BJP British Journal of Pharmacology

DOI:10.1111/bph.12979
www.brjpharmacol.org

REVIEW

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

M J Cross[1], B R Berridge[2], P J M Clements[3], L Cove-Smith[4], T L Force[5]*, P Hoffmann[6], M Holbrook[7], A R Lyon[8], H R Mellor[9], A A Norris[1], M Pirmohamed[10], J D Tugwood[4], J E Sidaway[11] and B K Park[1]

[1]*MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK,* [2]*Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC, USA,* [3]*David Jack Centre for Research & Development, GlaxoSmithKline, Ware, Herts, UK,* [4]*Clinical & Experimental Pharmacology, Cancer Research UK Manchester Institute, University of Manchester, Manchester, UK,* [5]*Center for Translational Medicine and Cardiology Division, Temple University School of Medicine, Philadelphia, PA, USA,* [6]*Preclinical Safety, Novartis Pharm Corp, East Hanover, NJ, USA,* [7]*Safety Pharmacology, Covance Laboratories, Ltd., Harrogate, North Yorkshire, UK,* [8]*NIHR Cardiovascular Biomedical Research Unit, Royal Brompton Hospital and Imperial College, London, UK,* [9]*Drug Safety Evaluation, Vertex Pharmaceuticals (Europe), Ltd., Abingdon, Oxfordshire, UK,* [10]*The Wolfson Centre for Personalised Medicine, Institute of Translational Medicine, University of Liverpool, Liverpool, UK, and* [11]*Innovative Medicines, AstraZeneca R&D, Macclesfield, UK*

Correspondence
Michael J Cross, MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, Sherrington Building, Ashton Street, The University of Liverpool, Liverpool L69 3GE, UK. E-mail: m.j.cross@liv.ac.uk

*Current address: Vanderbilt University School of Medicine, Cardiology Division, Nashville, TN, USA.

Received
17 June 2014
Revised
30 September 2014
Accepted
6 October 2014

The incidence of drug-induced structural cardiotoxicity, which may lead to heart failure, has been recognized in association with the use of anthracycline anti-cancer drugs for many years, but has also been shown to occur following treatment with the new generation of targeted anti-cancer agents that inhibit one or more receptor or non-receptor tyrosine kinases, serine/threonine kinases as well as several classes of non-oncology agents. A workshop organized by the Medical Research Council Centre for Drug Safety Science (University of Liverpool) on 5 September 2013 and attended by industry, academia and regulatory representatives, was designed to gain a better understanding of the gaps in the field of structural cardiotoxicity that can be addressed through collaborative efforts. Specific recommendations from the workshop for future collaborative activities included: greater efforts to identify predictive (i) preclinical; and (ii) clinical biomarkers of early cardiovascular injury; (iii) improved understanding of comparative physiology/pathophysiology and the clinical predictivity of current preclinical *in vivo* models; (iv) the identification and use of a set of cardiotoxic reference compounds for comparative profiling in improved animal and human cellular models; (v) more sharing of data (through publication/consortia arrangements) on target-related toxicities; (vi) strategies to develop cardio-protective agents; and (vii) closer interactions between preclinical scientists and clinicians to help ensure best translational efforts.

Abbreviations

ABPI, Association for British Pharmaceutical Industry; hESC-CM, human embryonic stem cell-derived cardiomyocyte; HF, heart failure; LVD, left ventricular dysfunction; LVEF, left ventricular ejection fraction; SCD, sudden cardiac death; TKI, tyrosine kinase inhibitor

Tables of Links

TARGETS		
Other protein targets[a]	**Catalytic receptors**[d]	**Enzymes**[e]
FABP	c-Met (Met)	ACE
TNF	Axl	AMPK
GPCRs[b]	EphA2	Brk
5-HT receptors	ErbB1 (EGFR)	ERK5
Angiotensin receptors	ErbB2 (HER2)	ILK
β-adrenoceptors	ErbB4 (HER4)	MEK1
NK_1 receptor	FGFR	MEK2
Ion channels[c]	FLT3	MMPs
hERG (K_V11.1)	Kit	p38α
	PDGFRα	PDE3
	PDGFRβ	PDK1
	Ret	PTEN
	TGFBR1 (ALK5)	a-Raf
	TIE2	b-Raf
	TrkB	c-Raf
	VEGFR-1	Src family
	VEGFR-2	
	VEGFR-3	

LIGANDS	
5-fluorouracil	Imatinib
Axitinib	Lapatinib
Bevacizumab	Neuregulin-1
BNP	Pazopanib
Cabozantinib	Pertuzumab
Carvedilol	Ponatinib
Casopitant	Regorafenib
Crizotinib	Sorafenib
Cyclophosphamide	Sunitinib
Dabrafenib	Trametinib
Dasatinib	Trastuzumab (Herceptin)
Dexrazoxane	Vandetanib
Doxorubicin	Vemurafenib
Enalapril	Vincristine
Erlotinib	

These Tables list key protein targets and ligands/inhibitors in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d,e]Alexander *et al.*, 2013a,b,c,d,e).

Introduction

Cardiovascular toxicities are observed with therapeutic agents used in the treatment of both cardiovascular and non-cardiovascular diseases and affect all components of the CVS. Cardiovascular adverse reactions can occur after acute or chronic treatment and can affect function (e.g. alteration of the mechanical function of the myocardium) and/or structure (e.g. morphological damage or loss of cellular/subcellular components of the heart) or vasculature. They remain a major cause of drug attrition during preclinical and clinical development, and drug withdrawals from the marketplace. This was highlighted in a scientific workshop on cardiovascular toxicity, which covered a wide range of potential liabilities, held at the Medical Research Council (MRC) Centre for Drug Safety Sciences (CDSS) in January 2010 (Laverty *et al.*, 2011). A recommendation from the workshop was to reconvene to discuss in greater detail a small number of selected cardiovascular liabilities.

On 5 September 2013, a workshop was hosted by the MRC CDSS (http://www.liv.ac.uk/drug-safety), University of Liverpool, in conjunction with the Association of the British Pharmaceutical Industry (ABPI) and the Medicines and Healthcare Products Regulatory Agency. It discussed current challenges in determining and understanding 'Structural Cardiotoxicity of Medicines' as a major and emerging issue in the development of new therapies – particularly oncology agents. The key aims of the workshop were to identify those areas of cardiovascular safety testing where our knowledge and understanding should be further strengthened and to recommend areas where collaborative efforts should be focused. The workshop was attended by representatives from pharmaceutical and biotechnology companies, contract research organizations, regulatory agencies and academia.

