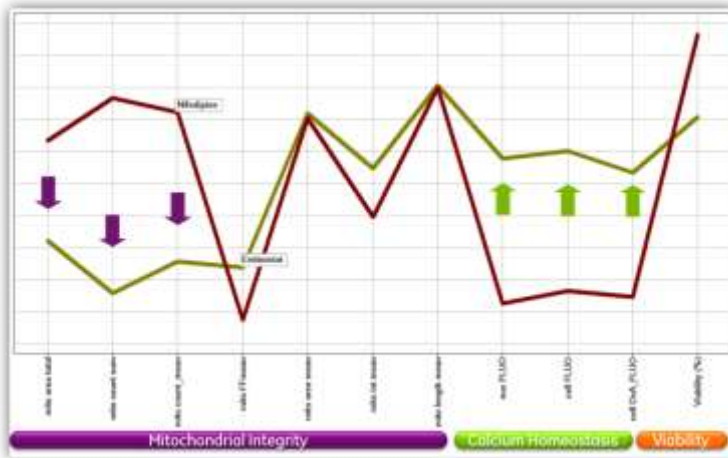


Color by Conc (uM):



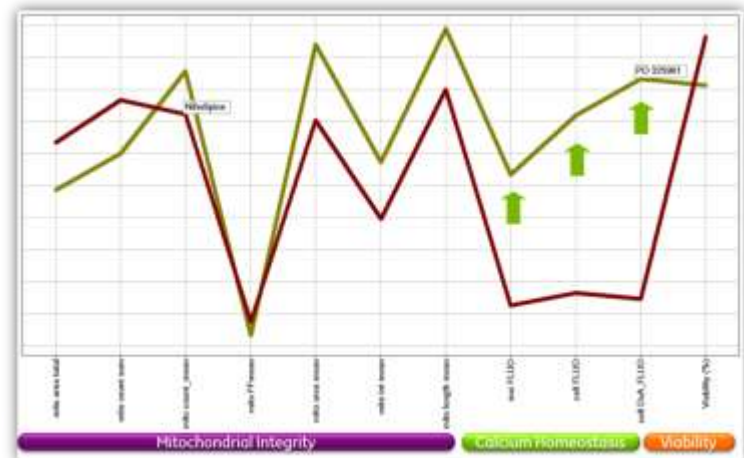
Entinostat (HDAC)

33.0 μ M – mitochondrial count and $\Delta[Ca^{2+}]$



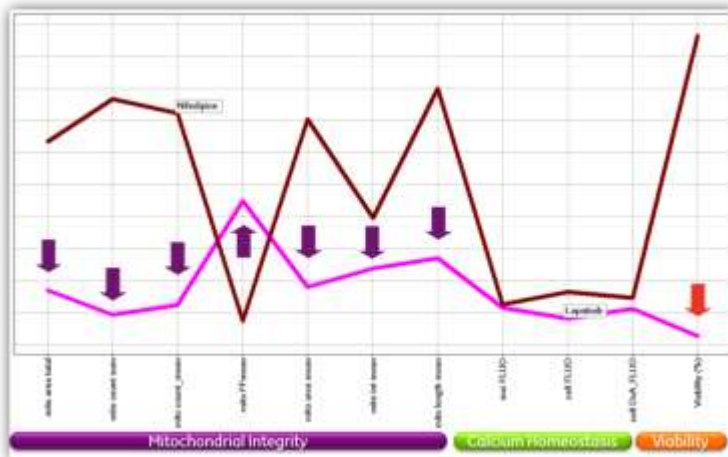
PD 325901 (MEK1)

33.0 μ M – $\Delta[Ca^{2+}]$ only



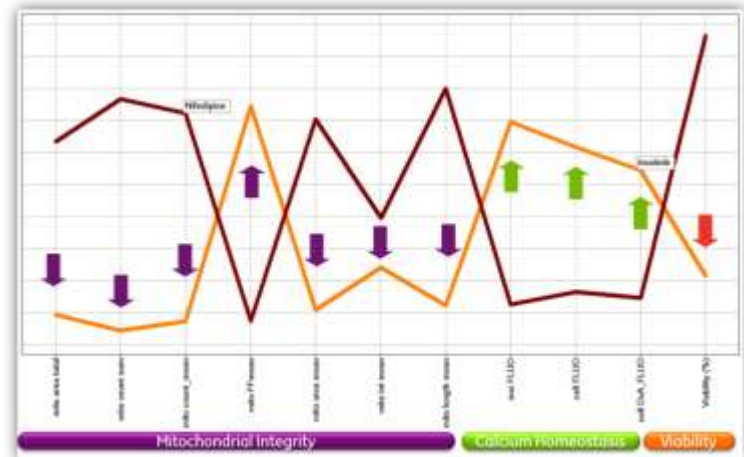
Lapatinib (TK – EGFR/HER2)

33.0 μ M – Mitochondrial count, morphology & viability



Imatinib (TK – PDGFR/KIT)

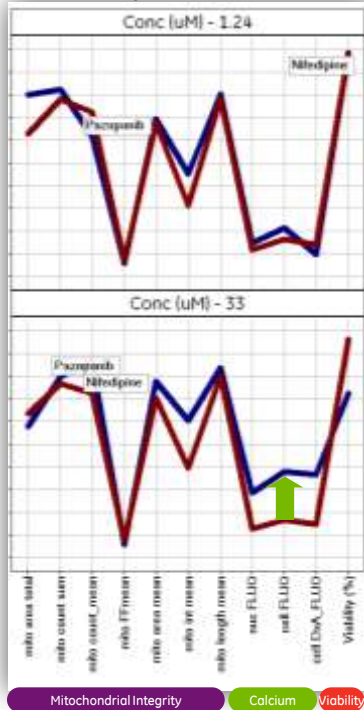
33.0 μ M – $\Delta[Ca^{2+}]$, mitochondrial count, morphology & viability



