Brunel University London

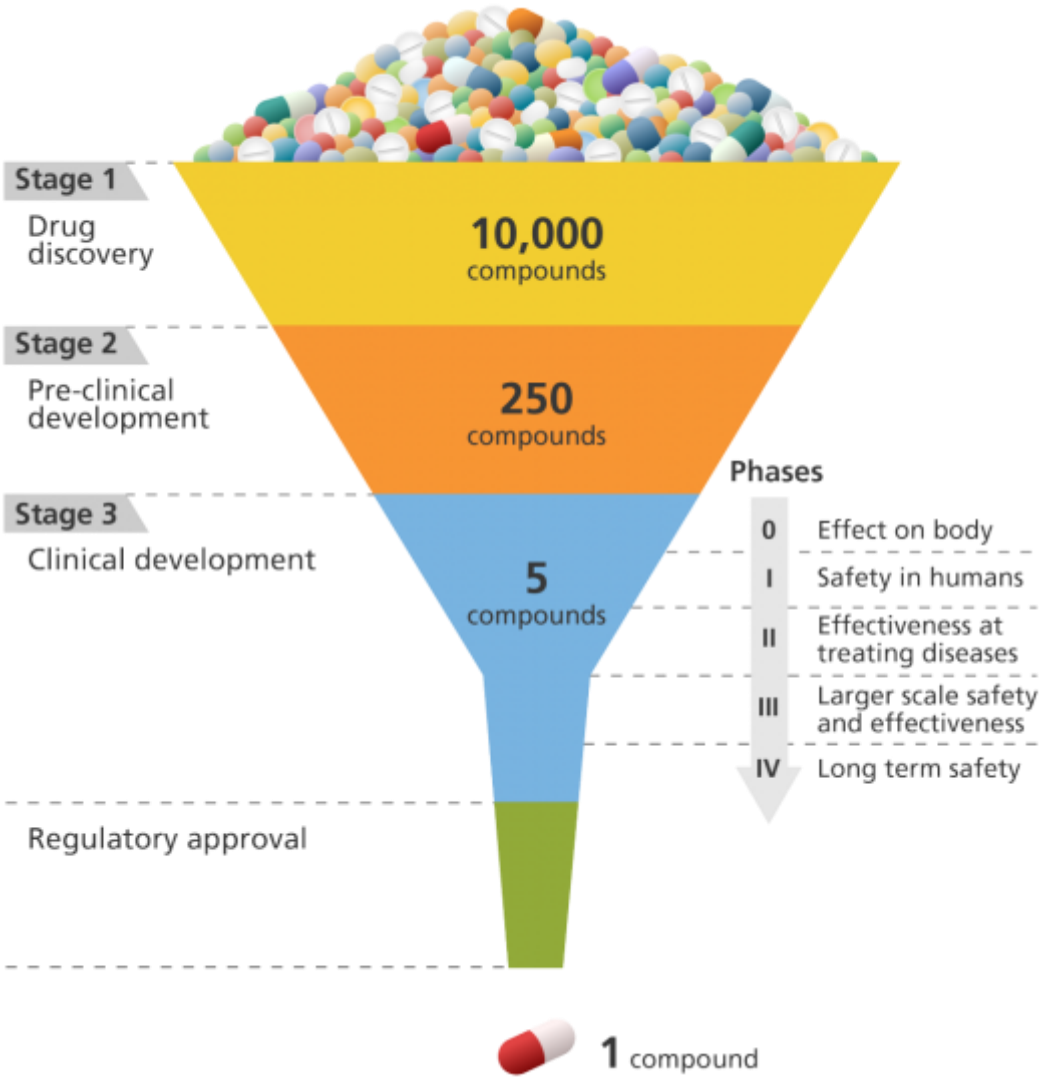
How do drugs work in the body?

Novel approaches to understand & predict non-obvious effects of pharmaceuticals

Luigi Margiotta-Casaluci

Biology@Brunel – 2 May 2018

Drug development is a risky & expensive process



$2.6 billion

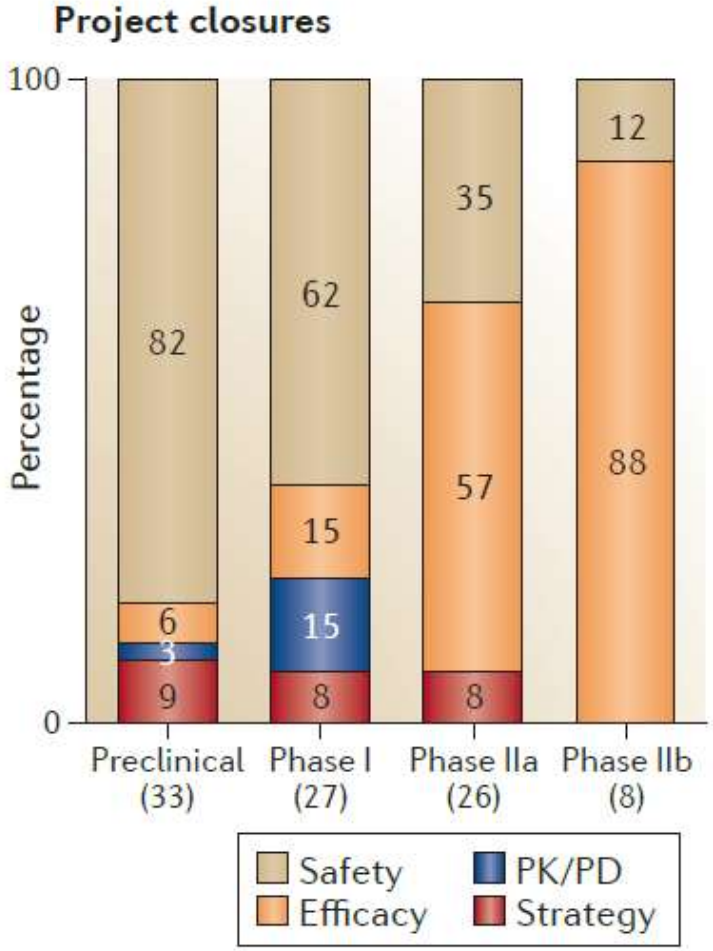
Tufts Centre for the Study of Drug Development 2014

Image credit: Genome Research Limited

Drug safety and project failures

82% of the failures in preclinical studies are due to safety issues

2005-2010



Cook et al. (2014) Nature Drug Discovery 13, 419-431

Hazard identification - Risk mitigation

GPCRs
Adenosine (A2a)
Adrenergic (α1a, α2a, β1, β2)
Cannabanoid (CB1, CB2)
Peptidergic (CCK-A, ET-A, V1a)
Dopamine (D1, D2)
Histamine (H1, H2)
Opioid (δ, κ, μ)
Muscarinic (M1, M2, M3)
Serotonin (5HT1a, 5HT1b, 5HT2a, 5HT2b)

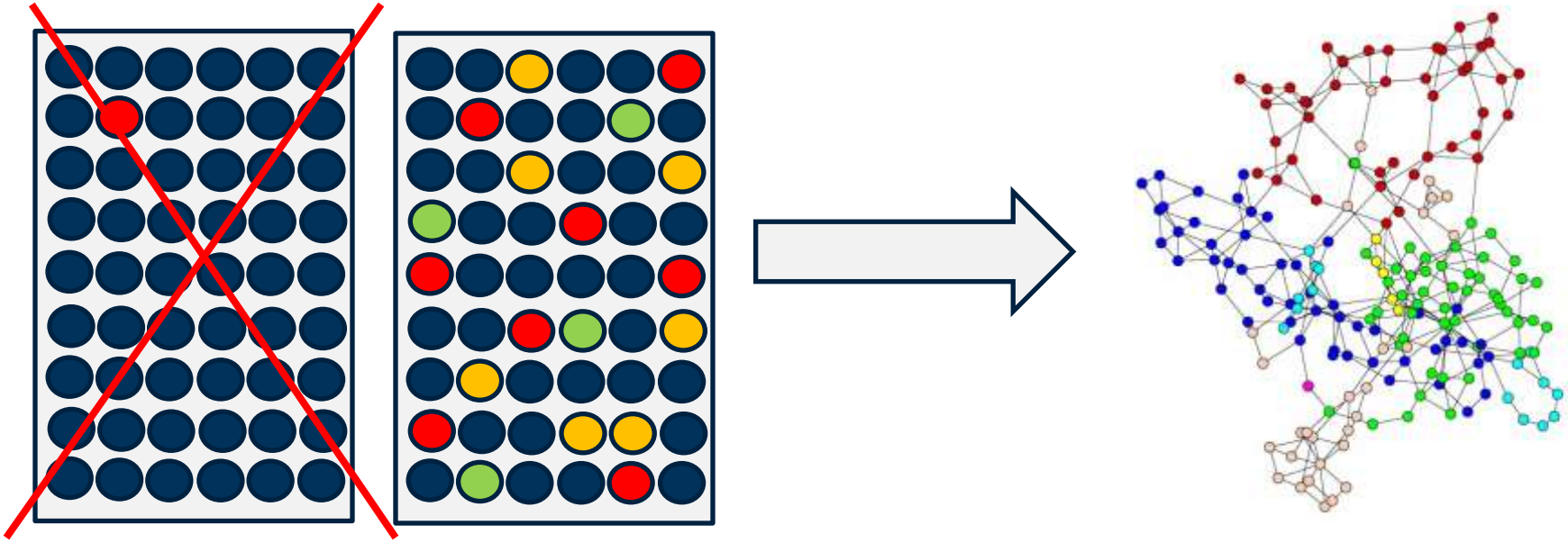
Ion channels
Voltage gated (Cav1.2, hERG, KCNQ1/KCNE1, Nav1.5)
Ligand gated (nACHR α1 or α4, GABA-a, NR1, 5HT3)

