Secondary pharmacology and off-target profiling as a way to provide mechanistic insights into drug-induced cardiovascular safety liabilities

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Inspired by patients.
Driven by science.

Cardiovascular safety - beyond QT

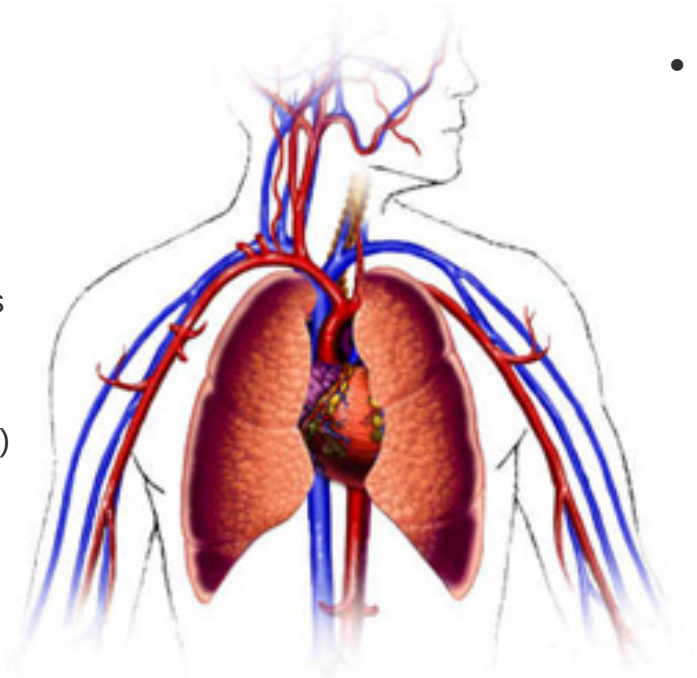
The breath of potential cardiovascular safety issues

A range of potential structural & functional effects impacting on the CV system

Heart

- Functional
  - Chronotropy
  - Dromotropy
  - Inotropy
  - Lusitropy
  - Arrhythmias (ECG)
- Structural
  - Cardiomyocyte hypertrophy,
  - Degeneration/Necrosis/apoptosis
  - Cardiomyocyte vacuolation,
  - Myocardial infarct/fibrosis
  - Inflammation (myocardium/valve)
  - Edema (myocardium/valve)
  - Mineralization/pigment/amyloid
  - Valvular stromal proliferation