Drug-induced structural cardiac damage is associated with changes in multiple cardiac cell types leading to cardiac fibrosis and cardiomyopathy (deterioration of the function of the myocardium due to injury) and subsequently, heart failure (HF). Structural cardiotoxicity is a concern with several classes of anti-cancer agents as any gain in life expectancy from therapeutic intervention might be countered by increased morbidity and mortality due to a variety of cardiovascular problems, including: heart muscle injury with cardiomyopathy and HF, complications of coronary artery disease leading to myocardial ischaemia, arrhythmias, hypertension and thromboembolism (Stortecky and Suter, 2010; Berardi *et al.*, 2013). Patnaik *et al.* (2011) showed that after 8–9 years following initiation of drug treatment, mortality in breast cancer patients as a result of cardiovascular toxicity overtakes risk of death from breast cancer recurrence. The incidence of structural cardiotoxicity depends on a number of different factors related to therapy (e.g. type of drug, dose administered during each cycle, cumulative dose, schedule of administration, route of administration, combination of other cardiotoxic drugs or association with radiotherapy covering the heart in the therapeutic field) and also to the patient phenotype based

on pre-existing cardiovascular risk (e.g. age, presence of 'traditional' cardiovascular risk factors, underlying cardiac dysfunction and any prior exposure to cardiotoxic chemotherapy or radiotherapy). Cardiotoxic effects can occur immediately following drug administration, or they may not manifest themselves until months or, in some examples, many years after the patient has been treated. There are a number of clinical challenges in managing cancer drug-treated patients and it is crucial that appropriate risk stratification based on previous drug exposure and patient phenotype is addressed. Significant challenges to preclinical assessment are also apparent as attempts to model the clinical factors, highlighted earlier, may have dubious translatable value. Although the use of traditional cancer therapies such as anthracyclines (Von Hoff *et al*., 1979) and radiation (Boivin *et al*., 1992) have long been associated with cardiac complications (up to a 20% risk of HF after 20 years following a period of treatment with chemotherapy and radiotherapy) (Hooning *et al*., 2007) other agents such as cyclophosphamide, 5-fluorouracil and paclitaxel are known to cause cardiac injury as well, albeit at lower rates than anthracyclines. Dosing regimens and newer agents, plus pre-screening to exclude patients with reduced cardiac function at baseline, have helped to reduce risk in current patients receiving anthracyclines, but in contemporary studies the rate of left ventricular dysfunction (LVD) is still between 5–20% (Shakir and Rasul, 2009) depending upon the definition applied and follow-up duration.

The more recently introduced 'targeted therapies', which inhibit various PKs have also been associated with cardiotoxicity, through both on-target and off-target effects. The toxicity of these agents is through different molecular and cellular mechanisms of cardiotoxicity to those caused by anthracyclines (Force and Kolaja, 2011; Mellor *et al*., 2011). However, in many cases, the adverse clinical cardiac events observed were not anticipated based on preclinical evaluation of these compounds. It is therefore important to identify new models/techniques, which can better predict adverse clinical outcomes with these agents.

We set out to address the following points at the workshop:

- What pathologies come under the banner of 'cardiovascular toxicity' and how well do we understand these as individual pathologies and components of a syndrome?
- What is the prevalence of the recognized individual pathologies, and how well do we understand their pathogenesis?
- How good is our mechanistic understanding of cancer therapeutics-induced structural cardiotoxicity?
- How well do we understand patient susceptibilities to cardiotoxicity and do we need animal models of 'disease' and/or 'physiological challenge'?
- How translatable are animal pathologies to the relevant human pathology?
- How can we share data on target-driven toxicities more efficiently to avoid a repetition of unnecessary animal research and preclinical toxicology studies?
- Can functional changes predict specific pathologies (and vice versa)?
- Can we identify/improve *in vitro* assays to model and predict specific animal/human pathologies?

This publication incorporates the key issues highlighted during the workshop along with the gap analysis and identifies key areas where a concerted effort could make a real difference by reducing cardiovascular liabilities of new medicines.

Clinical definitions of cardiovascular toxicity related to oncology therapies

Drug-induced cardiovascular toxicity may develop acutely or subacutely during or after a treatment period and effects may include disorders such as myocardial dysfunction, ischaemia, hypotension, hypertension, QT-interval prolongation, arrhythmias and thromboembolism. Chronic consequences of cardiomyocyte insult may manifest as an 'early' cardiomyopathy within the first year after treatment cessation or as a 'later' cardiomyopathy, occurring more than 1 year afterwards; these probably represent a continuous spectrum of the same pathophysiology, with dose and coexisting risk factors determining the rate of progression of cardiac dysfunction. Clinical presentation late in the course of the HF progression represents the most problematic type of injury. The most common initial feature of chronic cardiotoxicity is asymptomatic systolic LVD; left untreated, this may progress to congestive HF. This initial dysfunction may not be clinically apparent (i.e. asymptomatic) for many years because of attempted normalization of function by compensatory mechanisms, as seen following other forms of cardiac injury such as acute myocardial infarction. The incidence of chronic cardiotoxicity is influenced by a number of factors such as cumulative dose of chemotherapy administered, age of patient, cardiovascular disease history and prior radiation therapy, and can range from 5 to 65% of patients treated with anthracyclines (Dolci *et al*., 2008).

Anthracyclines produce a dose-related cardiac dysfunction, defined as type I cardiotoxicity (Ewer and Lippman, 2005), characterized by cardiomyocyte ultrastructural abnormalities, (vacuoles, myofibrillar disarray and dropout, necrosis), contractile abnormalities (dilated cardiomyopathy) and subsequent clinically evident dysfunction (Billingham *et al*., 1978). Some elements are initially reversible, but over time the burden of fibrosis and myocyte loss to apoptosis renders the dysfunction currently irreversible and more refractory to current HF therapy. In contrast, cardiac dysfunction not associated with ultrastructural change, described as type II, typically manifests as an asymptomatic decrease in left ventricular ejection fraction (LVEF) (expressed as a percentage of the total amount of blood in the left ventricle that is ejected in each heartbeat, with a range of 55–70% in healthy individuals) and less often by clinical HF (Ewer and Lippman, 2005). Agents such as trastuzumab (Herceptin®, Genentech/Roche, San Francisco, CA, USA) and the low molecular weight tyrosine kinase inhibitors (TKIs) for example sunitinib (Sutent®, Pfizer, New York, NY, USA), imatinib (Gleevec®, Novartis, Basel, Switzerland), lapatinib (Tykerb®, GlaxoSmithKline, London, UK) and sorafenib (Nexavar®, Bayer, Leverkusen, Germany) (see Table 1) are believed to cause type II cardiac dysfunction (Ewer and Ewer, 2010), although the cellular mechanism may be very drug-specific rather than a

Table 1

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	Taflinar® 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