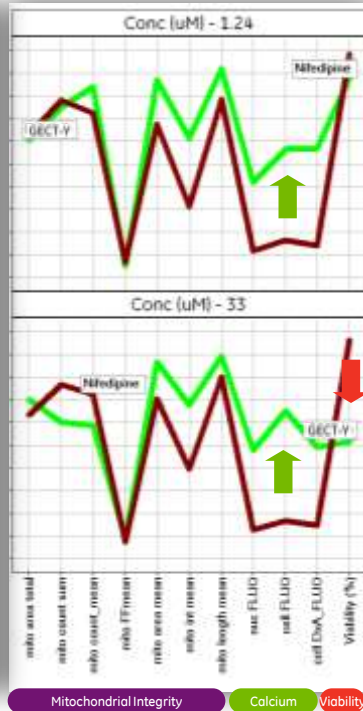
Selected Tyrosine Kinase Inhibitors

Range of MOAs

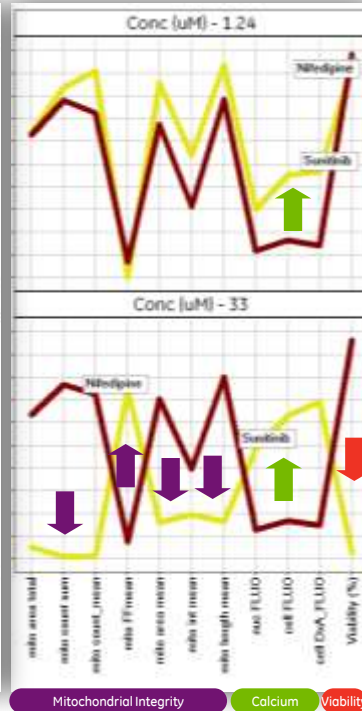
Pazopanib



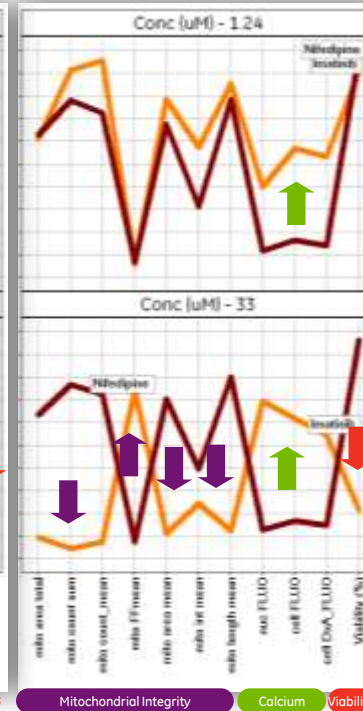
GECT-Y



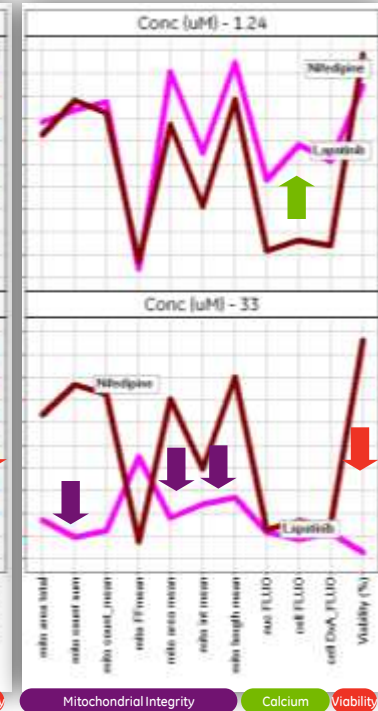
Sunitinib



Imatinib



Lapatinib



1.24μM no effect
33.0μM small Δ [Ca²⁺]

1.24μM Δ [Ca²⁺]
33.0μM Δ [Ca²⁺] and loss of viability

1.24μM Δ [Ca²⁺]
33.0μM Δ [Ca²⁺], changes in mitochondrial morphology and loss of viability

1.24μM Δ [Ca²⁺]
33.0μM Δ [Ca²⁺], changes in mitochondrial morphology and loss of viability