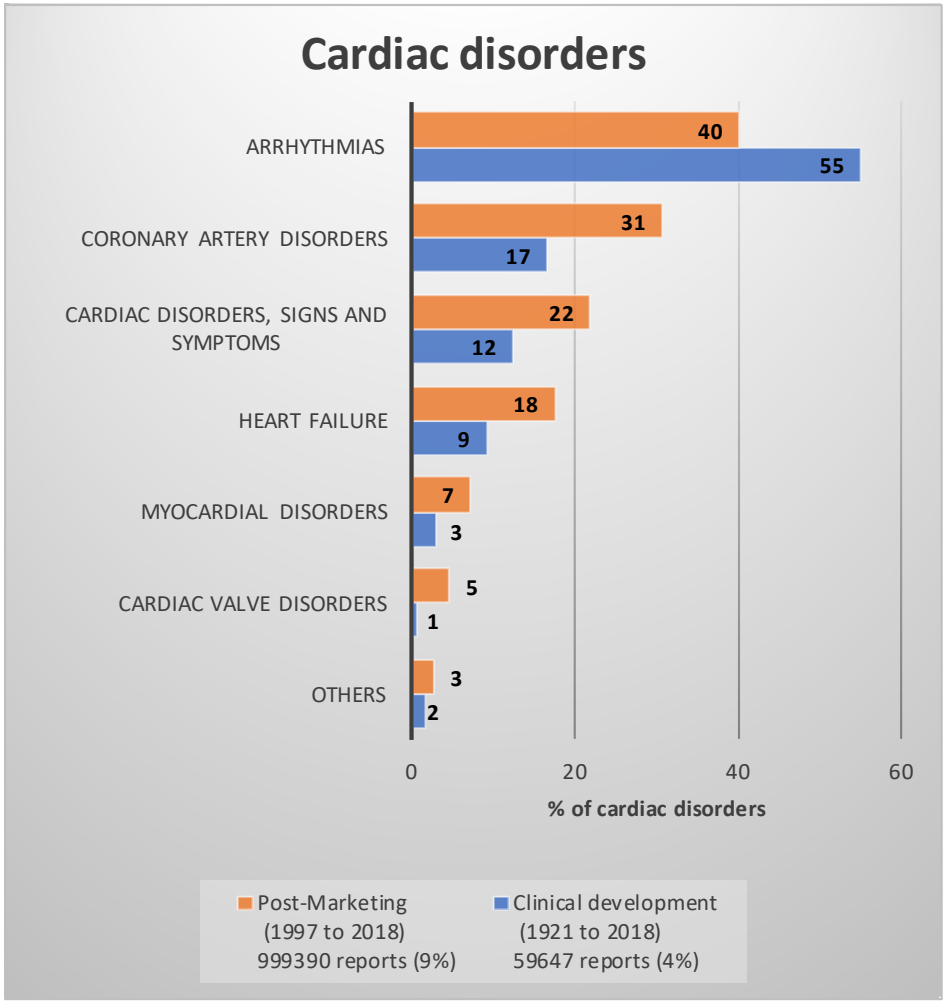
OTHER SYSTEMS

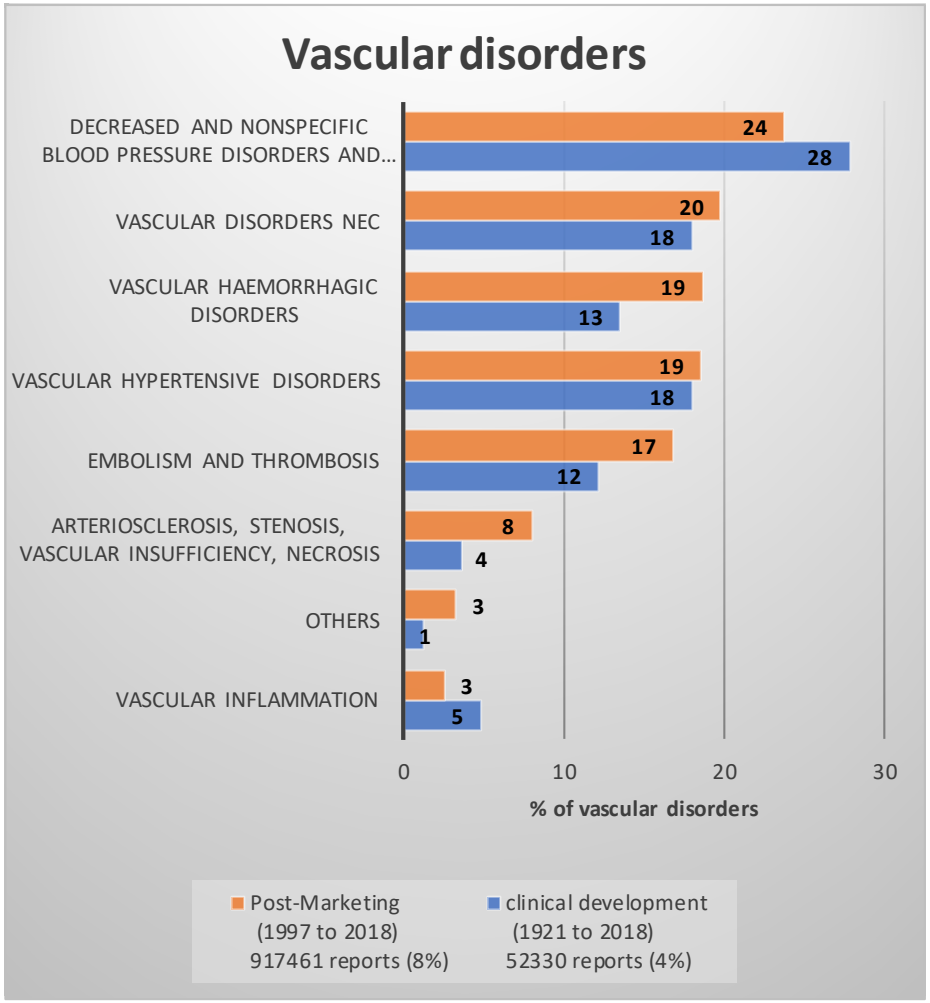


Blood Vessels

- Functional
  - Blood pressure changes
  - Orthostatic hypotension
- Structural
  - Endothelial hypertrophy,
  - Intimal thickening
  - Medial/mural hypertrophy
  - Medial/mural haemorrhage
  - Medial/mural degeneration /necrosis/inflammation
  - Medial/mural mineralization/amyloid
  - Medial/adventitial vacuolation
  - Perivascular inflammation/fibrosis
  - Aneurysm/angiostasis
  - Thrombus/embolus/intramural plaque

Cardiovascular adverse events in clinical development and post-approval

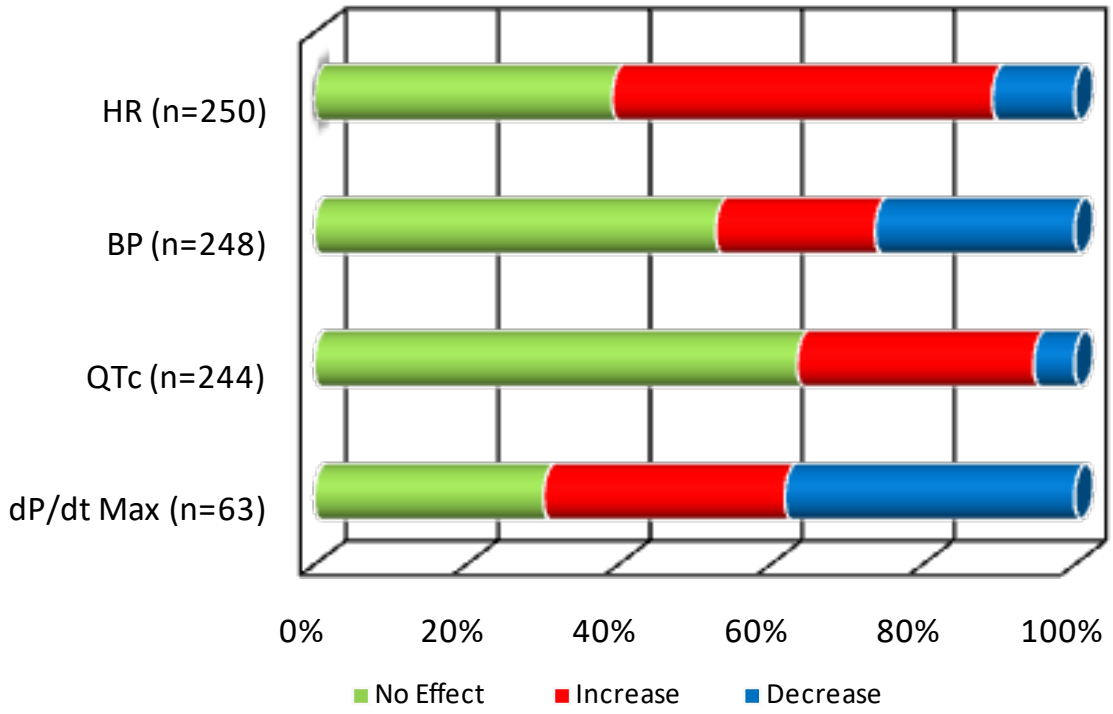




Source: Pharmapendium®; querry dated 2018-08-02 (FDA; EMA; Meyler's; Mosby's Drug Consult™; Pharmapendium Published Toxicity)