Table 1

Continued

Drug/ Biological	Target(s)	Oncology indications	Preclinical cardiac findings	Clinical cardiac findings	References
Pazopanib (Votrient®)	VEGFR1, VEGFR2, VEGFR3, PDGFRa/βKit	RCC	Acute increase in BP after dosing and decreased heart rate from 75 min to 24.5 h post-dose in monkeys	Cardiac dysfunction (congestive HF and decreased LVEF), QT prolongation, 2 cases of Torsades de Pointes in clinical programme, hypertension	Motzer *et al.* (2013) Votrient® FDA Pharm Review Votrient® Prescribing Information
Pertuzumab (Perjeta®)	HER-2 (ErbB2)	Breast cancer	None reported	Decreased LVEF, HF	Perjeta® Prescribing Information
Ponatinib (Iclusig®)	Bcr-Abl, Bcr-Abl T315I, VEGFR, PDGFR, FGFR, Eph, Src family kinases, Kit, Ret, TIE2 and FLT3	CML, Ph chromosome-positive ALL	Inhibition of hERG current in dose-dependent manner, systolic heart murmurs and myocardial necrosis in monkeys	HF, myocardial ischaemia, peripheral ischaemia (stroke, peripheral vascular disease)	Iclusig® FDA Pharm Review Iclusig® Prescribing Information
Regorafenib (Stivarga®)	VEGFR1/2/3, BCR-Abl, B-Raf, B-Raf (V600E), Kit, PDGFRα/β, Ret, FGFR1/2, TIE2 and EphA2	CRC	Dose-dependent increase in the finding of thickening of the atrio-ventricular valve in rats at 6 months	Hypertension, myocardial ischaemia and infarction	Stivarga® Prescribing Information
Sorafenib (Nexavar®)	Raf-1 (c-Raf), b-Raf , VEGFR1, 2 & 3, PDGFR family, Kit	HCC, RCC	hERG K-current and Ca-inward current inhibition. No ECG, BP or heart rate changes observed in 12 month dog study Autolysis, degeneration and inflammation in 3 month rat study. An increase in CK levels with haemorrhage and congestion of the heart in one animal in 12 month dog study	QT-interval prolongation, sudden death (possibly ventricular repolarization related), HF (cardiomyopathy), coronary vasospasm, arterial thrombosis	Choueiri *et al.* (2010) Escudier *et al.* (2009) Naib *et al.* (2011) Schmidinger *et al.* (2008) Uraizee *et al.* (2011) Veronese *et al.* (2006) Nexavar® FDA Pharm Review Nexavar® Prescribing Information
Sunitinib malate (Sutent®)	VEGFR1-3, PDGFRα and β, CSFR1, Ret kinase, Kit, FLT3 kinase	RCC, GIST	Potent hERG channel block and QT-interval prolongation and HR reduction at doses equivalent to human clinical exposures. Multiple ECHO parameter changes in primate including reductions in the ratio of right atrial to aortic diameter, LVEF time and LV area. Histopathological findings included capillary proliferation, myocardial vacuolization and pericardial inflammation	QT-interval prolongation, decreased LVEF, LVD, HF, increased BP, CHF linked to cardiovascular co-morbidities, arterial thrombosis	Bello *et al.* (2009) Choueiri *et al.* (2010) Chu *et al.* (2007) Faivre *et al.* (2006) Telli *et al.* (2008) Sutent® FDA Pharm Review Sutent® Prescribing Information
Trametinib (Mekinist®)	MEK1, MEK2, MEK1 kinase, MEK2 kinase	MM	Inhibition of hERG channel, cardiomyopathy (decreased LVEF, increased heart weight) in mice	Cardiomyopathy (cardiac failure, LVD, or decreased LVEF)	Mekinist® FDA Pharm Review Mekinist® Prescribing Information

Table 1

Continued

Drug/ Biological	Target(s)	Oncology indications	Preclinical cardiac findings	Clinical cardiac findings	References
Trastuzumab (Herceptin®)	HER-2 (ErbB2)	HER-2+ ve breast cancer	None reported. No evidence of toxicity in primate studies up to 6 months	Decreased LVEF, HF, increased risk if prior or concurrent anthracycline treatment.	Seidman *et al.* (2002) Herceptin® FDA Pharm Review Herceptin®Prescribing Information
Vandetanib (Caprelsa®)	EGFRs, VEGFRs, Ret, Brk, TIE2, Eph receptors and Src	Medullary thyroid cancer	Inhibition of hERG channel, increase in BP in rats,increased QTc and BP in dogs	QT-interval prolongation, Torsades de Pointes, acute cardiac failure, hypertension,	Scheffel *et al.* (2013) Caprelsa® Prescribing Information
Vemurafenib (Zelboraf®)	a/b/c-Raf and b-Raf	MM	Inhibition of hERG channel, increase in incidence of AV block in dogs, increased heart weight in rats	QT-interval prolongation	Zelboraf® FDA Pharm Review Zelboraf® Prescribing Information
Ziv-aflibercept (Zaltrap®)	VEGF	CRC	None reported	Hypertension	Zaltrap® Prescribing Information

ALL, acute lymphocytic leukaemia; AP, action potential; ASM, aggressive systemic mastocytosis; AV, atrioventricular; CEL, chronic eosinophilic leukaemia; CK, creatinine kinase; CRC, colorectal cancer; DFSP, dermatofibrosarcoma protuberans; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; HES, hypereosinophilic syndrome; HR, heart rate; MDS, myelodysplastic syndrome; MM, metastatic melanoma; MPD, myeloproliferative disorder; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

class effect, reflecting both on target and off-target toxicity. Typical features in this setting include lack of an obvious dose–relationship, increase in toxicity when given concurrently with anthracyclines, some reversibility after stopping treatment and restoration of normal cardiac function with appropriate medical management (Perik *et al.*, 2007; Slamon *et al.*, 2011). Many of these observations derived from cardiac safety analyses in oncology trials where patients were typically younger and pre-screened to exclude those with pre-existing cardiovascular disease, thereby preselected as more resistant to cardiotoxicity from that class of drugs. Cardiotoxicity rates tend to be higher in clinical practice compared with those reported in oncology trials (Farolfi *et al.*, 2013), reversibility is less common, and duration of treatment, and therefore dose, may contribute to a cumulative risk.

Early detection of subclinical cardiac dysfunction could lead to the identification, drug-intervention (e.g. ACE inhibitor and β-blocker) and prevention of late adverse cardiac events and this is ultimately the goal for both cardiologists and oncologists. Collection of endomyocardial biopsies to identify histopathological evidence of myofibrillar loss is an inaccurate and impractical form of monitoring heart damage. The use of LVEF as the only parameter of cardiac function is increasingly viewed, by the cardiology community, as an inadequate measure to predict and monitor cardiac damage. Nevertheless, LVEF is measured routinely as 2-D echocardiography (Echo) is the methodology of choice for frequent monitoring and is cost-effective and has fairly widespread availability, despite its known higher method variability. Other screening modalities, such as cardiac MRI, are gaining in popularity, because of low inter-scan variability and ability to offer virtual histology, which is capable of detecting signs of fibrosis when combined with the contrast agent gadolinium (Tandri *et al.*, 2005).