1.24μM Δ [Ca²⁺]
33.0μM changes in mitochondrial morphology and loss of viability

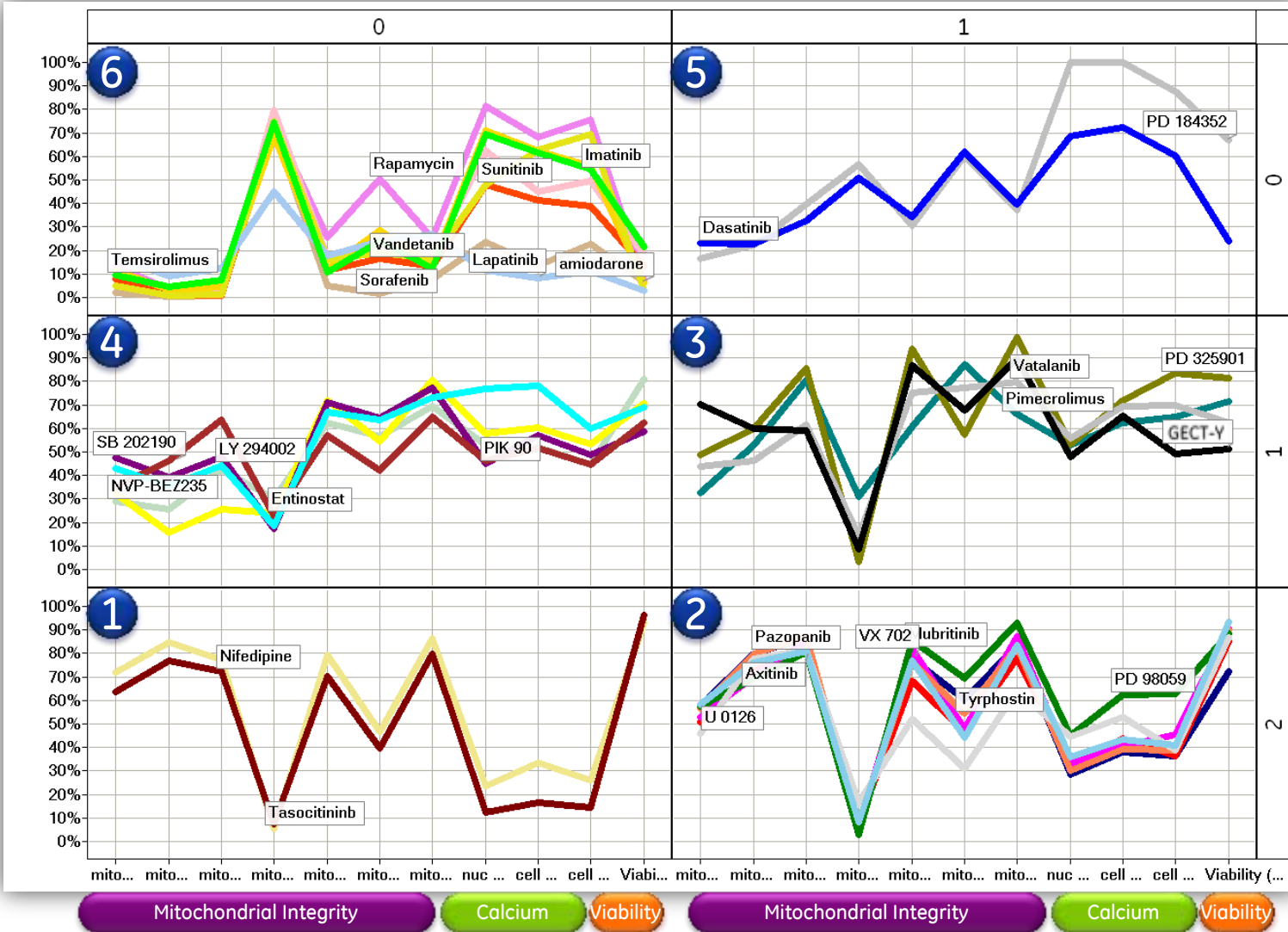
SOM Clustering

72h all compounds @ 1.24μM



SOM Clustering

72h all compounds @ 33.0μM



Clustering of Anti-Cancer Drugs

Classification by automated profile matching

Compound	Cluster
Nifedipine	1
Tasocitinib	1
Axinitib	2
Mubritinib	2
Pazopanib (Votrient)	2
PD 98059	2
Tyrphostin	2
U 0126	2
VX 702	2
GECT-Y	3
PD 325901	3
Pimecrolimus	3
Vatalanib	3

Compound	Cluster
Entinostat	4
LY 294002	4
NVP-BEZ235	4
PIK 90	4
SB 202190	4
Dasatinib (Sprycell)*	5
PD 184352	5
Amiodarone*	6
Imatinib (Gleevec)*	6
Lapatinib (Tyverb)*	6
Rapamycin	6
Sorafenib (Nexavar)*	6
Sunitinib (Sutent)*	6
Temsirolimus	6
Vandetanib (Zactima)	6