Incidence of cardiovascular side effects in preclinical studies

High incidence in safety pharmacology & toxicology studies



Data from 273 candidate drugs (CDs) evaluated in 36 anesthetised dog and 214 dog telemetry studies from 1999 to 2010. *

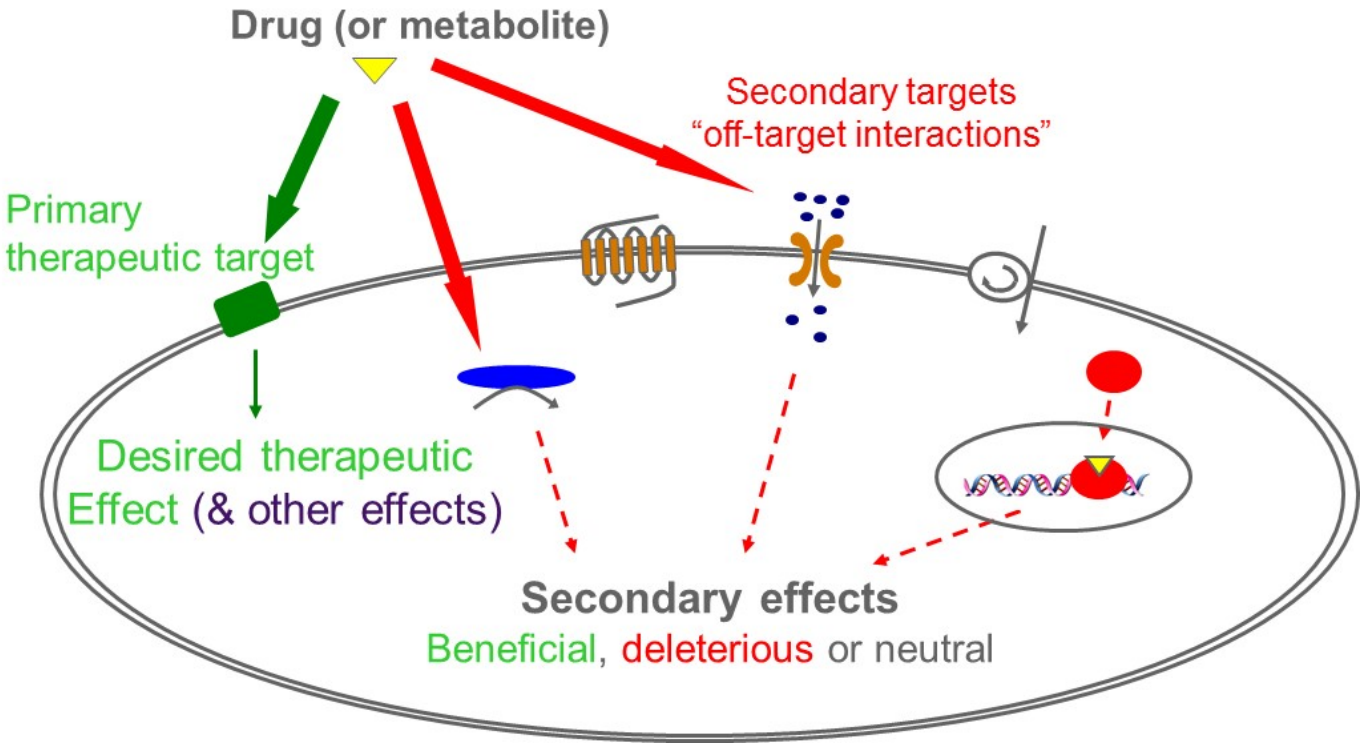
Out of 135 CDs: Cardiac pathologies were identified in 12% of dog toxicology studies of up to 1 month duration. Areas included the myocardium, AV/aortic valves, and atrium, with morphologic changes that included degeneration, vacuolation and inflammation. **