Although there is no clear definition of cardiotoxicity, a practical and easily applicable definition was created by a panel of investigators involved in the clinical development of trastuzumab (Seidman *et al.*, 2002), which considered chemotherapy-induced cardiotoxicity as either a 5% decline from baseline LVEF to less than 55% overall with accompanying signs or symptoms of HF, or asymptomatic decrease in LVEF in the range of equal to or greater than 10% to less than 55%. Other trials have used 50% as the threshold to define cardiotoxicity, but given the potential variability of Echo-based LVEF measurements as discussed earlier, in reality, this depends upon the low limit of normal (LLN) for a healthy population for individual centres, and therefore current guidance is to determine LLN and interpret the guidance using local cut-off values. The extent to which this is practised in the real world has yet to be clarified.

Medical management of anthracycline-induced HF is based largely on the use of agents used to treat HF, including ACE inhibitors, β-blockers and aldosterone antagonists, with loop diuretics for decreasing fluid retention. In a recent study of patients with an anthracycline-induced decrease in LVEF ≤ 45%, treatment with enalapril and carvedilol resulted in normalization of LVEF in 42% of patients (Cardinale *et al.*, 2010). These responders had a higher LVEF after HF treatment compared with partial responders (whose LVEF increased >10%, but did not normalize) and nonresponders who were most resistant to HF treatment and failed to improve ventricular function. A striking observation was that the patients in the responder group all had their 'rescue' HF therapy initiated within 4 months of the chemotherapy, whereas if it was

initiated beyond 4 months then response was considerably less. This is particularly important in light of recent data that indicates that only 31% of patients receiving chemotherapy with an asymptomatic decrease in LVEF receive an ACE inhibitor or angiotensin receptor blocker, 35% receive a β-blocker and 42% are referred for cardiology consultation (Yoon *et al.*, 2010). This emphasizes the importance of appropriate communication between the oncologist and cardiologist and highlights that early detection and treatment of cardiac injury is critical to a successful outcome.

Drug-induced myocardial injury: pathogenesis and manifestations

For preclinical structural cardiotoxicity, in the absence of a clear target-driven liability, the underlying molecular mechanism(s) are rarely known. It is crucial therefore to build an understanding of the pathogenesis of the toxicity, the monitorability and safety margin based on efficacious exposures, all to inform assessment of the potential risk to man. Understanding the temporal progression of the lesion provides valuable information in understanding the pathogenesis. It is also important to consider drug–target relationship, any functional correlates which may be driving the structural changes, the interplay between the cardiac and vascular systems, translational relevance to patients and to recognize that results of a repeat-dose general toxicity study (mainly macroscopic and histological data at the end of the treatment period) provide only a static picture of a process that may be temporally dynamic. The best understanding therefore comes from integrating all relevant pieces of data together to reveal a wider picture. Ultimately, a better understanding of the pathogenesis may help with the development of a risk mitigation strategy to include a biomarker component for clinical use. Although an understanding of pathogenesis can help in the management of liabilities, the identification of defined (and ideally common) molecular mechanisms of structural cardiotoxicity are required to aid in better drug design.

Cardiac cell injury is a continuum (Figure 1) as in many other organs and normally progresses from degeneration, necrosis, responding inflammatory changes and eventually fibrosis, which can be considered the repair process although it does not result in functional contractile tissue. The ultimate impact of cellular injury on myocardial contractile function is highly dependent on the number and distribution of cells involved.

A non-lethal cell injury, generally considered 'degeneration', can be characterized by cytoplasmic vacuolation of cardiac myocytes and may be caused by lipid accumulation, mitochondrial swelling or dilation of sarcoplasmic reticulum. Although a non-lethal injury suggests there is an opportunity for some reversibility of the condition, this can only be viewed in the context of the tissue; the low inherent regenerative capability of the heart suggests that any sort of adaptive response mounted to the injury may become a source of subclinical or occult change in cardiovascular function. This condition may predispose to an impaired ability over time to cope with stressors such as hypertension or treatment with cardiotoxic agents leading potentially to the development of HF.

A lethal injury to the myocardium results in myocellular necrosis characterized by a terminal irreversible stage of cell injury (cardiomyocyte death), loss of membrane integrity and release of cytosolic proteins (potential biomarkers of cardiac injury such as troponin), in which the adverse morphological change is temporally progressive and includes an inflammatory cell infiltrate and regions of myocardium replaced by fibrosis. Extensive fibrosis can affect myocardial compliance and contractility and play a direct role in the development of chronic progressive cardiomyopathies. It is important

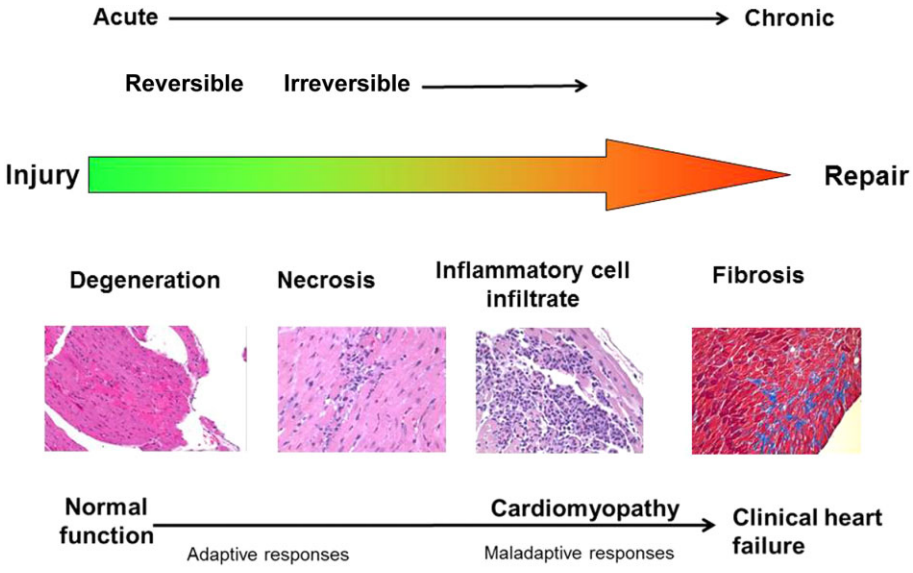

Figure 1
Schematic representation of the morphological continuum of myocardial injury and repair.

however to recognize the difference between extensive regional myocardial necrosis (i.e. infarct) and the multifocal lesions often seen in response to drug-induced injury.