Enzymes
AchE
COX1
COX2
MAOa
PDE3A
PDE4D
LCK

Nuclear Hormone Receptors
AR, GR

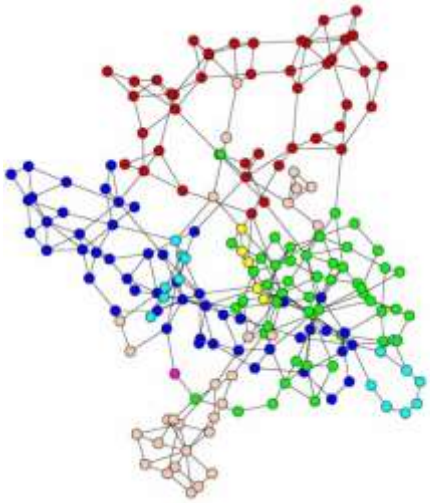
Transporters
DAT, NET, SERT

Bowes et al. Nature Reviews Drug Discovery 11, 909 (2012)

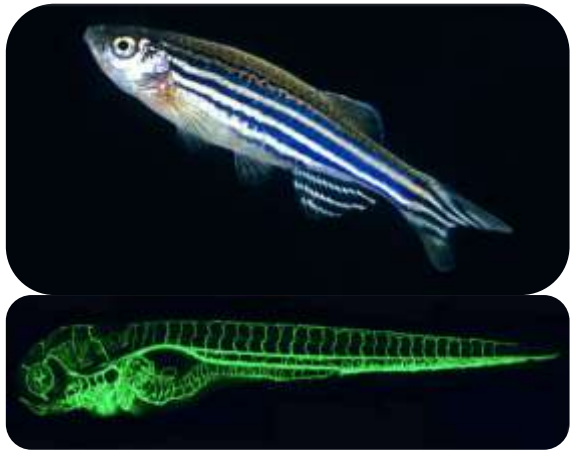


Our research vision

Computational biology, multi-scale modelling & network pharmacology

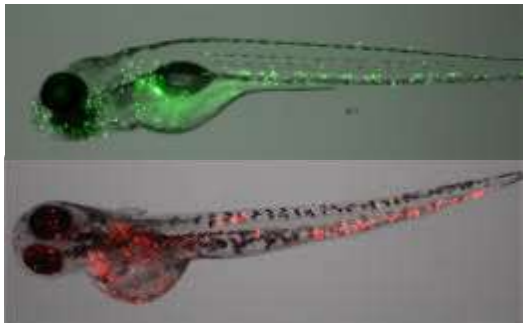


Zebrafish model



Supporting pre-clinical & clinical drug safety assessment

Our tools



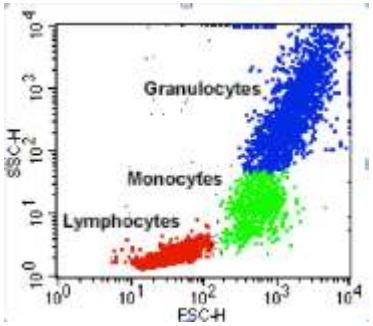
- *Neutrophils*
- *Interleukin-1b*

- *Macrophages*

Automated system for behavioural profiling

(temporary courtesy of Dr Winter, U. of Exeter)

- *-omics outsourced*



- *Advanced flowcytometry @ Queen Mary University*

Fluorescence microscope *(Leica DMi8)*

BBSRC bioscience for the future

NC 3Rs

Brunel University London

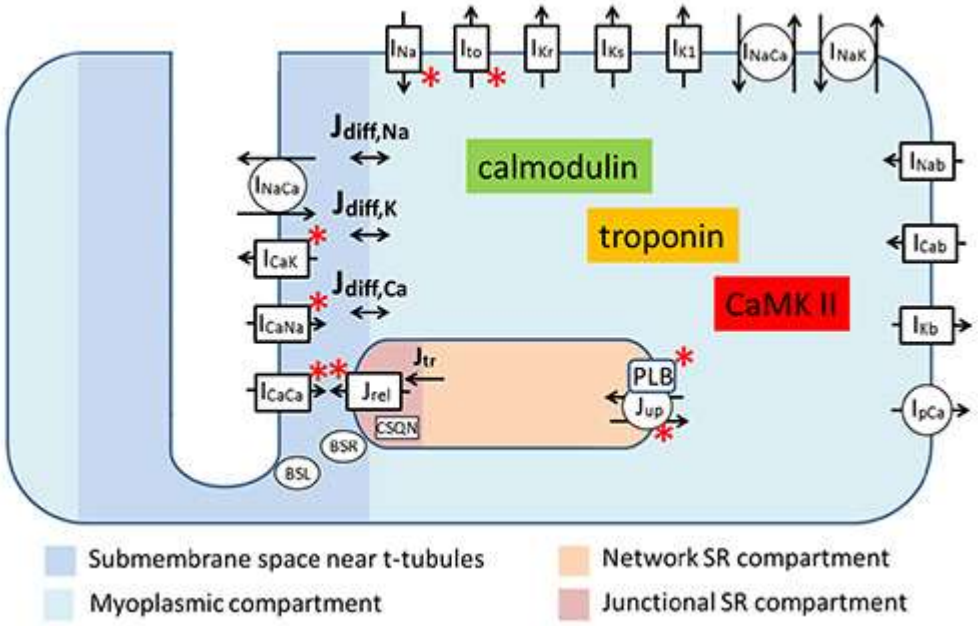
AstraZeneca

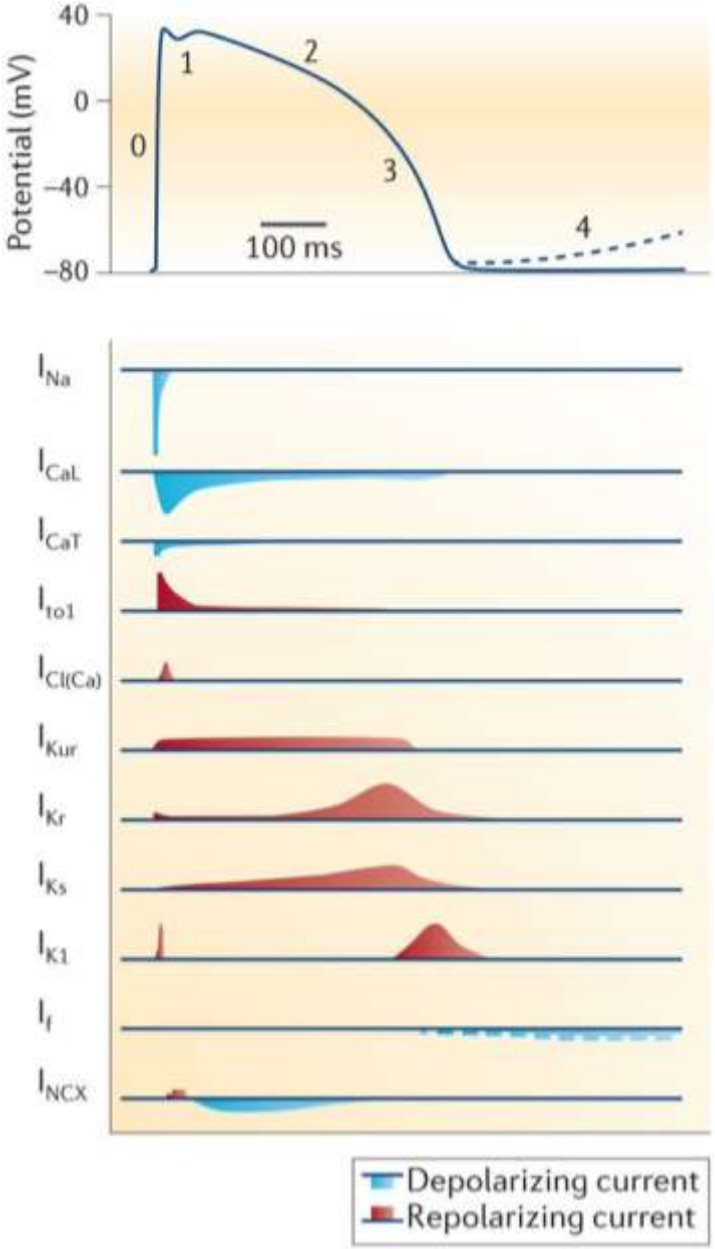
NC3Rs Strategic Award 2017-2018

National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Development of an Adverse Outcome Pathway for cardiotoxicity mediated by the blockade of L-type calcium channel