* Courtesy from Dr Pierre Lainée, AstraZeneca – Sanofi

** Milliken P et al., manuscript in preparation

Pharmacology mediated side effects

Approaches to pharmacology studies in drug discovery

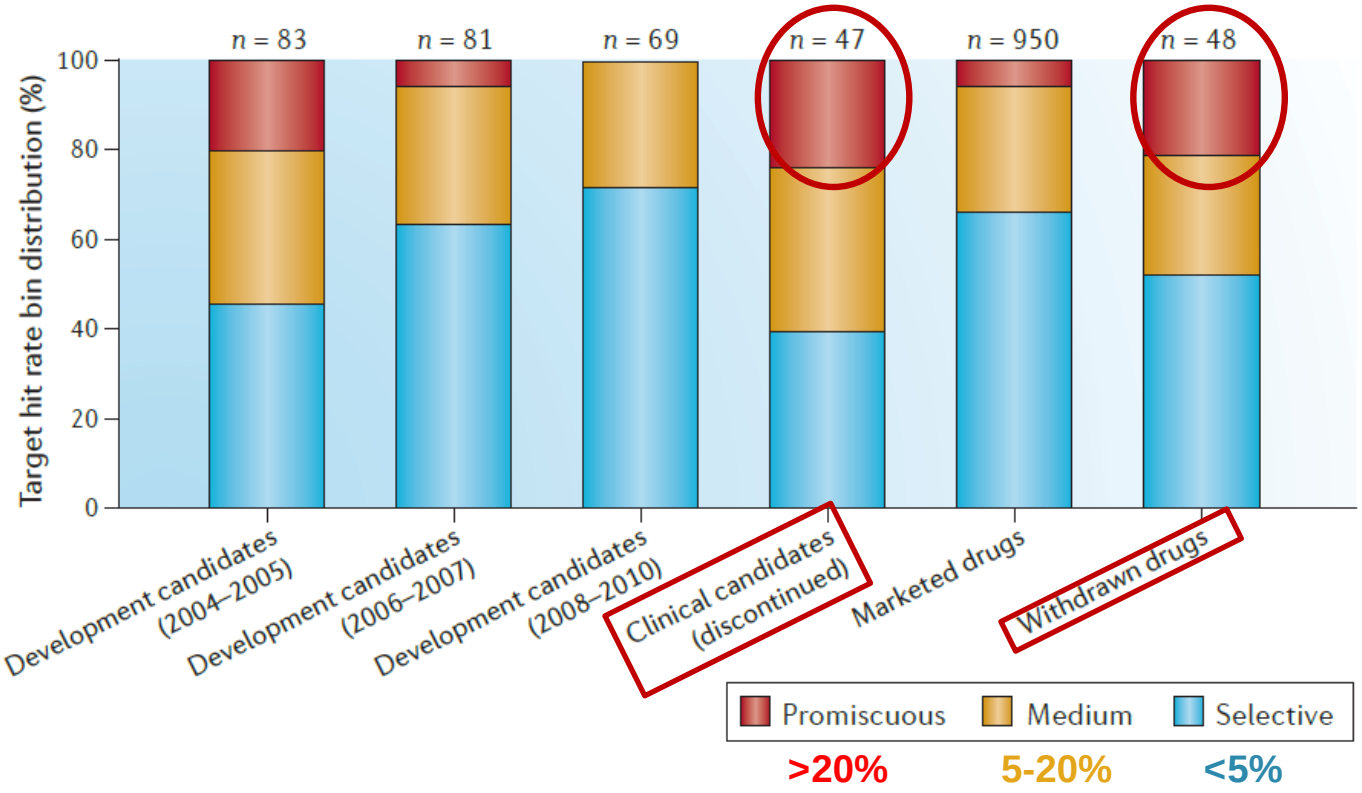


- **Side effects may result from activity at primary or secondary targets**
- **ICHS7A secondary pharmacodynamics definition: '*Studies on the mode of action and/or effects of a substance not related to its desired therapeutic target*'**

Adapted from Valentin & Hammond, JPTM, 2008; and Bowes et al (2006) in The Process of New Drug Discovery and Development, 2nd Edition Smith & O'Donnel Ed. CRC Press p104

Definition, target panel and profiling strategies

Rationale for a strategy: A high promiscuity rate translates into a high attrition rate



Promiscuity = target hit rate (% of targets with >50% binding at 10µM); *Data set excludes antipsychotics*

- **In vitro off-target promiscuity is a valuable measure of "probability of success" in early discovery**

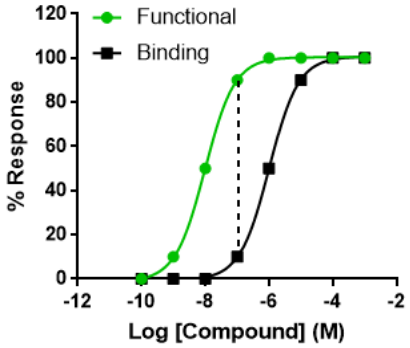
Bowes et al 2012 *Nature Rev Drug Discov*; 11:909-922. Figure 2.

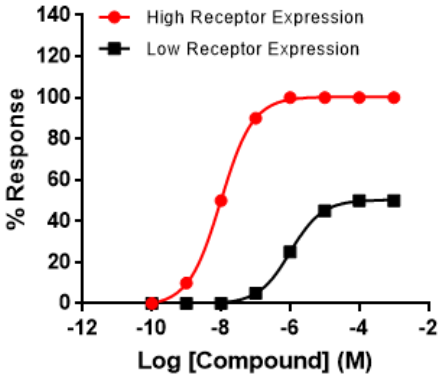
Methodology: Data generation and analysis

Binding versus functional: impact on data generated

- **Binding and functional assays are routinely used**
- **Each format has strengths and limitations with respect to risk assessment**

Factor	Binding	Functional
Affinity measurement	Direct	Indirect or none
Site on the target	Single, defined	Any (even allosteric)
Technology	Widely established	Diverse, require extensive technical expertise
Identification of mode of action (ago, antago)	No	Yes
Radioligand	Needed	Usually not needed
Possible gaps	High-efficacy/low affinity agonists or allosteric compounds	Potency and efficacy dependent on receptor expression/coupling





Adapted from Bowes et al 2012 *Nature Rev Drug Discov*; 11:909-922.

Discussion: Data interpretation and contextualisation

Assessment of individual target effects

Literature evidence for each target/adverse drug reaction (ADR) pair from:

- Online Mendelian Inheritance in Man (OMIM)
- Knock-out/in animals
- IUPHAR Guide to Pharmacology
- Pharmacological reference compounds
- Marketed/withdrawn drugs (primary target or known off-target)
- FDA Adverse Event Reporting System (FAERS)
- Databases e.g. Pharmapendium, Off-X,...