*Reported Clinical Cardiotoxicity

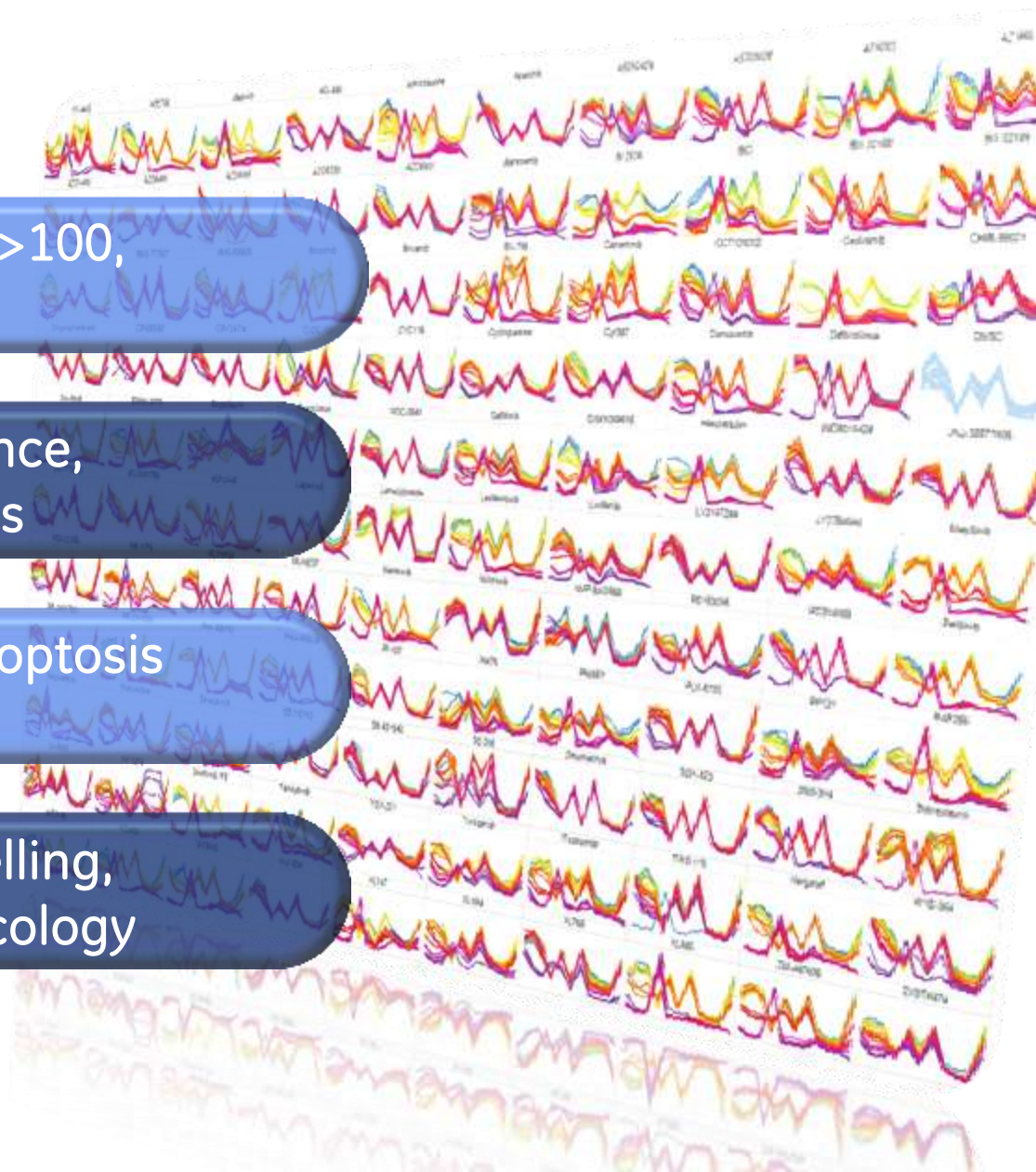
Further data under analysis

Extension of compound set to >100, including biologics

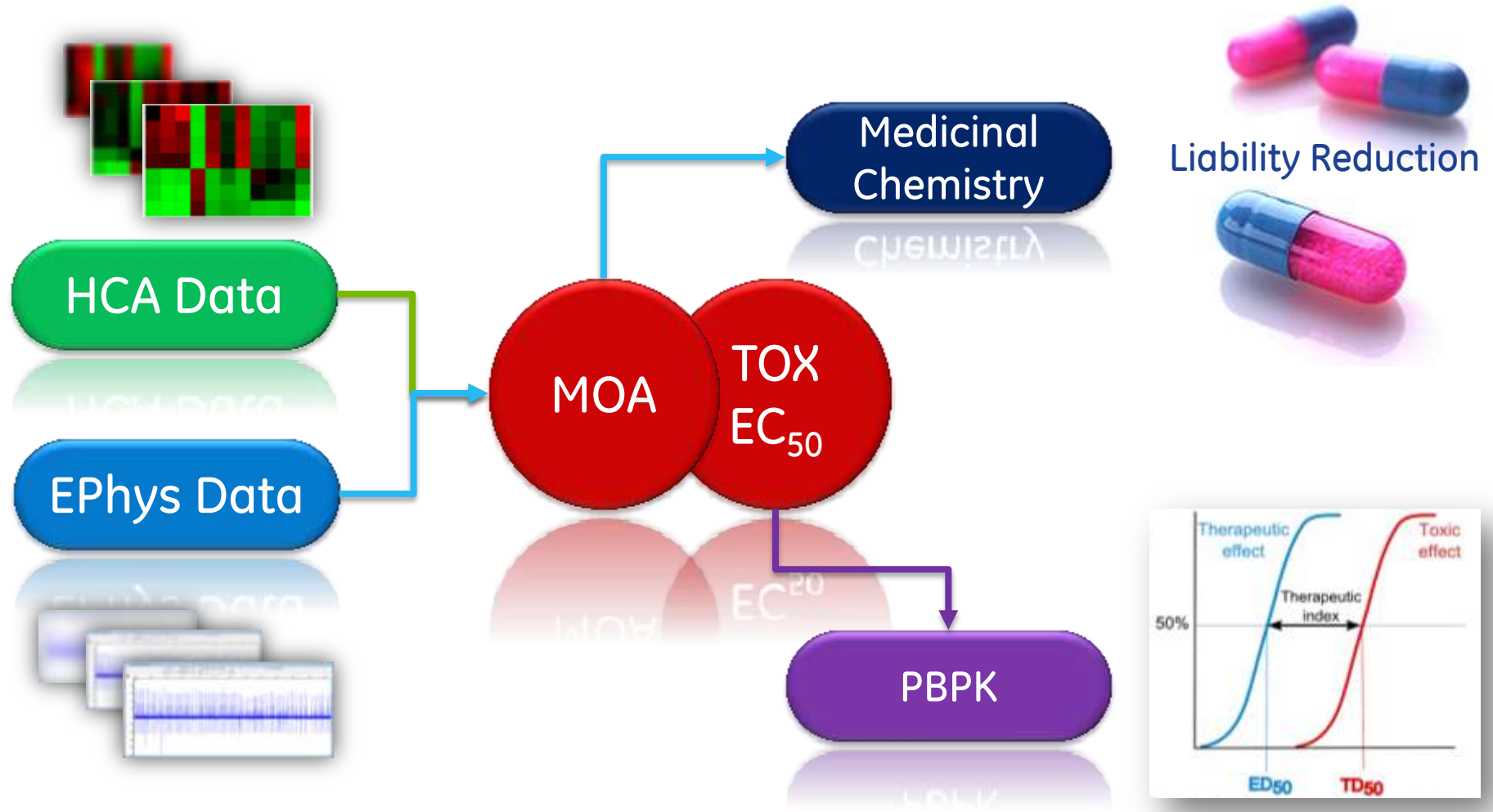
Complementary data; impedance, biochemistry and bioenergetics