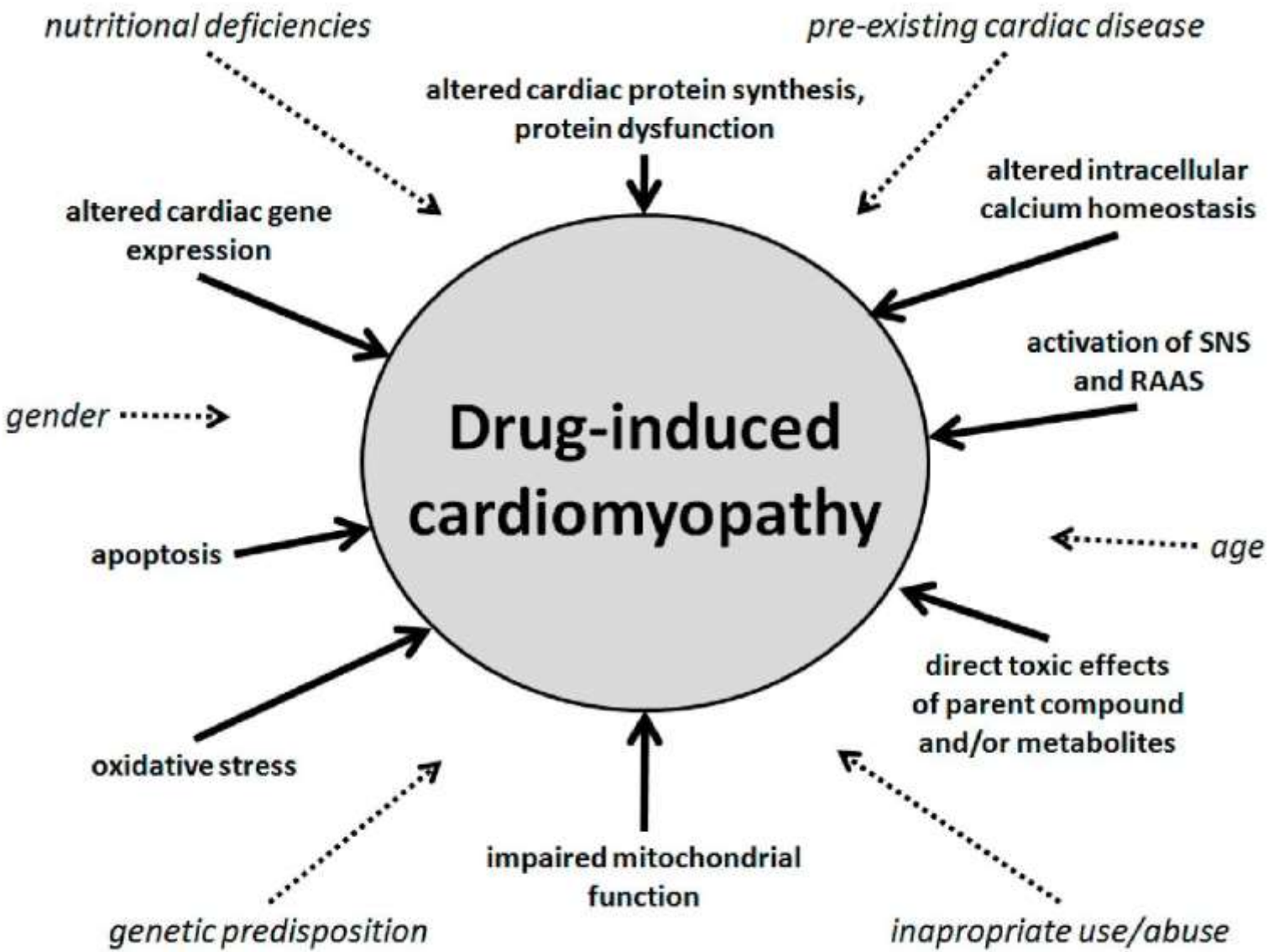
Unpredicted cardiotoxicity is a major cause of drug failure





O'Hara et al. 2011. PLoS Comput Biol 7(5): e1002061

Image adapted from Gintant et al. 2016 Nature Reviews Drug Discovery 15, 457–471


nutritional deficiencies
pre-existing cardiac disease
altered cardiac protein synthesis, protein dysfunction
altered cardiac gene expression
altered intracellular calcium homeostasis
activation of SNS and RAAS
gender
Drug-induced cardiomyopathy
apoptosis
age
direct toxic effects of parent compound and/or metabolites
oxidative stress
impaired mitochondrial function
genetic predisposition
inapropriate use/abuse

L-type calcium channel blockade as molecular initiating event (MIE)

- Calcium ions play a vital role in cellular and organism physiology
  - E.g. mediation of ***muscle contraction***, hormone secretion, and neuronal transmission
- L-type calcium channels are responsible for the excitation-contraction coupling of skeletal, smooth, and cardiac muscle
- Perturbation of calcium dynamics in the heart may impair organ function and health

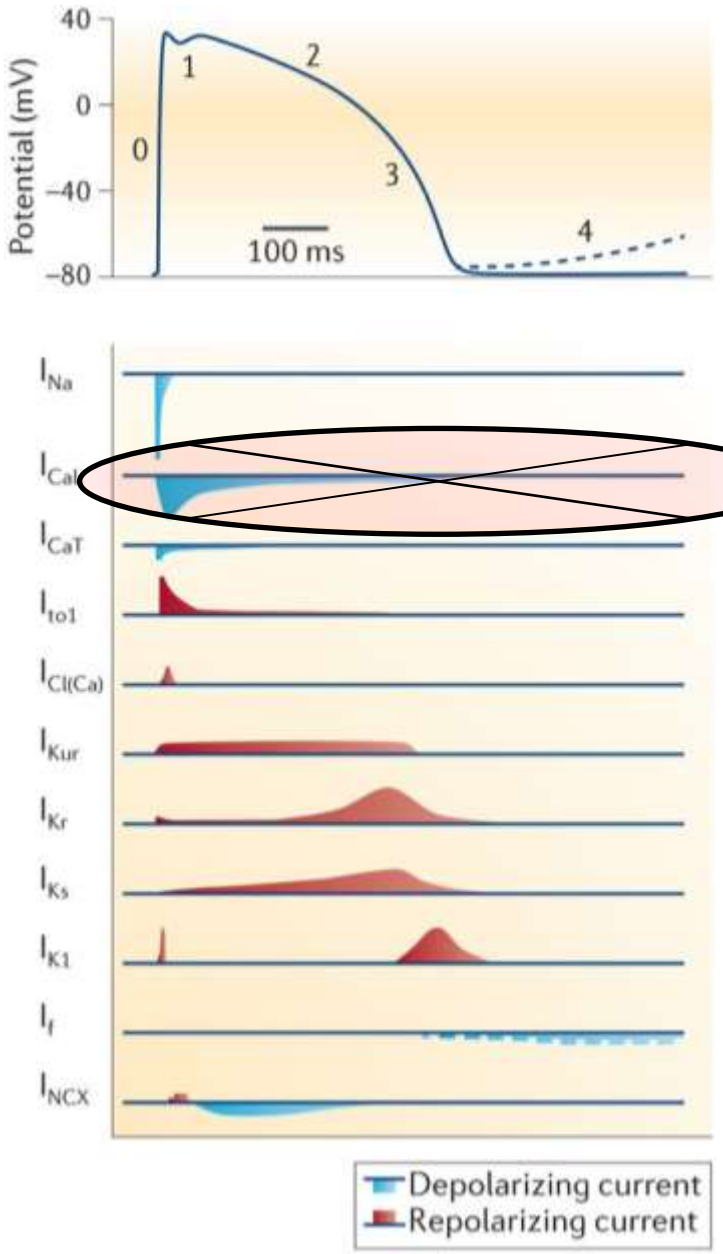
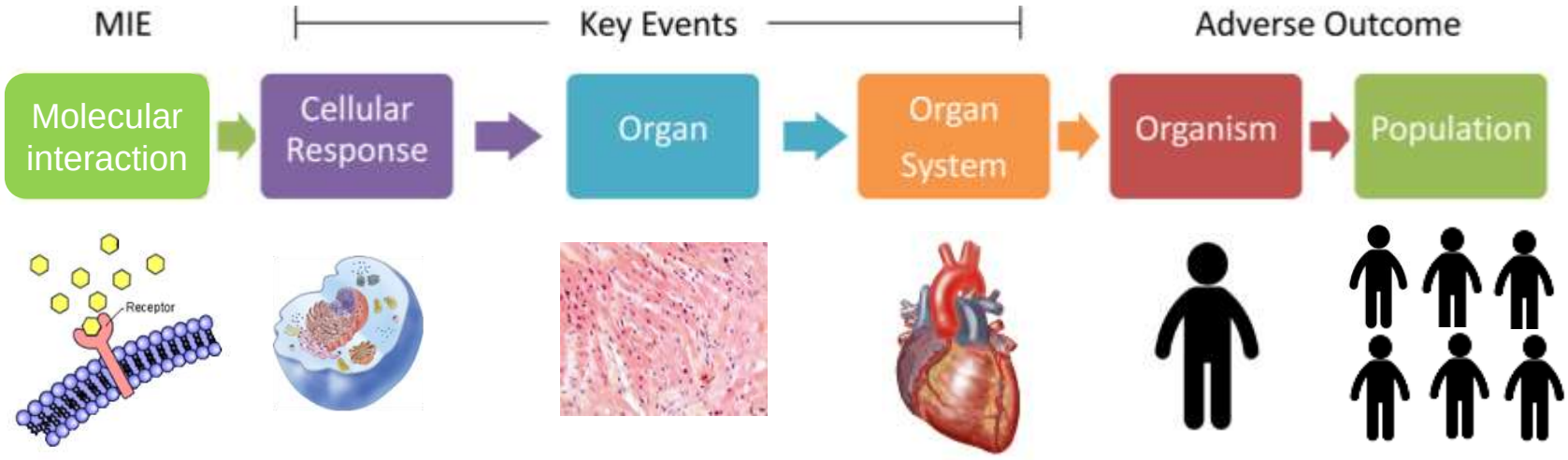