Cognisant that polypharmacology could lead to different responses

Build understanding of physiological function of target and effects of disruption

- Lynch et al (2017) *J Pharm Tox Method* S1056-8719(16):30147-30152.
- Bowes et al (2012) *Nature Rev Drug Discov* 11:909-922.
- Hamon et al (2009) *Future Med Chem* 1:645-665
- Whitebread et al (2005) *Drug Discovery Today* 10(21):1421-1433.

No centrally / publicly available repository

Discussion: Data interpretation and contextualisation

Examples of « off-target » side effects

Effects can be on organ system function or structure, such as:

Target	Organ	MOA	Potential physiological effects	Potential structural effects
hERG	CVS	Inhibitor	Increased APD, prolonged QTc, TdP	Embryonic malformations and death due to reduced cardiac output and hypoxia
5-HT$_{2B}$	CVS	Agonist	Pulmonary hypertension	Cardiac valvulopathy
Adrenergic beta 2	CVS CVS	Agonist Antagonist	Tachycardia, hypotension Bronchospasm	Vascular smooth muscle necrosis, cardiac lesions (dogs)
Transporter, Dopamine (DAT)	CNS Repro Skin	Inhibition Inhibition Inhibition	Effects on cognition & locomotor activity, drug abuse, depression	 Rat specific uterine tumours Acne
COX1	Upper GI	Inhibition	Increased acid secretion and reduced mucus production	Erosion/ulceration of gastric mucosa
COX2	CVS Immune	Inhibition Inhibition	Hypertension Anti-inflammatory effect	Myocardial infarction, atherothrombosis

MOA, Mechanism Of Action

Bowes et al 2012 *Nature Rev Drug Discov* 11:909-922.
Lynch et al 2017 *J Pharm Tox Method* S1056-8719(16)30147-2.
Papoian et al 2015 *Nature Rev Drug Discov* 14(4):294-296
Hamon et al 2009 *Future Med Chem* 1:645-665.

Source of information: the IUPHAR database

Search Database

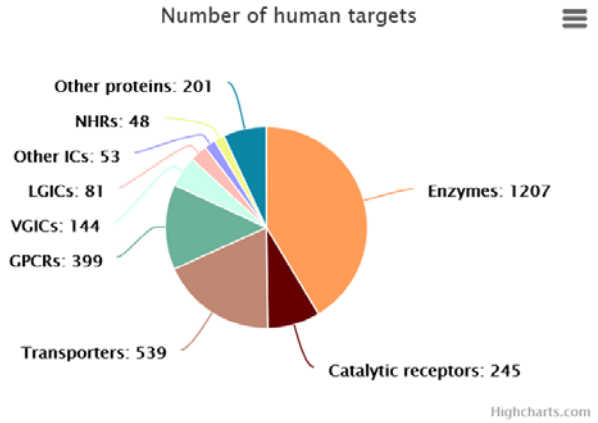
IUPHAR/BPS
Guide to PHARMACOLOGY

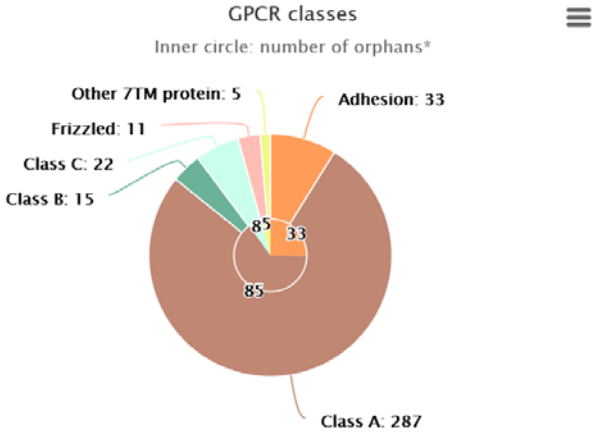
Home | About | Targets | Ligands | Diseases | Resources | Advanced search | Guide to IMMUNOPHARMACOLOGY Portal

An expert-driven guide to pharmacological targets and the substances that act on them.

Out of 2917 targets, 221 are associated with 'cardiac' and 272 with 'vascular'
=> Does not necessarily mean causal relationship with adverse events

Target Class	Number of human targets‡
7TM receptors	399
G protein-coupled receptors including orphans	394
Orphan G protein-coupled receptors*	126
Other 7TM proteins	5
Nuclear hormone receptors	48
Catalytic receptors	245
Ligand-gated ion channels	81
Voltage-gated ion channels	144
Other ion channels	53
Enzymes	1207
Transporters	539
Other protein targets	201
Total number of targets	2917





http://www.guidetopharmacology.org/

Physiological, pharmacological and toxicological considerations of drug-induced structural cardiac injury

Adverse preclinical and clinical cardiac effects – approved kinase inhibitors used in oncology (adapted from Mellor *et al.*, 2011)