To demonstrate the usefulness of various modalities for characterizing the pathogenesis of cardiovascular injury, Casartelli *et al*. (2011) reported an integrated and longitudinal study to investigate the onset, progression, and reversibility of an off-target cardiac lesion caused by a neurokinin (NK)-1 receptor antagonist (casopitant) in dogs, after long-term (6 months) administration with intermittent data collection. Transmission electron microscopy examination revealed changes in cardiac cells with multi-lamellar bodies in sarcoplasm (associated with a progressive impairment and perturbation of cardiomyocytes) after only 6 weeks of treatment and this coincided with an initial rise in cardiac troponin I (cTnI). After 20–26 weeks, some necrotic myofibres, filled with multi-lamellar bodies, were also observed at a time when light microscopy observations were first made. The most informative picture of cardiac changes was obtained by integrating cTnI alterations (as a biomarker of cardiac damage) with EM findings as these changes preceded evidence of injury at the light microscopic level. Elevations were also seen in N-terminal pro-brain natriuretic peptide (NT-pro BNP), a biomarker for the onset and evolution of cardiac hypertrophy, which started to increase after 2 weeks of treatment, preceding most, if not all, the anatomical and functional (ECG) changes. Thus, the integration of different investigative tools (in addition to the standard regulatory requirement of histopathology) provided early evidence of cardiac cell injury and a means of accurately characterizing the onset and progression of the lesion with a clear translatability to a clinical setting (i.e. cTnI and NT-pro-BNP increases). Early recognition of important liabilities facilitates early decision-making around progression or strategies for mitigating risk in patients. It is however unfeasible to perform EM routinely as part of a high-volume toxicity screening process and measurement of circulating predictable and specific biomarkers remains the goal.

Preclinical cardiotoxicity (by light microscopy) was not apparent during the development studies with the Abelson murine leukemia viral oncogene homologue 1 (c-Ab1), PDGF and stem cell factor receptor (CD117) (c-Kit) inhibitor, imatinib although clinical findings suggestive of decreased LVEF and HF in patients without previous heart disease were reported after launch (Kerkelä *et al*., 2006). Taking a more translational and innovative approach, Kerkelä *et al*. (2006) demonstrated, using transmission EM, that mitochondrial abnormalities and accumulation of membrane whorls in both vacuoles and the sarco-(endo-)plasmic reticulum of human and mouse cardiomyocytes, *in vitro*, were suggestive of the clinical presentation of toxic myopathy. Similarly, the cardiac dysfunction (LVD, LVEF and HF) seen in patients with the multi-targeted receptor tyrosine kinase inhibitor (TKI) sunitinib (Chu *et al*., 2007), was subsequently ascribed, using transmission EM, to depletion of coronary microvascular pericytes resulting in changes such as increased endothelial permeability in the coronary microvasculature. Pericyte loss is not a feature of cardiotoxicity reported by other agents in the TKI class and this observation indicates that injury to non-contractile elements can still progress to cardiomyopathy. These findings also highlight the need for *in vitro* screens, which reconstitute different cellular components to aid in specific liability identification (see section 'Novel human cell-based models to predict cardiac microvascular toxicity').

Although there is currently significant cancer biomarker trial activity, most is related to prognostics and pharmacodynamics with relatively few studies in the area of safety biomarkers. To date, little has been done to assist general practitioners in identifying cardiovascular adverse effects of cancer treatments, although there is a general awareness regarding growing late toxicity from cancer treatment as patient survival increases. Investigators at the Cancer Research UK Manchester Institute (University of Manchester), in collaboration with scientists at AstraZeneca (Alderley Park, UK), have initiated a study to identify predictive biomarkers of safety in rodents in one of the few studies to capture functional change, biomarkers and histology in a longitudinal fashion. Rats showed a significant reduction in LVEF after 43 days during an 8 week continual dosing study with doxorubicin (iv), which continued after cessation of dosing with no evidence of reversibility although the point at which irreversibility occurred was not determined (Cove-Smith *et al*., 2014). Overt histological changes were observed after 29 days dosing although EM changes (mitochondrial damage and myocyte ultrastructural changes) occurred after a single dose to rats. Functional decline (decrease of LVEF and diastolic dysfunction measured by E/A ratio) preceded the rise in cTnI and histological damage (light microscopy). However, despite the incremental decline in systolic function, the LVEF remained above the normal clinical threshold of 55% until the end of study. It is envisaged that a future panel of biomarkers will help determine when cardiac damage presents initially and resolves.

Mechanisms of structural cardiotoxicity

Understanding the mechanisms behind the cardiomyopathies that arise as a result of targeted cancer therapies and developing strategies to treat these complications are important for the cardiovascular care of the cancer patient as well as to enable future development of non-cardiotoxic drugs. Furthermore, an understanding of these cardiomyopathies may also have implications for more common types of HF and may provide unexpected insights into the biology of the heart. After many years of little advance in the understanding of the mechanism of toxicity of anthracyclines, recent novel findings point to a role for topoisomerase II β in inducing DNA damage in cardiomyocytes, mitochondrial dysfunction and generation of reactive oxygen species leading to cardiotoxicity (Zhang *et al*., 2012).

There are currently over 100 TKIs in discovery or development (Broekman *et al*., 2011). Approximately 100 genes have been implicated in driving cancers with ~50 being potential anti-cancer targets and a proportion are also likely to play an important role in cardiomyocyte homeostasis (see Table 2). Off-target toxicity is also a major issue as ATP competitive inhibitors demonstrate significant kinase promiscuity leading to undesirable off-target effects. This lack of

Table 2

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 cardiomypathy 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.

selectivity is not limited to kinases, but includes non-kinase targets, which also bind ATP. This general problem and apparent 'class effect' has been observed for a number of approved kinase inhibitors, as summarized in Table 1. See Force and Kolaja (2011) for a comprehensive review of kinase cardiac biology and potential mechanistic links to cardiotoxicity.

Mechanisms of functional and/or structural cardiotoxicity may fall into several major categories:

(a). Electrophysiological perturbations, mediated via ion channel inhibition (Na, K or Ca channel interactions; e.g. hERG/*KCNH2*) and represents a significant cause of early compound attrition as a result of the implementation of early screening strategies).
(b). Cytotoxicity [molecular inactivation of cell processes, altered energetics, oxidative stress/free radical generation, may be primary (target) or secondary (off-target) pharmacology related].
(c). Primary pharmacology (an undesirable target-mediated activity and a common preclinical and clinical mechanism, e.g. hyper-pharmacology of cardio- and vasoactive drugs).

The workshop focused largely on cytotoxic and pharmacological mechanisms of drug-induced on- and off-target cardiotoxicity as primary mediators of structural injuries. In the pharmaceutical industry, to identify potential safety liabilities early in drug development, initiation of new discovery programmes includes a review of the published target biology information. This enables identification of potential toxicological issues because of primary pharmacology, planning of hypothesis-based experiments to confirm or refute potential issues and generation of toxicological data to support or reject target validity. Secondary pharmacology screening for compound interactions with key cardiovascular homeostatic proteins and receptors is also becoming increasingly important for identifying off-target liabilities associated with a particular compound or series.