Image adapted from Gintant et al. 2016 Nature Reviews Drug Discovery 15, 457–471

Adverse Outcome Pathway concept



Cardiotox AOP - Project workflow

Literature review and data extraction

Review papers
Genetic manipulation studies
CCBs exposure studies

In vivo, ex vivo, in vitro

148 experimental papers + reviews

Plausibility, essentiality, & weight of evidence analysis
(e.g. dose response concordance, reproducibility, etc)

AOP development

Outputs:

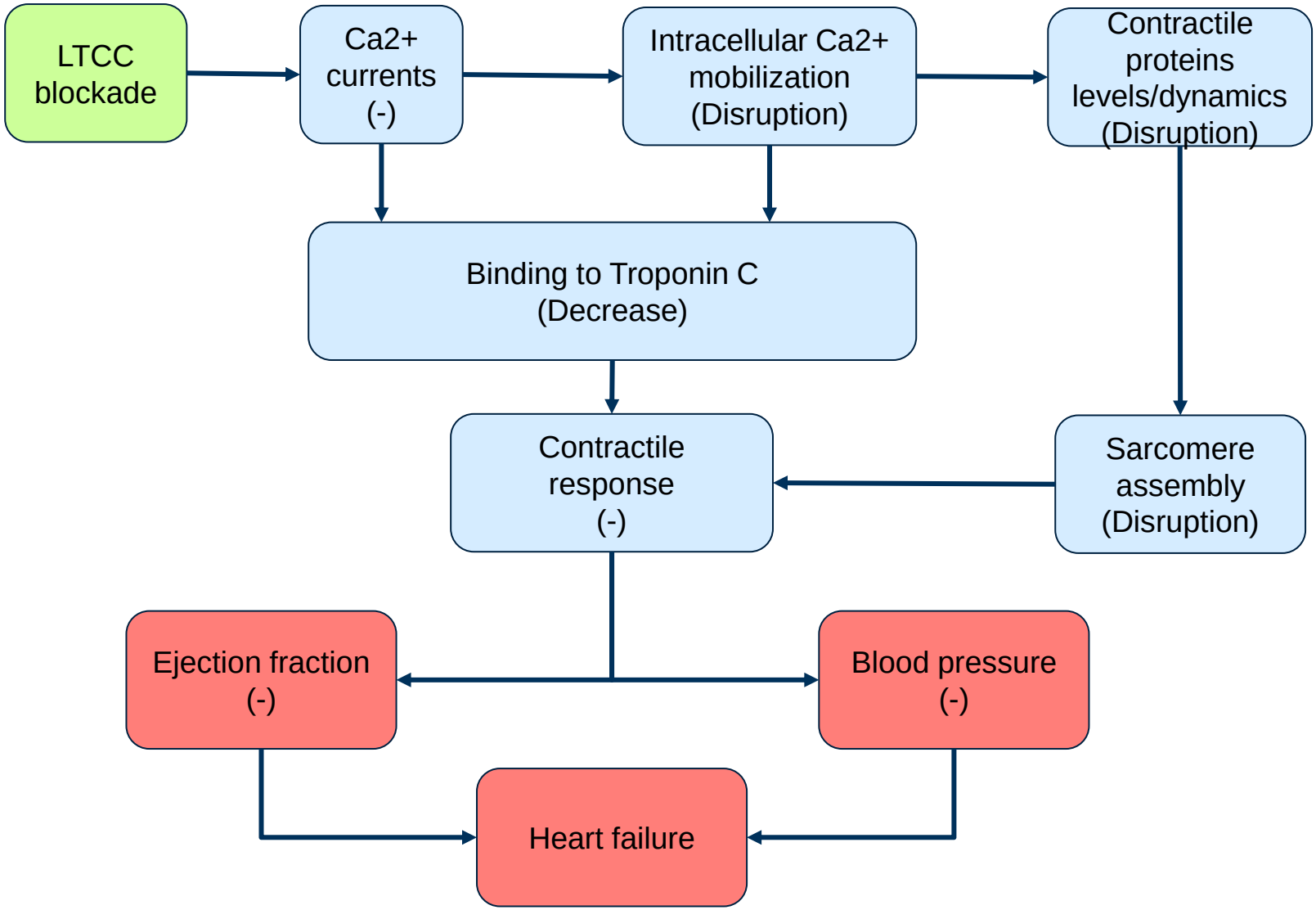
- Database of LTCC-mediated effects
- #1 report for OECD submission
- #1 manuscript

Literature review and data extraction
Empirical evidence
CCBs exposure studies

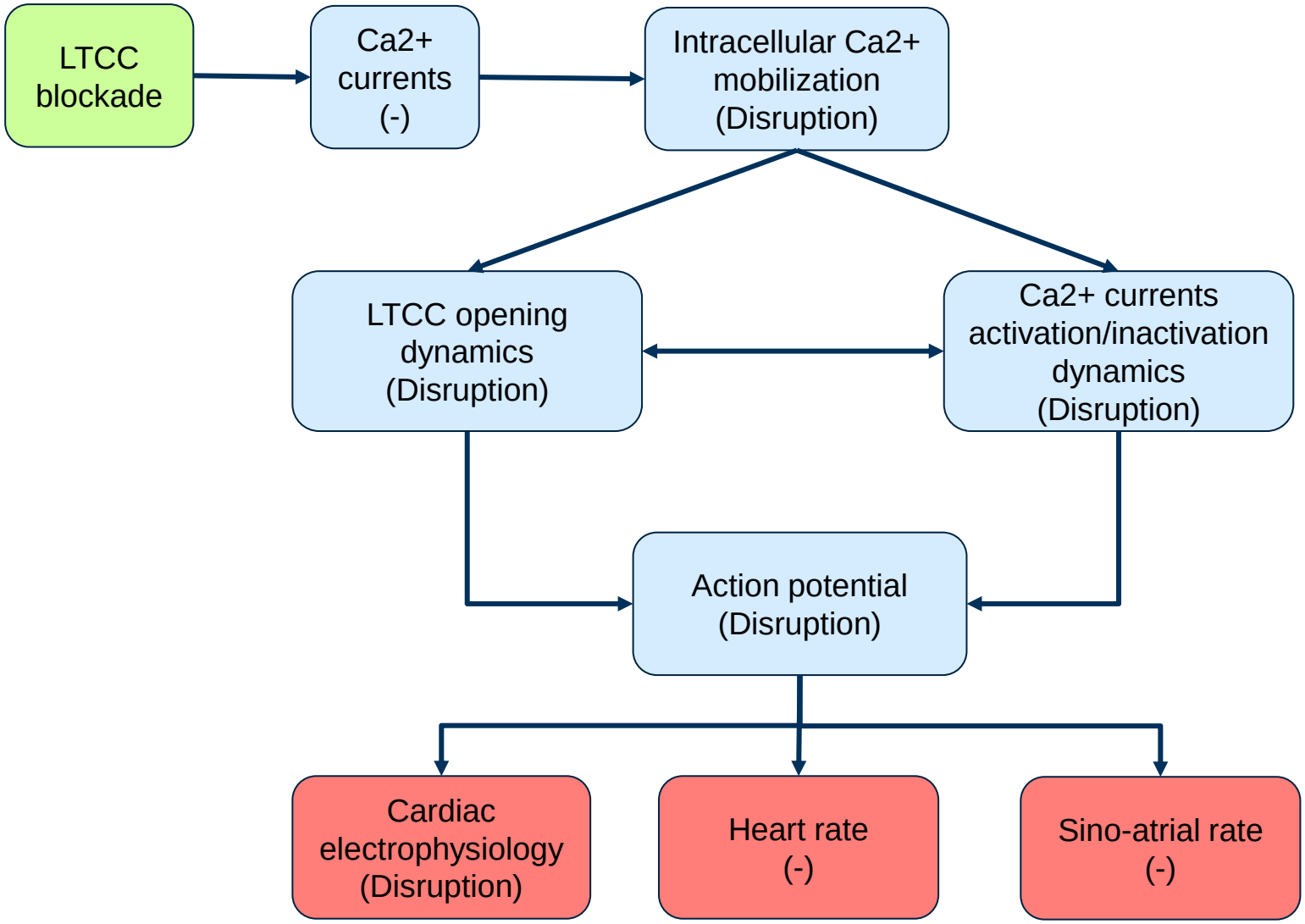
AOP developed using data extracted from exposure studies involving 10 CCBs and healthy biological models (i.e. non-disease models)

Drug	Class	Total no. of data points	Affinity to LTCC (1C) (Lowest Ki, nM)*	Species	Data source
Nifedipine	Dihydropyridine CCB	345	0.5	Rat	ChEMBL
Amlodipine	Dihydropyridine CCB	114	20	Rat	ChEMBL
Felodipine	Dihydropyridine CCB	14	0.053	Rat	ChEMBL
Nisoldipine	Dihydropyridine CCB	2	0.476	Rat	ChEMBL
Nimodipine	Dihydropyridine CCB	2	0.156	Rat	ChEMBL
Nitrendipine	Dihydropyridine CCB	1	0.246	Rat	ChEMBL
Diltiazem	Benzothiazepine CCB	123	16 nM	Rat	ChEMBL
Verapamil	Phenylalkylamine CCB	272	12 nM	Rat	ChEMBL
Fendiline	Phenylalkylamine/non-selective CCB	17	17000 (**IC50, Ki n/a)	Rat	ChEMBL
Mibefradil	Non selective CCB	44	156 nM (**IC50, Ki n/a)	Human	ChEMBL