Drug/ Biological	Target(s)	Oncology indications	Preclinical cardiac findings	Clinical cardiac findings	References
Axitinib (Inlyta®)	VEGFR1/2/3	RCC	Modest dose-dependent elevation in systolic BP in rats	Hypertension	Inlyta® FDA Pharm Review Inlyta® Prescribing Information
Bevacizumab (Avastin®)	VEGF	CRC, NSCLC; breast cancer;	None reported	HF, hypertension, ischaemia	Choueiri *et al.* (2011) Chen *et al.* (2013) Avastin® Prescribing Information
Cabozantinib (Cometriq®)	Ret, Met, VEGFR1/2/3, Kit, trkB, FLT3, Axl, TIE2	Metastatic medullary thyroid cancer	Cardiac inflammation noted in a single female dog when administered for a 6 month period	Hypertension	Cometriq® Prescribing Information
Crizotinib (Xalkori®)	ALK, c-Met (HGFR), and ROS	ALK-positive NSCLC	Dose-dependent inhibition of the hERG current, decrease in HR and increase in left ventricular end-diastolic pressure in dogs, myonecrosis in rats	QT-interval prolongation, bradycardia	Xalkori® FDA Pharm Review Xalkori® Prescribing Information
Dabrafenib (Tafinlar®)	B-Raf	MM	Adverse cardiovascular effects in dogs consisting of coronary arterial degeneration/necrosis and haemorrhage, as well as cardiac atrio-ventricular valve hypertrophy/haemorrhage	QT-interval prolongation, decreased LVEF	Tafinlar® Prescribing Information
Dasatinib (Sprycel®)	Bcr-Abl, Src family, Kit, PDGFRβ, EphA2	CML, ALL	QT prolongation, increased BP. Vascular and cardiac fibrosis, cardiac hypertrophy, myocardial necrosis, haemorrhage of the valves, ventricle and atrium and cardiac inflammation	QT-interval prolongation, HF, pericardial and pleural effusion, pulmonary hypertension	Brave *et al.* (2008) Montani *et al.* (2012) Sprycel® Prescribing Information
Erlotinib (Tarceva®)	ErbB1 (EGFR)	RCC	None reported	Myocardial infarction/ischaemia	Tarceva® Prescribing Information
Imatinib mesylate (Gleevec®)	Bcr-Abl, PDGFRα and β, Kit	CML, ALL, GIST, MDS/MPD, ASM, HES, CEL, DFSP	Reversible hypertrophy in rats. Decrease in arterial BP after single i.v. dose in rats. No effect on the rate of beating or force of contraction in the isolated atria of guinea pigs	Decreased LVEF, LVD, rare frequency of HF	Kerkelä *et al.* (2006) Gleevec® FDA Pharm Review Gleevec® Prescribing Information
Lapatinib (Tykerb®)	EGFR (ErbB1), HER-2 (ErbB2)	HER-2+ ve breast cancer	Dose-responsive increase in BP in dog. Focal fibrosis and myocyte degeneration in rat and dog. No QT changes in rat and dog	Decreased LVEF, HF, asymptomatic cardiac events, QT-interval prolongation.	Perez *et al.* (2008) Tykerb® FDA Pharm Review Tykerb® Prescribing Information
Nilotinib (Tasigna®)	Bcr-Abl, PDGFRα and β, Kit	CML	QT-interval prolongation	QT-interval prolongation, sudden death (possibly ventricular repolarization related) Ischaemia, peripheral ischemia	Kantarjian *et al.* (2007) Tefferi (2013) Weisberg *et al.* (2005) Tasigna® Prescribing Information

Kinase/phosphatase conditional knockout mouse models associated with cardiovascular functional effects (adapted from Mellor *et al.*, 2011)