Mitochondrial function and apoptosis protein biomarkers

Data integration & PBPK modelling, incorporating clinical pharmacology



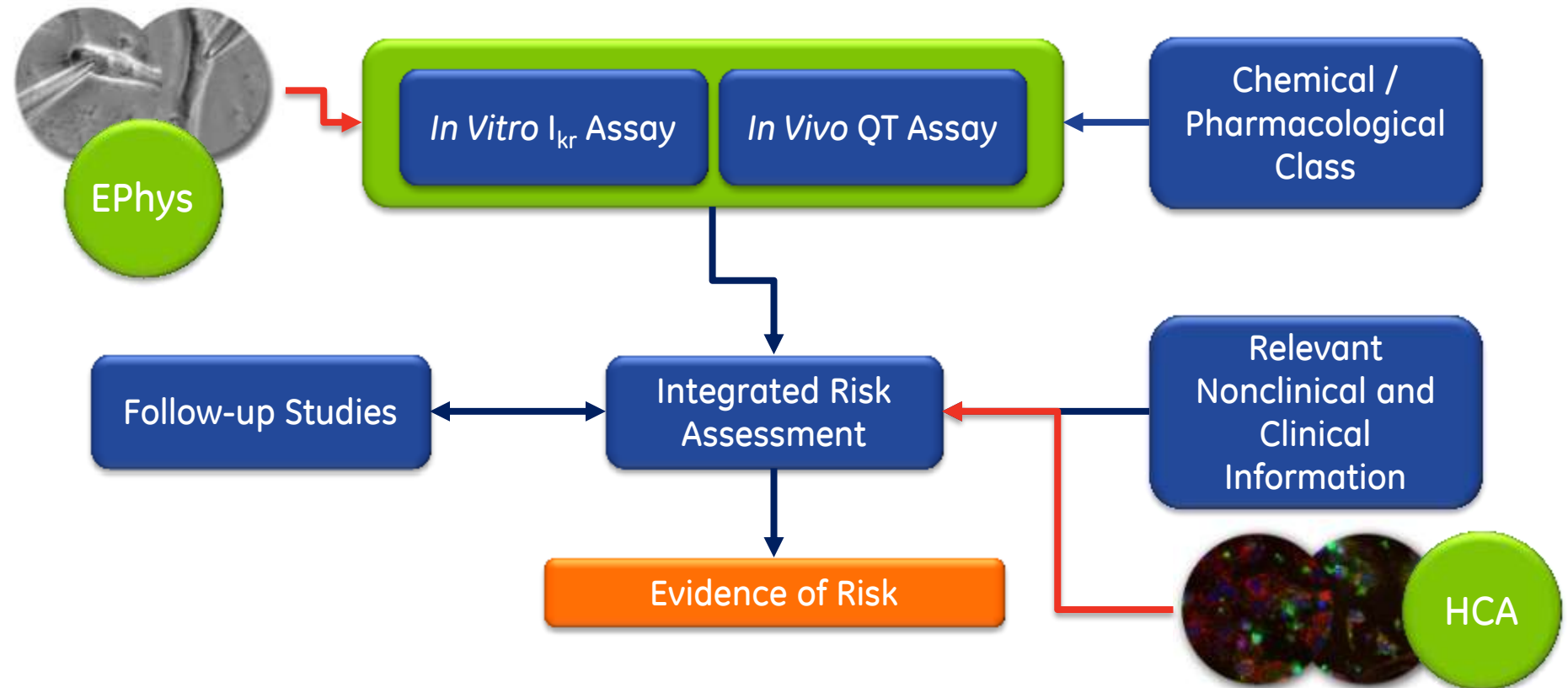
Integrated Cardiotoxicity Assessment



Therapeutic index

Cardiotoxicity ICH S7B

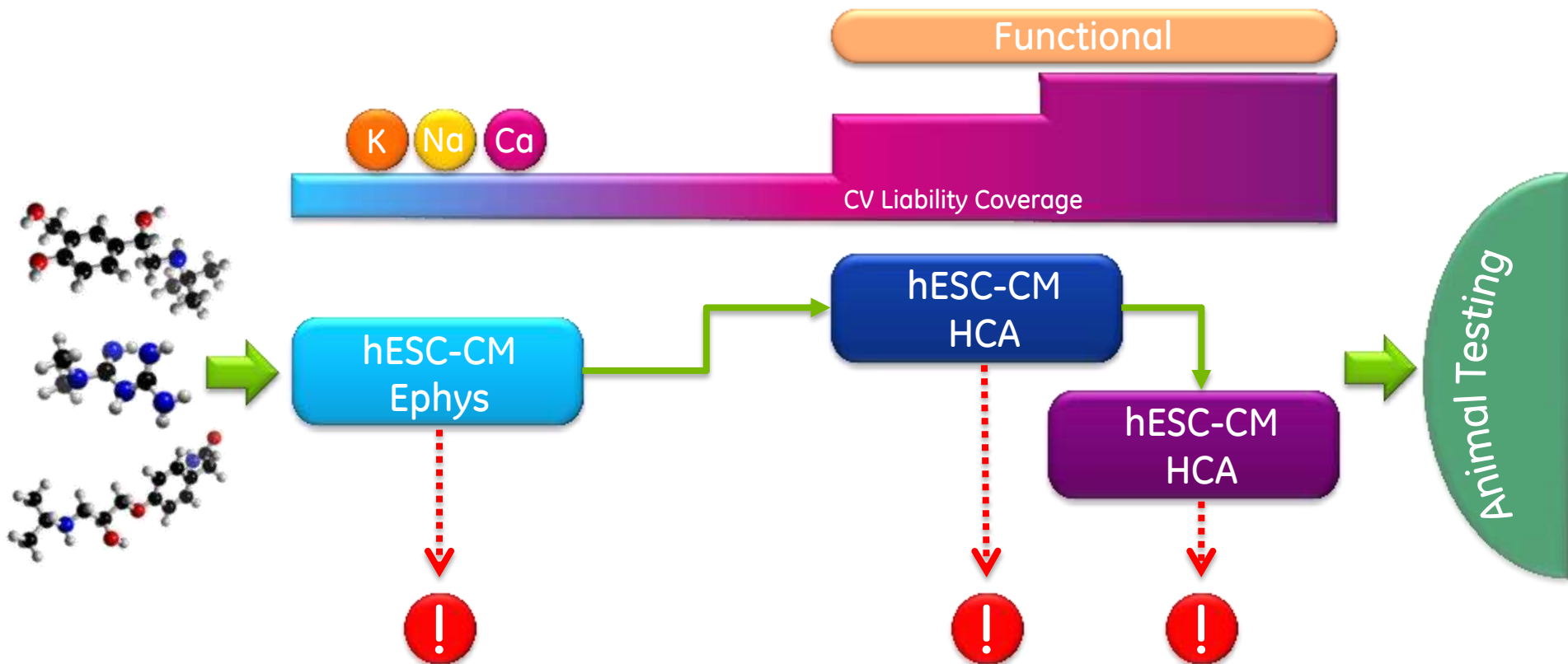