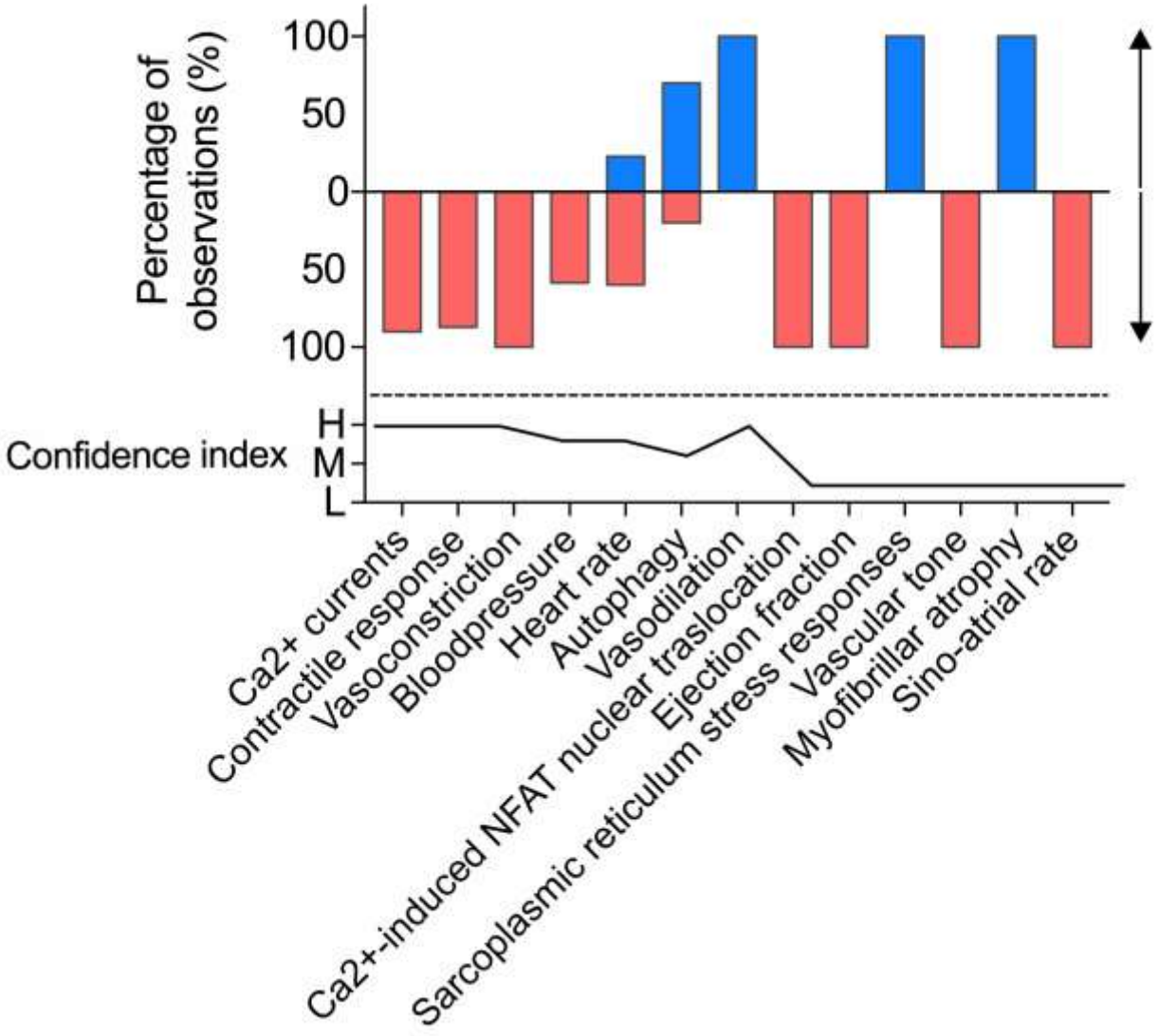
AOP1: Disruption of cardiac contractility



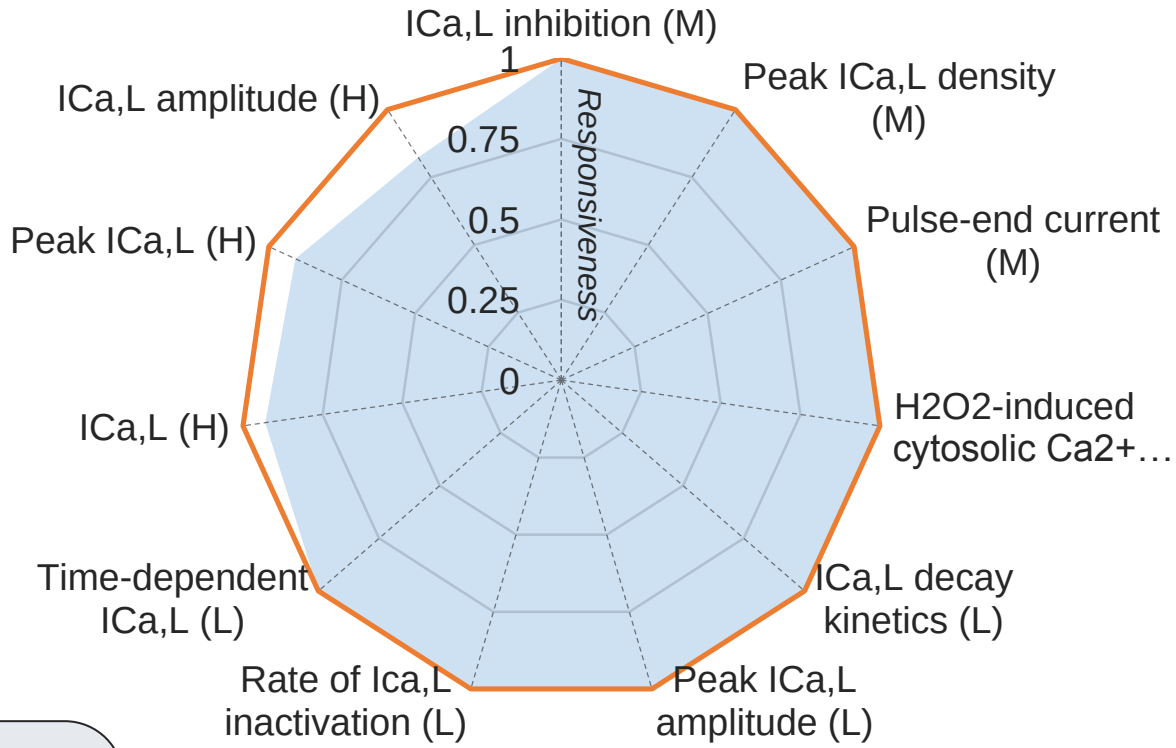
AOP2: Disruption of cardiac electrophysiology