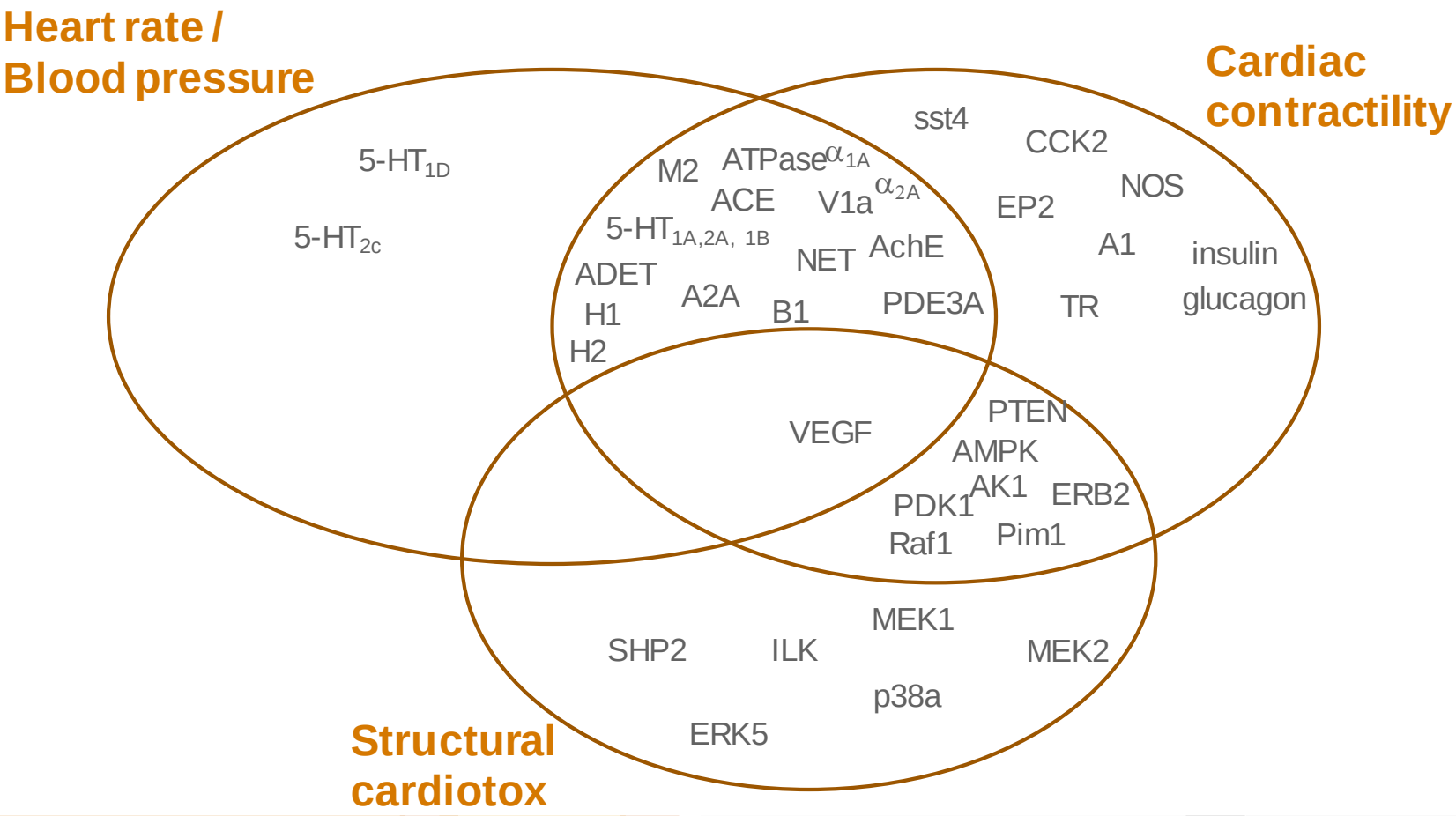
Protein	Signalling role	Knockout animal model	Effect on cardiac function	Reference
PTEN	Lipid phosphatase Negative regulator of PI3-kinase signalling	Muscle-specific PTEN knockout mouse	Basal hypertrophy Mild reduction in contractility Reduced hypertrophy in response to pressure overload compared with wt	Crackower *et al.* (2002) Oudit *et al.* (2008)
AMPK	Serine/threonine kinase Activated by increase in AMP: ATP Acts to preserve/generate ATP	Heterozygous AMPKα2 knockout mouse	Mild reduction in contractility Worsened hypertrophy in response to pressure overload compared with wt	Zhang *et al.* (2008)
SHP2	Tyrosine phosphatase Regulates leptin and insulin signalling	Muscle-specific Shp2 knockout mouse	Severe dilated cardiomyopathy HF and premature death	Kontaridis *et al.* (2008) Princen *et al.* (2009)
ERB2	Receptor tyrosine kinase Co-receptor in neuregulin/EGRF signalling	Ventricular myocyte-specific ERB2 knockout mouse	Severe dilated cardiomyopathy Decreased contractility HF and sudden death	Crone *et al.* (2002) Ozcelik *et al.* (2002)
PDK1	AGC serine/threonine kinase Activates AKT and p70S6K	Muscle-specific PDK1 knockout mouse Tamoxifen-inducible heart-specific PDK1 knockout mouse	Apoptotic death of cardiomyocytes Impaired LV contractility Severe and lethal HF	Ito *et al.* (2009) Mora *et al.* (2003)
Pim1	Serine/threonine kinase Acts downstream of AKT to block apoptosis Induction and stabilization of c-myc	Cardiac-specific Pim-1 dominant-negative in mouse	Progressive dilation Reduced contractility Increased LVEDP Decreased LVDP Alterations in Ca^{2+} handling	Muraski *et al.* (2008)
Raf-1 (c-Raf)	Serine/threonine kinase Involved in the ERK signalling pathway	Cardiac-specific Raf-1 knockout mouse	Reduced contractility Increased heart size Decreased posterior wall thickness	Yamaguchi *et al.* (2004)
ILK	Serine/threonine kinase Phosphorylates Akt and GSK-3β	Muscle-specific ILK knockout mouse	Increased heart size Dilated cardiomyopathy Cardiac fibrosis Sudden death	White *et al.* (2006)
AK1	Kinase/phosphotransferase Adenine nucleotide homeostasis	AK1 knockout mouse	Reduced contractility – coronary flow relationship Recovery of flow after I/R was compromised	Dzeja *et al.* (2007)
p38α	MAPK phosphorylates MAPKAP kinase 2, ATF-2, Mac and MEF2	Cardiac-specific p38α dominant-negative in mouse	Cardiac hypertrophy reduced fractional shortening LV and septal wall thinning Lethal cardiomyopathy	Braz *et al.* (2003)
ERK5	MAPK (serine/threonine kinase) phosphorylates MEF2C, Sap1a, p90RSK	ERK5 knockout mouse ERK5 –/– cardiomyocyte knockout	Embryonically fatal at E9.5–10.5 Defective cardiac development, heart looping, angiogenesis and vascular maturation Mice develop normally but have reduced cardiac hypertrophic remodelling	Regan *et al.* (2002) Kimura *et al.* (2010)

AMPK, AMP-activated protein kinase; ATF-2, activating transcription factor 2; GSK-3β, glycogen synthase kinase 3 β; ILK, integrin-linked kinase; I/R, ischaemia/reperfusion; LVDP, left ventricular diastolic pressure; LVEDP, left ventricular end-diastolic pressure; MEF2, myocyte enhancer factor 2; wt, wild type; PDK1, 3-phosphoinositide-dependent PK-1; PTEN, phosphatase and tensin homolog; Shp2, src homology 2 region.