Case examples of pharmacological mechanisms (on-target and off-target) of structural cardiotoxicity

On-target

A number of companies have pursued activin receptor-like kinase 5 (ALK5 also known as TGFBR1) as an oncology and fibrosis target. It has previously been shown that the TGF-β superfamily signalling pathways play a key role in cardiac development, and that ablation of ALK5 in the endocardium of mice results in defects in epithelial-mesenchymal transformation and an early stage of cardiac valve formation (Sridurongrit *et al.*, 2008). Nevertheless, a role for ALK5 in the developed heart was poorly understood, but a potential role in cardiac valve homeostasis represented a safety concern. As a result, safety scientists at AstraZeneca (Anderton *et al.*, 2011) undertook an acute (5–8 days) study in rodents using selective inhibitors of ALK5 to assess this potential target liability. Importantly, early ALK5 inhibitors were available from different chemical scaffolds, enabling clear separation of target/off-target effects. A comprehensive evaluation of the heart was performed to assess all four heart valves in each animal. Histopathological heart valve lesions were observed in all animals, in all heart valves and from two distinct chemical series. Valves were distorted with severe haemorrhage, fibrin deposition and neutrophil infiltration and valvular interstitial cells were enlarged with increased cytoplasm. Immunohistochemistry analysis revealed the heart valve, but not the myocardium was positive for ALK5 expression. The compounds were inactive against 5-HT receptors, previously implicated in drug-induced valvulopathy. As a result of these findings, the project was terminated in the discovery phase because of unacceptable target-related toxicity. No safety margin was expected, the lesion was considered to be un-monitorable and there was no defined hypothesis to support humans being different from rodents in respect of the ALK5 inhibitor-mediated pathology. Anecdotally, this experience was shared by at least two other pharmaceutical companies, but not published. The publication by Anderton *et al.* (2011) alerted other organizations working in this area or considering initiating discovery efforts on this target to the safety implications. This represents an excellent example of the benefit of sharing adverse target safety information in order to reduce further animal experimentation, resource and industry attrition, and is a position encouraged greatly by all of the workshop representatives.

Off-target

A safety concern relating to compound promiscuity is that off-target pharmacological activity unrelated to the primary drug action might be associated with adverse cardiac effects. An example is the c-Met inhibitor (PF-04254644), which leads to myocardial pathological changes in rats within 6 h after a single dose, resulting in replacement fibrosis after 7 days (Hu *et al.*, 2012). Myocardial EM changes (necrosis of myofibres, intra-mitochondrial densities and lipid deposition) were detected at very early time points post-dosing (within 2 h). These time points were coincident with peak elevations of serum troponin and an associated functional increase in heart rate and BP within 2–7 h post-dose. As other c-Met inhibitors currently in clinical use are not associated with adverse cardiac effects, it was clear this represented an off-target effect of the compound. Wide ligand binding profiling revealed that PF-04254644 is a potent inhibitor of PDE3 and also 2, 5, 10 and 11 (Aguirre *et al.*, 2010). It is well recognized that inhibition of multiple PDEs leads to increased heart rate, contractility and sheer stress force and may result in secondary myocardial degeneration (Larson *et al.*, 1996). These observations enabled the identification of alternative c-Met inhibitors without off-target PDE activity and the associated cardiovascular liability.

New approaches to the mechanistic understanding of cardio-protection

Dexrazoxane is the only clinically approved cardioprotective agent used to reduce the cardiotoxicity associated with anthracyclines such doxorubicin (Lipshultz *et al.*, 2004). The

cardioprotective effect was initially thought to be due to its ability to chelate iron and reduce the number of metal ions complexed with anthracycline, leading to decreased formation of superoxide radicals (Sterba *et al.*, 2013). However, more recent data suggest that dexrazoxane antagonizes doxorubicin-induced DNA damage through interference with topoisomerase II β (Lyu *et al.*, 2007; Ky *et al.*, 2013). Despite its cardio-protective effect, there is persistent concern that dexrazoxane may cause myelosuppression, reduce the anti-cancer effectiveness of anthracyclines and promote secondary malignancies in patients. Both the latter concerns were addressed and refuted by randomized clinical trials and their meta-analyses (Jones, 2008). A variety of different iron-chelators and antioxidants, such as vitamin E and N-acetylcysteine, have been studied in animal models and clinical trials. However, these agents failed to provide cardioprotection against anthracycline chemotherapy (Sterba *et al.*, 2013).

Cardioprotective/pro-survival mechanisms exist in the heart [e.g. ErbB2 (Her2)-induced pro-survival signalling] (Force and Wang, 2013) and the blockade of this receptor on cardiomyocytes by trastuzumab (Herceptin) may explain in part the cardio-toxicity of this agent. Neuregulin-1β is an agonist at the ErbB2 receptor and has been shown to offer cardio-protective effects (Fukazawa *et al.*, 2003) as a result of activation of cell survival pathways in cardiomyocytes. However, there would clearly be issues in successful management of the concomitant administration of a neuregulin-1β receptor agonist and trastuzumab in order to achieve maximum therapeutic value with minimal adverse effects. This paradox has recently been addressed by using a modified bivalent neuregulin-1β ligand, which promotes cardioprotection, via ErbB4 homodimers, but minimizes proneoplastic potential in cancer cells (Jay *et al.*, 2013). Recent preclinical studies have also determined a cardio-protective role of Cdk 4/6 inhibitors in anthracycline-induced cardiotoxicity suggesting a new potential treatment option (McClendon *et al.*, 2012). The area of cardio-protection is currently under-served and there is an urgent need for better protection strategies in order to allow maximally effective therapies to be administered with minimized risk of cardiotoxicity.

Predicting structural effects from functional changes

A UK pharmaceutical consortium in collaboration with ABPI carried out an analysis of single-dose Good Laboratory Practice (GLP) safety pharmacology (cardiovascular function; heart rate, BP and QT-interval) data from telemetered dogs and 28 day repeat-dose toxicology (morphology) data from rodents in an attempt to understand if there are any trends in the acute study that predict the longer-term outcome (Milliken *et al.*, 2012). Relationships between these data sets has been largely undetermined and there is increasing interest in utilizing data such as this for predictive associations, for earlier detection of compounds with risk for cardiovascular pathologies. One hundred thirty-five compounds (all low molecular weight) were included in the data set and when normalized for C_{max} exposure concordance the number of compounds for inclusion in the data set was 126. Cardiovascular histopathology changes were seen in 15 compounds of which the highest incidence of pathology (eight compounds) occurred in the myocardium and presented with degenerative and/or inflammatory events. Six of the eight compounds with pathologies showed a peak increase >40 bpm and duration ≥5 h. There were no instances of vessel pathology without a change in systemic BP and if compounds showed no change in HR, there was a high degree of assurance that there would be no morphology findings (at least up to the time of investigational new drug enabling studies in the same species). This initial analysis reinforces a growing general impression that cardiovascular injury has a haemodynamic driver element and suggests that functional changes observed in acute single-dose studies could prompt moving (sub) chronic toxicology studies forward, to identify cardiovascular liabilities earlier in development. These findings suggest it may be worth including more end points in future toxicity studies (e.g. telemetered animals and/or pathology readouts in safety pharmacology studies), although issues in translatability need addressing given that an ABPI-sponsored Animal Model Framework (Valentin *et al.*, 2009) analysis showed a weak relationship between dogs and humans in terms of haemodynamic changes. In general, dogs are hypersensitive for haemodynamic changes and rodents appear hypersensitive for vascular injury-raising issues of comparative physiology/pathophysiology. In terms of future studies, it would be valuable to assess the panel of compounds used in this analysis in relevant *in vitro* pharmacodynamic screens (e.g. rodent, dog, human cardiomyocytes) to understand better the role of functional changes (cause or effect) in structural cardiovascular toxicity.