Effect direction and confidence assessment of bi-directional KEs

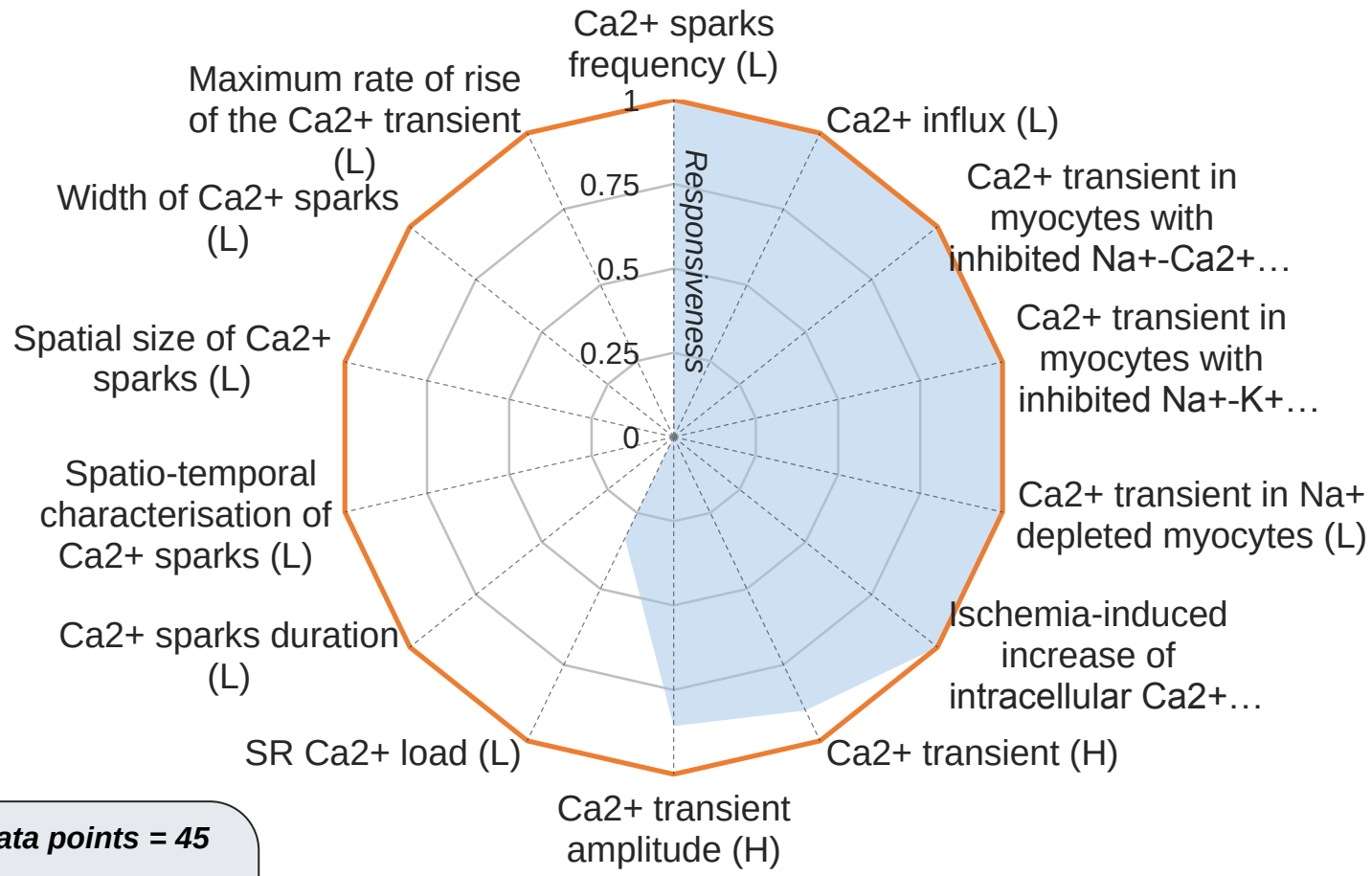


KE: Calcium current, Decrease



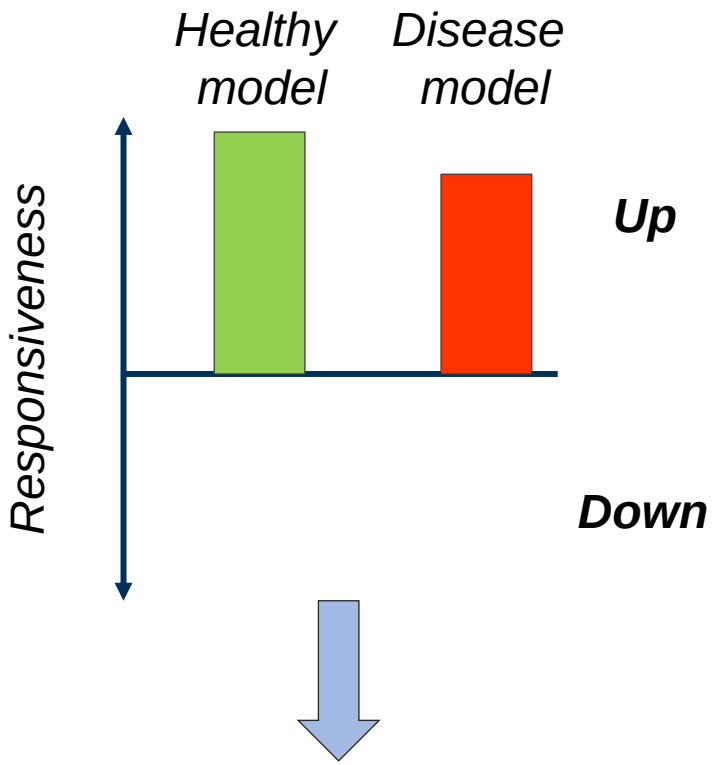
Total no. of data points = 91
Nifedipine: 37
Verapamil: 25
Diltiazem: 17
Fendiline: 6
Felodipine: 3
Amlodipine: 2
Semotiadil: 1

KE: Intracellular calcium mobilization, Disruption

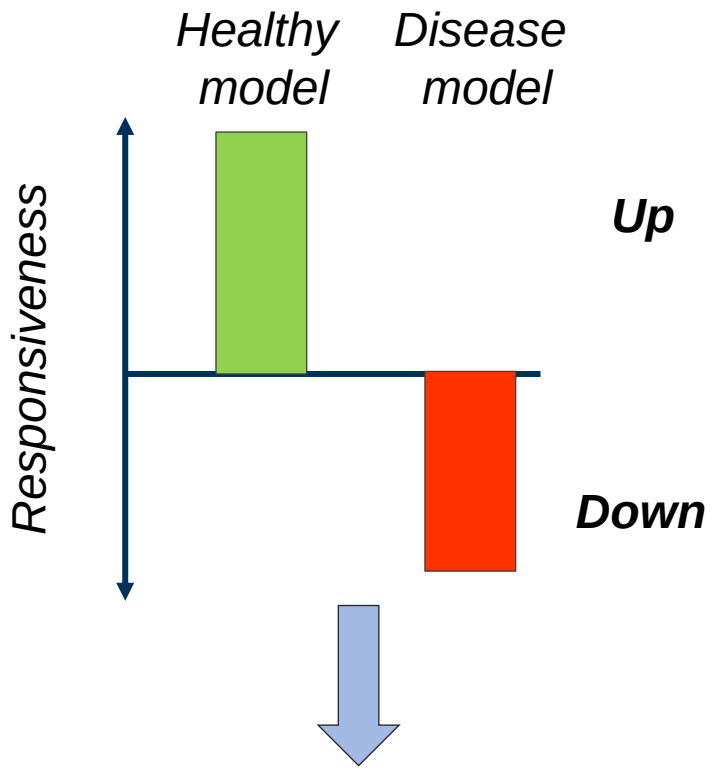


Total no. of data points = 45
Nifedipine: 24
Verapamil: 8
Amlodipine: 7
Mibefradil: 3
Felodipine: 2
Fendiline: 1