Vision for integrating hESC-Cardiomyocytes



- hESC-CM complementing/replacing in-vitro hERG assay with global ion channel liability surveillance
- HCA assays complementing EPhys for functional cardiac liabilities

DDT Context of Use Workflow

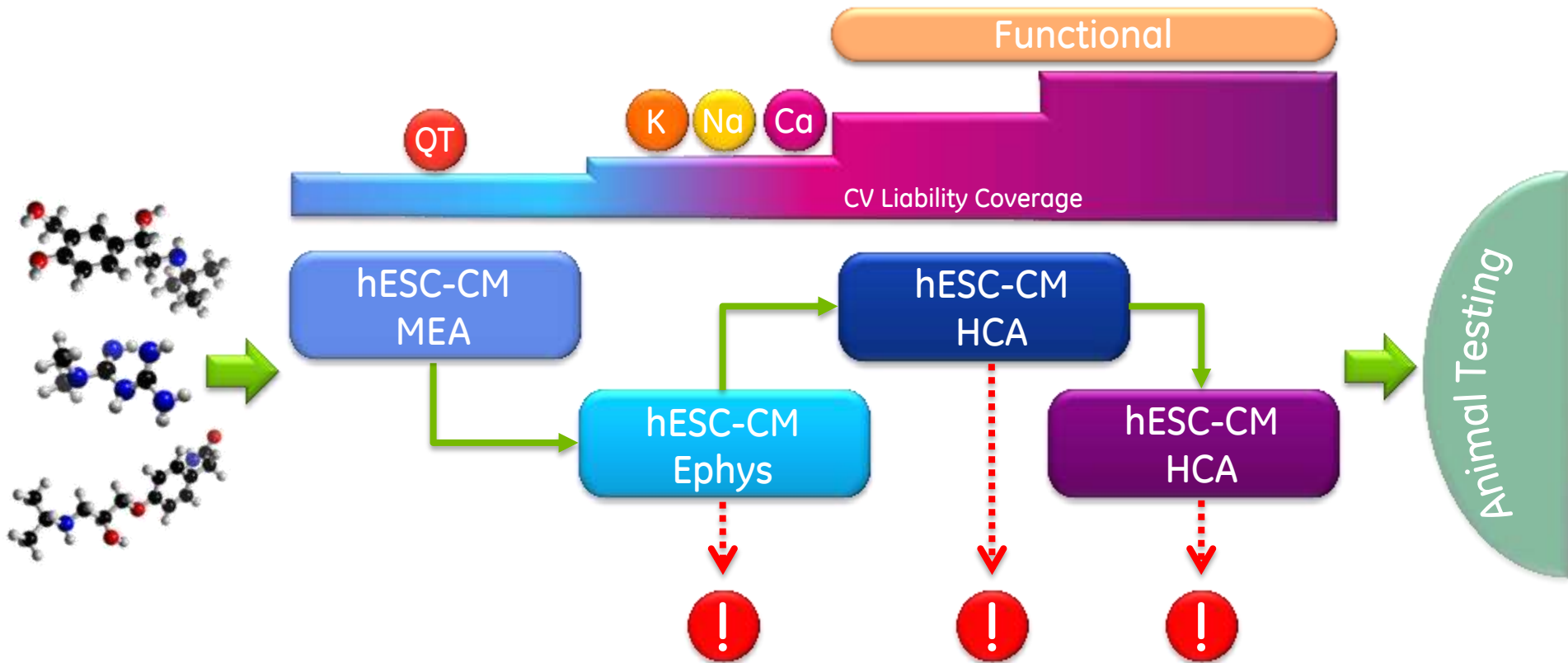
Triage of liability surveillance – medium term