Better predictability in man

Although LVEF is an important prognostic factor in dilated cardiomyopathy, effective risk stratification remains challenging, particularly with respect to sudden cardiac death (SCD) as most patients who experience SCD do not have severely reduced LVEF, and many patients with significant impairment of LVEF may still be at low risk for SCD (Dagres and Hindricks, 2013). Identification of better independent prognostic factors is necessary to enable clinicians to more accurately stratify risk in patients with dilated cardiomyopathy and to implement early (preventative) medical management. Measurement of cardio-specific biomarkers represents a valid diagnostic approach for early identification, assessment and monitoring of cardiotoxicity. Cardinale *et al.* (2004) showed that early detectable levels of circulating cTnI after high-dose anthracycline chemotherapy predicted both occurrence and severity of LVEF impairment. A higher rate of major cardiac events (and morbidity) within the first year of follow-up was also noted in patients who demonstrated elevated levels of cTnI for more than a month after the last chemotherapy administration. Equally important, they reported a high negative predictive value for cTnI (99%), which identified low-risk patients who would most likely not encounter subsequent cardiac complications. Cardinale *et al.* (2010) also reported an elevation in cTnI in 72% of patients who subsequently developed trastuzumab-induced cardiotoxicity compared

with only 7% of patients who showed normal cTnI. A number of studies suggest that cTnI determination is able to predict the occurrence of clinically significant LVD, at least 3 months in advance (Cardinale *et al.*, 2010; Sawaya *et al.*, 2012).

An ongoing biomarker study at the Cancer Research UK Manchester Institute (University of Manchester) in a cohort of 30 patients with lymphoma and breast cancer, treated with anthrayclines, is measuring both circulating (cTnI, ILs, TNF, fatty-acid-binding protein, myeloperoxidase, MMPs, NT-proBNP) and imaging (cardiac MRI sequences including volumetric analysis, T1 mapping, T2 mapping, late gadolinium enhancement and myocardial strain) biomarkers of cardiotoxicity in an attempt to correlate biomarkers with clinical data. Although the data are immature, it has shown that LVEF steadily decreases over the time course; however, no circulating biomarker data are available yet (Cove-Smith *et al.*, 2014).

In summary, strategies are urgently required to detect cardiotoxicity in patients by focusing on pathways common to all or many cardiotoxicities. This in turn would be expected to lead to the identification of novel biomarkers, allowing earlier detection and potential intervention. Novel potential robust approaches to address this issue include measurement of circulating miRNAs (De Rosa *et al.*, 2014) and the use of metabolomics to identify metabolic signatures in the circulation which are indicative of injury. These may help to detect very early changes of cardiotoxicity (Roberts *et al.*, 2012).

New clinical directions

A cardio-oncology day-case assessment service at the Royal Brompton Hospital in London has been established recently, acting as a 'one-stop shop', in which serum biomarkers, resting and stress echocardiography, cardiac MRI, research (e.g. genomics), and a clinical review are carried out at baseline and during the treatment phase in patients at risk or who have potential cardiotoxicity detected on current surveillance strategies. Given the limitations in the sensitivity of biomarker (particularly Echo) strategies without appropriate baseline measurements, this approach offers a means of monitoring any changes as they occur and with a higher degree of certainty. A key element of this approach is a close working relationship between cardiology and oncology colleagues, to balance the risks and benefits of the cancer treatment with any cardiotoxicity detected. It is critical to remember that this increasing problem of cardiotoxicity has arisen out of the remarkable success of modern cancer treatment, and it would be paradoxical and clinically inappropriate to withhold potentially life-saving cancer treatments because of the detection of subclinical cardiotoxicity using more and more sensitive biomarkers and imaging technologies. Instead the philosophy of early detection is to stratify cardioprotection to those in need, and equally to reassure those without evidence of cardiac injury, given the negative predictive value is the most powerful. This initiative has also led to the development of a UK-wide consortium of seven cardiac and eight oncology centres that offers coordinated assessment of patients and follows efforts in other European countries and North America to better coordinate cardio-oncology care.

Preclinical considerations – bridging back to man

A key challenge for the pharmaceutical industry is to not only detect (or ideally predict) preclinical drug-induced changes in cardiac structure or function, but also to understand the relevance of these cardiotoxic effects to humans and, more specifically, the potential impact within the clinical setting for which the drug is intended. In order to avoid cardiotoxicity emerging at the later stages in the drug development pathway, it is vital that the potential for structural cardiovascular toxicity, associated with either a target or a molecule, can be identified as early as possible. Failure to do so results in drug attrition, limits the availability of new treatment options for patients and is extremely costly financially. The cardiac electrophysiological effects of a molecule can be detected routinely through a combination of *in silico*, *in vitro* approaches (utilizing cell lines overexpressing certain ion channels) and supported via short-duration *in vivo* studies. However, the assessment of structural cardiovascular toxicity currently involves longer-term repeat-dose *in vivo* toxicity models (which are cost and labour intensive) primarily focussed on histopathological end points. In most chronic toxicology studies (e.g. GLP regulatory studies), left ventricular function, electrocardiogram and biomarkers of cardiac injury are not routinely measured (Mellor *et al.*, 2011). It is clear that the area of structural cardiotoxicity could also benefit enormously from a more predictive and integrated tiered approach (e.g. *in silico/in vitro/in vivo*).

Novel *in vivo* models of structural cardiotoxicity

One *in vivo* approach to providing better strategies for predicting structural cardiotoxicity involves the measurement of cardiomyocyte loss after drug treatment in zebrafish (Cheng *et al.*, 2011). The most common gross morphological defect induced by cardiotoxic drugs was an associated swelling of the heart chambers. If the ventricle stops beating, blood often pools and clots in the chamber, but this does not immediately kill the fish as there is sufficient oxygenation acquired through passive diffusion. Rates of pericardial oedema are noted, as well as the presence of thrombi and their location. Most thrombi occur at the yolk sac just posterior to the entrance to the atrium. Drugs known to have adverse cardiotoxic effects in humans also demonstrate cardiotoxicity in zebrafish. Because zebrafish can survive in the absence of cardiac output and in the presence of major vascular defects for several days, abnormalities can be studied that would be rapidly fatal in mammals. In this model, sorafenib was shown to reduce the numbers of cardiomyocytes (Cheng *et al.*, 2011) and both sorafenib and sunitinib caused a marked reduction in total myocyte number per heart, contractile dysfunction and ventricular dilatation.