Influence of disease state on the KEs



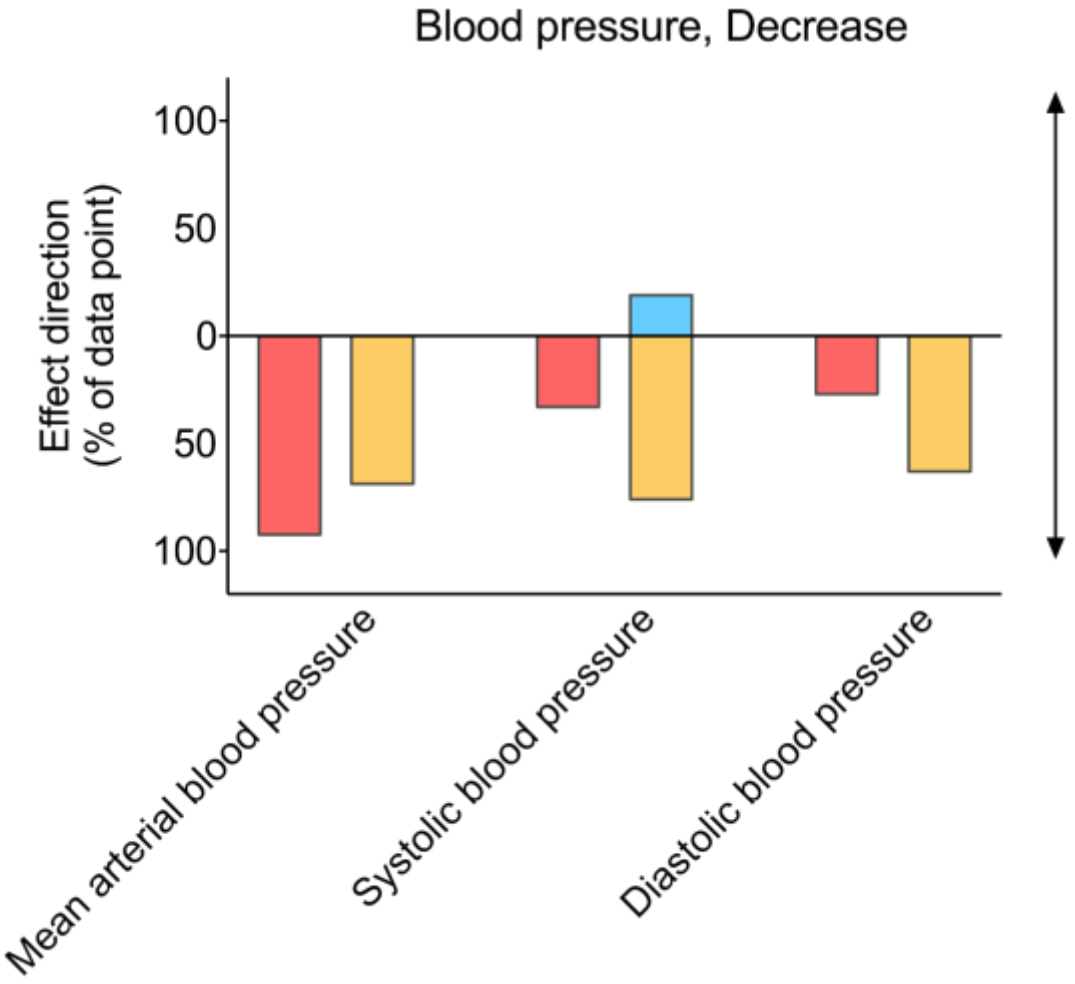
Empirical support
+
Weight of Evidence



Identification of modulating factors

+300 data points

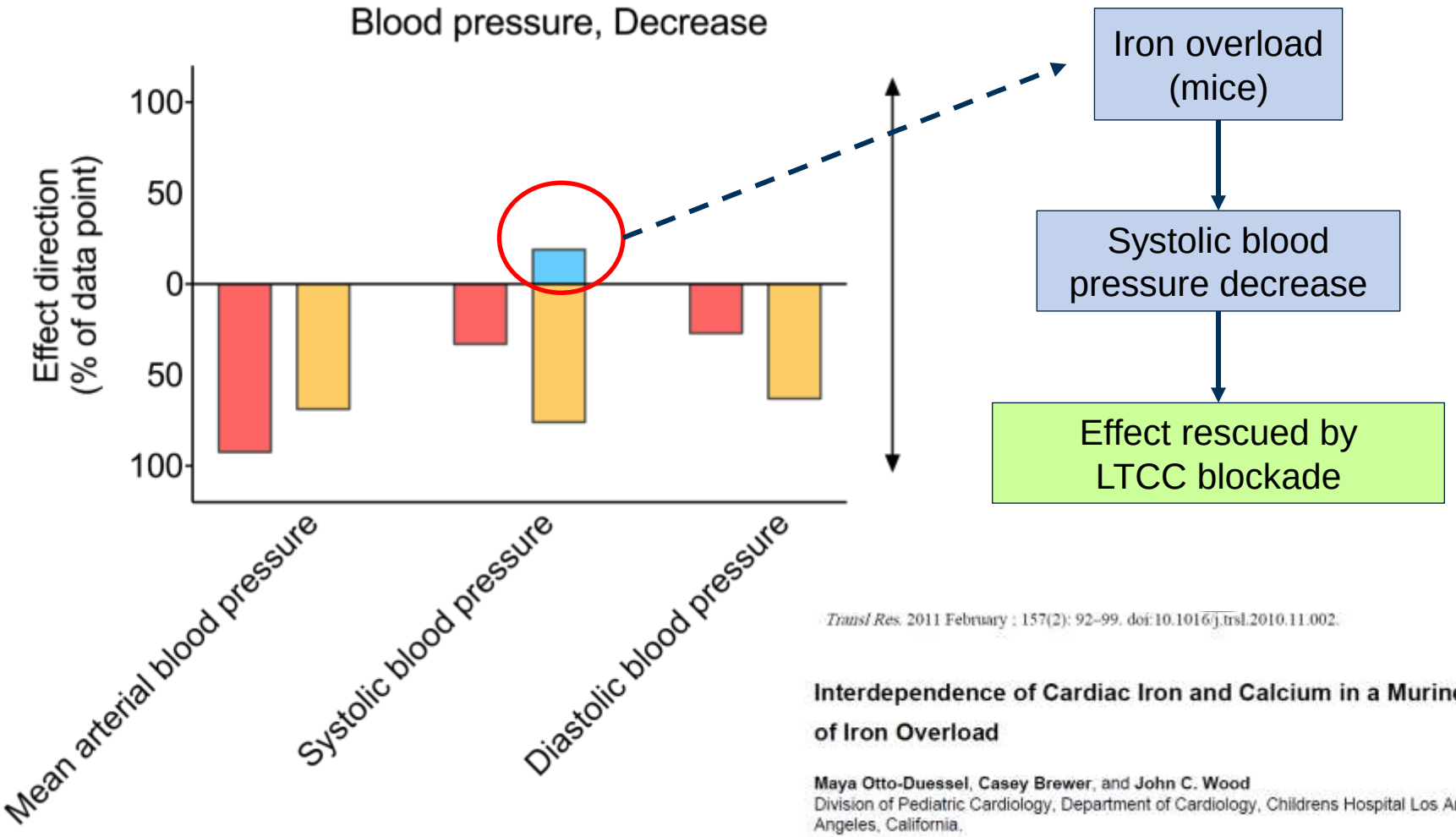
Influence of disease state on the KEs



Disease type

- *Alcohol dependence*
- *Balloon injury of the carotid artery*
- *Chronic atrioventricular block*
- *Heart failure*
- *High salt diet*
- *Hypertension*
- *Iron overload*
- *Ischemia*
- *Myocardial infarction*
- *Rapid atrial pacing*
- *Patients undergoing coronary angiography with or without percutaneous coronary interventions*
- *SHR hydronephrotic model*

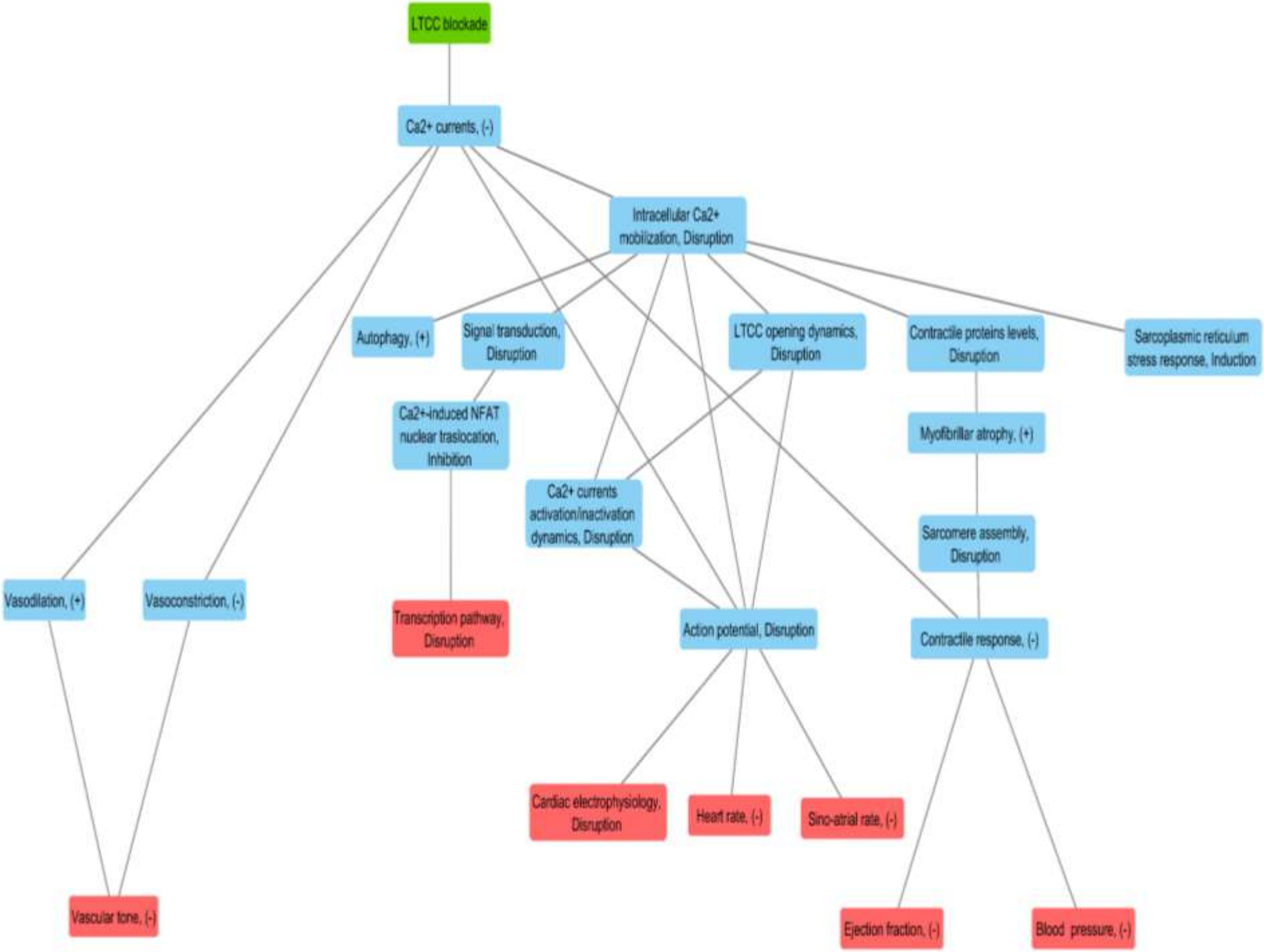
Influence of disease state on the KEs



Transl Res. 2011 February ; 157(2): 92–99. doi:10.1016/j.trsl.2010.11.002.