- hESC-CM replacing in-vitro hERG assay and ex-vivo systems for global ion channel liability surveillance
- HCA for integrity profiling and mitochondrial/apoptosis markers

DDT Context of Use Workflow

Triage of liability surveillance – long term



- hESC-CM provides common cross platform model for integrated in-vitro human CV liability surveillance

Validation & Qualification

Complement or Replace ?

Different validation criteria and hurdles

- size and focus of study ?
- compound class/treatment class based ?

What decisions are enabled, aided or improved;

- for Pharma ?
- for Regulators ?

Equivalence or superiority to existing systems in specificity & sensitivity

Different levels of assay;

- single target checkpoint; CHO-hERG
- integrated ; CM or PF APD, MEA QT interval
- holistic; EPhys, structural and functional

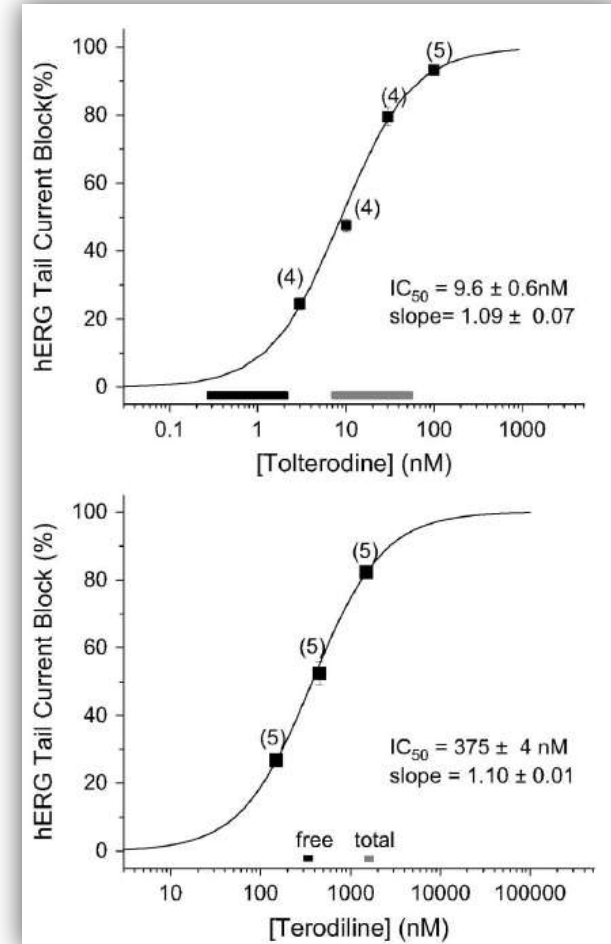


Validation & Qualification

Discordance Resolution ?

Discordance in Clinical QT Risks Observed with Terodiline and Tolteridone

- Anticholinergic compounds used for the treatment of urinary incontinence. Compounds potent hERG blockers.
- Terodiline withdrawn from clinic due to drug-induced proarrhythmia. Tolterodine has a generally benign clinical cardiac safety profile,
- Measurement of hERG channel blockage alone is insufficient to predict cardiac safety
- Literature Ephys studies with range of experimental models including HEK-hERG cells, CHO-hERG cells, guinea pig myocytes and canine Purkinje fibres, i.e. human non-integrated or non-human integrated systems.
- Integrated cross-platform (HCA, MEA, IMP etc.) study with Cytiva cardiomyocytes to determine if multi-factorial Ephys, functional or structural mechanisms contribute to Tolterodine and Terodiline clinical discordancy