Cross et al., BJP, 2015

Targets associated with cardiovascular safety liabilities

Based on genetic, pharmacological and clinical (adverse events) evidences



Targets associated with cardiotoxicity: OFF-X

Research by targets, by drugs, or by adverse events
Out of 7,446 targets, 286 targets were reported to be associated with cardiotoxicity

cardiotoxicity (cardiac)

TARGET - ACTION		ALERT TYPE				ALERT PHASE				NUMBER OF ALERTS	
		CLASS ALERT		DRUG ALERT		TARGET DISCOVERY / PRECLINICAL		CLINICAL / POST MARKETING			
All Target - Actions	286	279	37	408	108	382	77	305	79	687	124

Filters can be applied to the following criteria

ALERT TYPE
- Class Alert
- Drug Alert

ALERT PHASE
- Target Discovery
- Preclinical
- Clinical
- Postmarketing

LEVEL OF EVIDENCE
- Confirmed/Reported
- Suspected
- Refuted/Not Associated

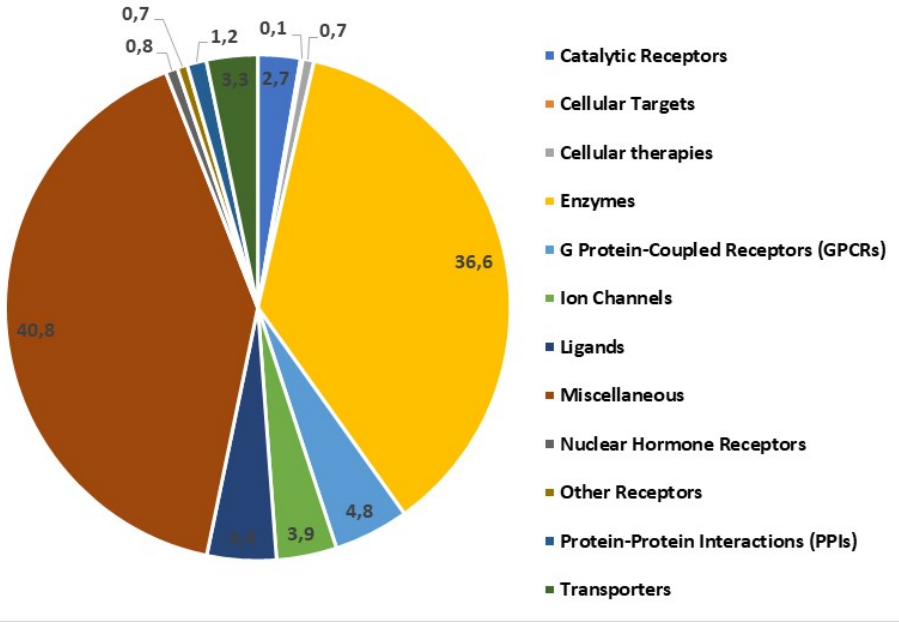
ON/OFF-TARGET
- On-Target
- Off-Target
- Not Specified

SOURCE TYPE
- Regulatory Agency Communication
- Congress Alert
- Company Communication
- Journal
- Other

PERIOD
- Full Database
- Last Year
- Last Month

cardiotoxicity (cardiac)

187 – on-target; 21 – off-target; 100 – not specified
77 – Confirmed/Reported
66 – Clinical
29 – Regulatory Agency Communication



https://www.targetsafety.info/

OFF-X The translational safety intelligence portal

Discussion: Data interpretation and contextualisation

How to estimate safety margins

- **Can we use exposure data and ADR or efficacy data from marketed and withdrawn drugs to estimate appropriate safety margins?**
  - It can be challenging to generate clear and robust translation due to
    - Limited availability of clinical data (especially PK)
    - Differences in in vitro results between laboratories
      - Reference compounds must be tested in the same in vitro assay
    - Polypharmacology of reference and test compounds
    - Physiological compensation mechanisms (e.g. reflex tachycardia)

  - Examples of successful translational safety studies include

• hERG	30x free Cmax	(Redfern 2003)
• hNav1.5	30-100x Free Cmax	(Harmer 2011)
• 5-HT_{2B}	Ki/FCmax ratio or Ki relative to 5-HT	(Papoian 2017)
• ENT-1	4-13x disease state dependent	(Rosenbrier Ribeiro 2017)

- **Using legacy safety studies; Sharing pre-competitive data**
  - 1000 publications / year on hERG/QT/TdP to understand the issue

New technologies and future focus

- **Accessing a wider range of targets**
  - Implementing different screening technologies e.g. proteomics
  - Understanding previously poorly characterized targets
- **Defining the strategic and scientific approach to explore novel mechanisms of action, novel modalities, new classes or proteins**
- **Increasing understanding of pathway-level impact**
  - E.g., potential for additive, synergistic, antagonistic effects within a pathway (kinases pathways)
- **Increasing understanding of pharmacokinetic/pharmacodynamic relationships and of target safety liabilities (and annotation)**
- **In silico approaches**
  - Individual target quantitative structure-activity relationship (QSAR) models
  - Profile prediction tools
  - Machine Learning
    - Natural language processing to mine literature, FAERS, etc

Summary and conclusion

- **In vitro profiling is an integral part of the drug discovery and development process**
- **Implemented widely across medium/large pharma**
- **Well established technologies and operating principles**
- **Can be deployed to deconvolute drug-induced cardiac toxicity**
- **Future challenges focus on:**
  - Increased biological understanding for individual targets
  - Generating quantitative translation for each target
  - Accessing wider range of targets (technology shifts)
  - Improving predictivity
  - Improving understanding of the “fingerprint” vs individual targets

Thanks to:

Colleagues from Toxicology, Development Science, UCB-Biopharma in particular Annie Delaunois, Vitalina Gryshkova and Julia Roquigny

Colleagues from the IQ-DruSafe In vitro Secondary Pharmacology WG

Questions?