Interdependence of Cardiac Iron and Calcium in a Murine Model of Iron Overload

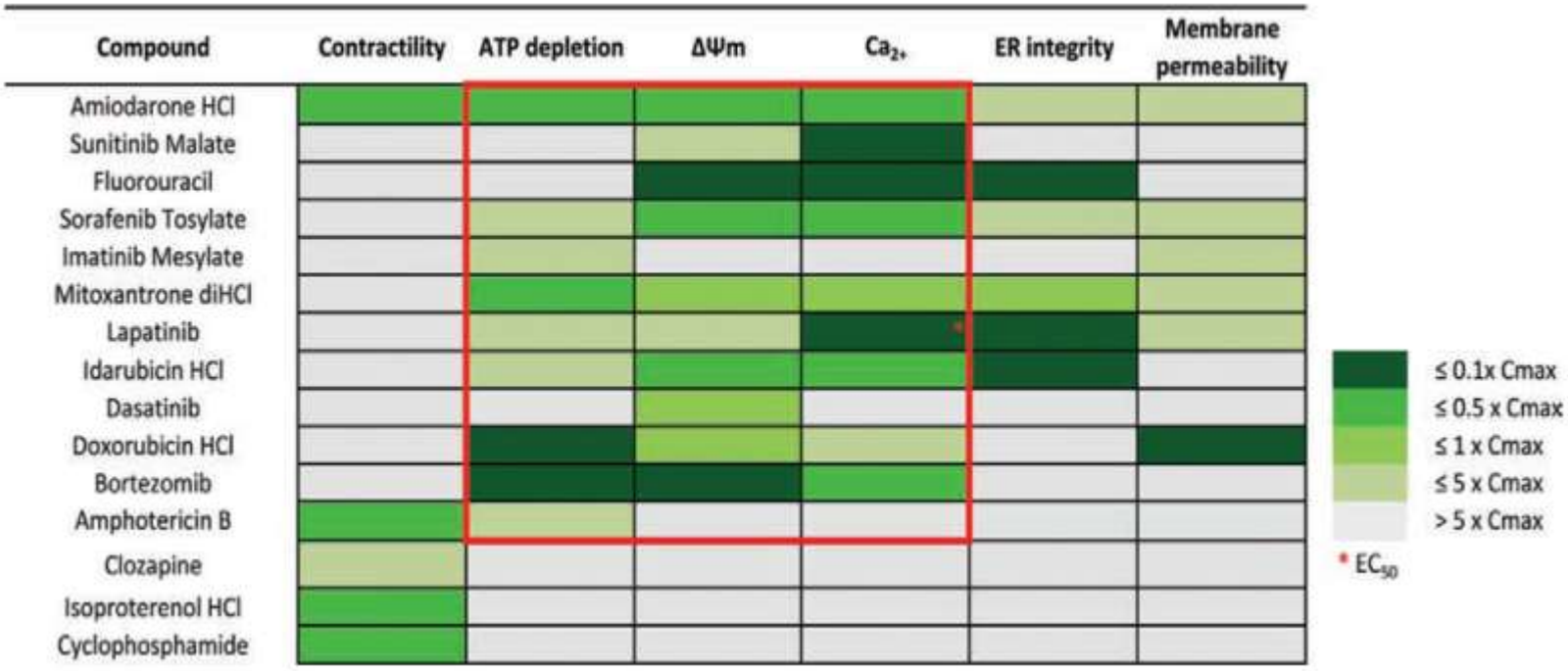
Maya Otto-Duessel, **Casey Brewer**, and **John C. Wood**
Division of Pediatric Cardiology, Department of Cardiology, Childrens Hospital Los Angeles, Los Angeles, California.


LTCC blockade
Ca2+ currents, (-)
Intracellular Ca2+ mobilization, Disruption
Autophagy, (+)
Signal transduction, Disruption
LTCC opening dynamics, Disruption
Contractile proteins levels, Disruption
Sarcoplasmic reticulum stress response, Induction
Ca2+-induced NFAT nuclear trasiocation, Inhibition
Myofibrillar atrophy, (+)
Ca2+ currents activation/inactivation dynamics, Disruption
Sarcomere assembly, Disruption
Vasodilation, (+)
Vasoconstriction, (-)
Transcription pathway, Disruption
Action potential, Disruption
Contractile response, (-)
Cardiac electrophysiology, Disruption
Heart rate, (-)
Sino-atrial rate, (-)
Vascular tone, (-)
Ejection fraction, (-)
Blood pressure, (-)

In vitro phenotypic profiling of structural cardiotoxins

- Human embryonic stem cell–derived cardiomyocytes
- H9c2 cell line
- Canine cardiomyocytes

AstraZeneca



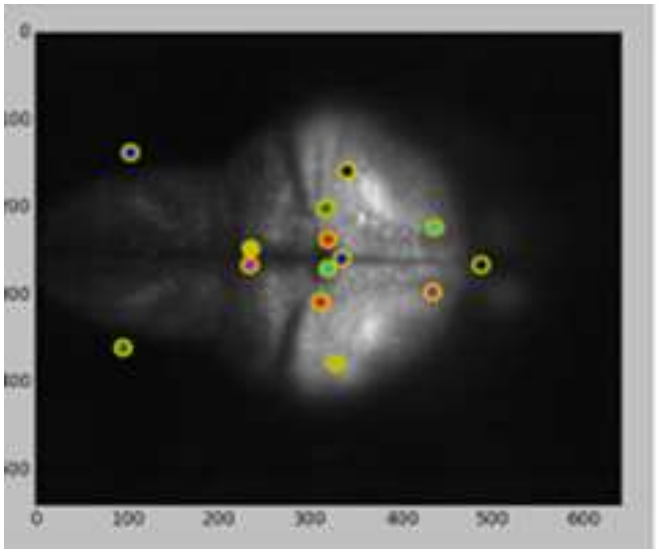
Pointon et al. (2013) Toxicological sciences 132(2), 317–326

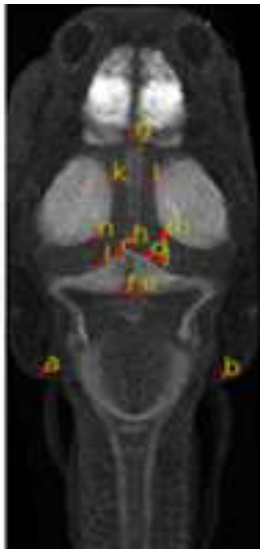
Future developments

4-dimensional functional profiling of larval zebrafish brain

Winter et al. (2017) Scientific Reports 7, 6581

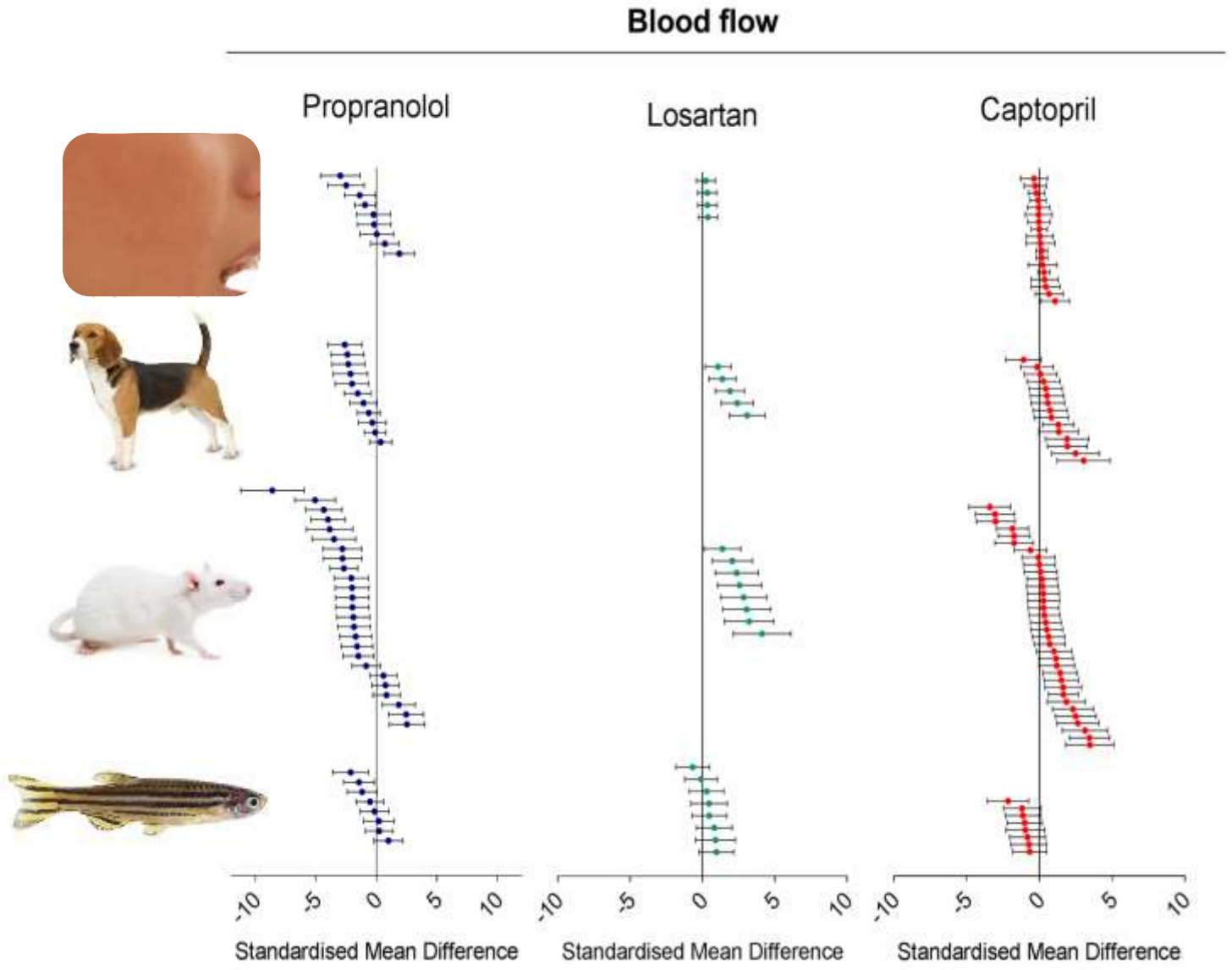
UNIVERSITY OF EXETER





Zebrafish model to investigate the coupling between calcium transient disruption and structural/functional cardiotoxicity

Establishing the translational value of the zebrafish model



Margiotta-Casaluci et al., in preparation

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