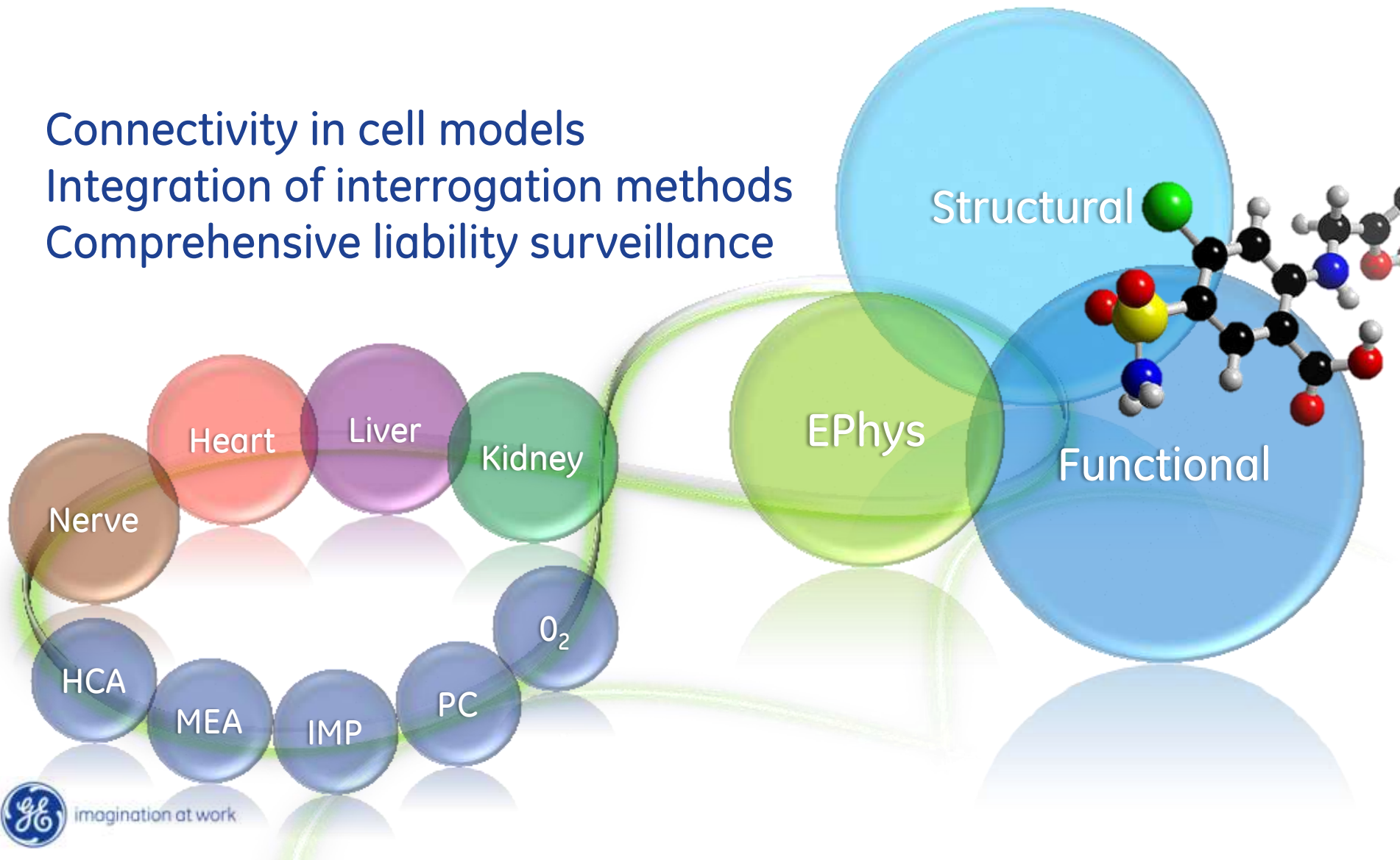


Martin et al J Cardiovasc Pharmacol 2006

Stem Cell Models in Toxicology

Vision for future development

Connectivity in cell models
Integration of interrogation methods
Comprehensive liability surveillance





Andrew Bruening-Wright & Arthur Brown



Lars Sundstrom



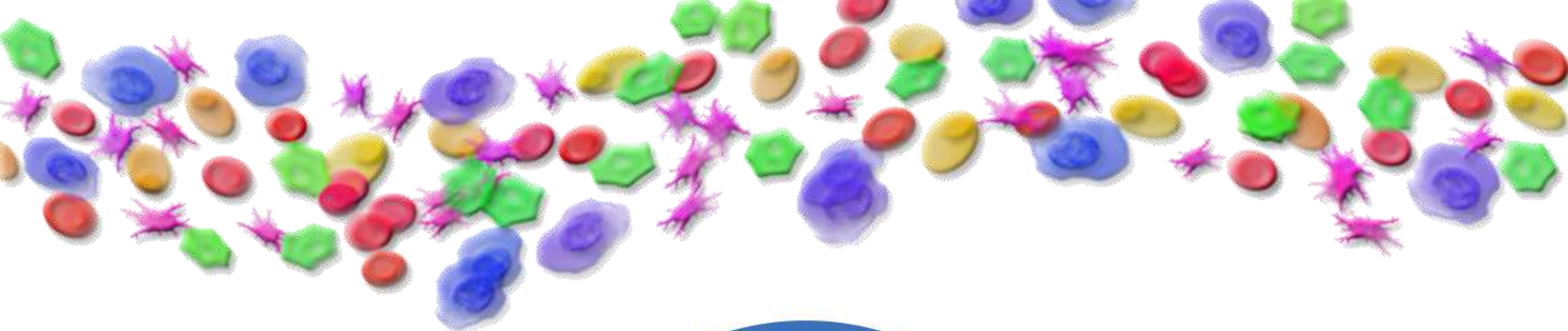
Hirdesh Uppal & Ariel Kauss



Cardiff GE Cell Technologies Team



imagination at work



The IN Cell Analyzer system and the In Cell Investigator software are sold under use license from Cellomics Inc. under US patent numbers US 5989835, 6365367, 6416959, 6573039, 6620591, 6671624, 6716588, 6727071, 6759206, 6875578, 6902883, 6917884, 6970789, 6986993, 7060445, 7085765, 7117098, 7160687, 7235373, 7476510 ; Canadian patent numbers CA 2282658, 2328194, 2362117, 2381344; Australian patent number AU 730100; European patent numbers EP 0983498, 1095277, 1155304, 1203214, 1348124, 1368689; Japanese patent numbers JP 3466568, 3576491, 3683591, 4011936 and equivalent patents and patent applications in other countries.

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