Novel human cell-based models to predict structural cardiotoxicity

Currently, *in vitro* screening strategies are predominately focused on identifying functional cardiotoxicity through the detection of ECG abnormalities and QT-interval prolongation by ion channel screening and measurement of cardiac action potentials and changes in Echo through cardiomyocyte contractility abnormalities (Pollard *et al.*, 2010; Harmer *et al.*, 2012). Predictive *in vitro* assays to detect structural cardiac toxicity in man have been lacking. This has mainly been due to the apparent complexity and diversity of the underlying mechanisms and resulting pathologies and a lack of reproducible human cardiomyocyte cells. Recent advances in the development and production of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) has facilitated the development of human *in vitro* cell models of cardiotoxicity (Jonsson *et al.*, 2009). Pointon *et al.* (2013) utilized hESC-CMs and the rat myoblastic H9c2 cell line to phenotypically profile a panel of structural cardiotoxins (34 clinical and AstraZeneca internal drug candidates with known *in vivo* structural cardiotoxic liabilities and 32 clinical and AstraZeneca internal drug candidate non-structural cardiotoxins). High content screening was carried out using live-cell fluorescent imaging of mitochondrial membrane potential, endoplasmic reticulum integrity, Ca^{2+} mobilization, and membrane permeability combined with an assessment of cell viability, via ATP content. Results showed that the hESC-CM screen shows greater sensitivity and specificity compared with the immortalized rat cardiomyocyte cell line (H9C2). Data suggested that hESC-CMs in combination with imaging parameters and assessment of cell viability were able to predict the *in vivo* (animal) outcome (structural cardiotoxicity) with an overall sensitivity and specificity of 74%. Importantly, this study also demonstrated that a beating phenotype is important for the development of structural toxicity, highlighting the intricate relationship between cardiac functional and structural toxicity.

The challenge for *in vitro* cell models with acute drug treatment is the direct predictability of the chronic forms of toxicity, which may take years to manifest in patients because of cumulative dosing with drugs such as anthracyclines. The development of 3-D stem cell-based cardiac microtissues/spheroids may offer the potential for repeated drug dosing and cumulative effects with more predictive chronic toxicity potential (Thavandiran *et al.*, 2013).

Novel human cell-based models to predict cardiac microvascular toxicity

The cardiac myocardium is composed of cardiomyocytes, which constitute approximately 30% of the total cells, and non-myocytes (fibroblasts, endothelial), which constitute approximately 70% of the total cells (Brutsaert, 2003). Cardiomyocytes generate the contractile force while fibroblasts secrete extracellular matrix and paracrine factors. Endothelial cells line the coronary vasculature and allow delivery, via the bloodstream, of the free fatty acids and oxygen required to meet the high metabolic demands of the contractile myocytes (Brutsaert, 2003; Tirziu *et al.*, 2010). Endothelial cells also form a barrier to thrombus formation and regulate the adherence of immune cells. There is a growing awareness that cardiotoxic anti-cancer drugs can also adversely affect cardiac vascular function. Tubulin binding drugs, such as vincristine, have been shown to adversely affect rat cardiac microvascular endothelial cells (Mikaelian *et al.*, 2010) while doxorubicin has recently been shown to affect VEGF signalling in rat cardiac microvascular endothelial cells (Chiusa *et al.*, 2012). Recent data have shown that sunitinib (Sutent), which targets a range of different receptor tyrosine kinases [VEGF receptors, Kit, PDGF receptors and Fms-like tyrosine kinase-3 (FLT3)] can adversely affect cardiac pericytes resulting in cardiac toxicity (Chintalgattu *et al.*, 2013). Collectively, these data show that drug-induced cardiac toxicity can have a multicellular component. This has profound implications for the development of *in vitro* preclinical cardiovascular toxicity screens within the pharmaceutical industry, which are currently focused solely on detecting adverse effects in cardiomyocytes. There is a need for multicellular models that reconstitute cardiac physiology to allow researchers to simultaneously investigate structural changes and functional changes in different cardiac cell types.

Conclusions and recommendations

Although significant progress has been made in better understanding the pathogenesis and mechanisms of structural cardiotoxicity caused by a number of novel therapeutics, the ultimate goal is to identify more comprehensive *in vivo* screens and, in particular, *in vitro* assays, which are reliable predictors of clinical cardiotoxicity, as achieved with implementation of hERG-centred screening to identify risks of drug-induced QT-interval prolongation. Efforts to address the following issues are encouraged and supported:

(a). Standard histopathological (light microscopic) assessment may not identify early changes in cardiovascular morphology and without the aid of additional, sensitive, evaluative tools such as transmission EM or measurement of appropriate early biomarkers (Casartelli *et al.*, 2011), longer-term studies (with associated patient risks, increased costs and lengthening of project timelines) may be required to determine any liabilities. Although impractical to propose routine EM in early toxicity studies, there have been encouraging signs of early detection of cardiac toxicities using translatable biomarkers (e.g. cTnI) and further research in this area is expected to provide significant benefits. More studies are required also on mechanistic understandings and functional correlates that can act as better translational biomarkers (e.g. serological, functional, histopathological, imaging). An industry consortia approach has demonstrated trends towards a relationship between functional changes in acute, single-dose studies in dogs and cardiovascular pathologies seen in (28 day) repeat-dose toxicology studies. Cell-based studies using cardiomyocytes (Pointon *et al.*, 2013) have shed light also on relationships between a beating phenotype and mitochondrial

toxicity and calcium disruption as common mechanisms for structural toxicity.

(b). The identification and prediction of drug-induced structural cardiotoxicity in patients is complicated by the fact that in many cases where the left ventricle is mechanically disadvantaged, there may be no clinically apparent manifestations for many years because of an optimization of function by compensatory mechanisms. Thus, better independent prognostic factors are needed to enable clinicians to more accurately stratify risk in patients with cardiomyopathy and to implement early preventative drug treatment. Although there have been major advances in the area of functional assessment clinically, it is currently widely accepted that change of function is preceded by damage to the myocardium, which can be detected by biomarkers thus allowing a more rapid and cheaper way to monitor patients on a regular basis. Indeed, a number of studies suggest that cTnI determination is able to predict the occurrence of a clinically significant LVD, at least 3 months in advance (Cardinale *et al.*, 2010; Sawaya *et al.*, 2012). As it is now becoming apparent from ultrastructural assessment that toxicities can affect a range of different cardiac cell types, it is important also therefore to identify early markers which help determine the specific liability in patients.

(c). The choice of translatable animal models (and *in vitro* screens) remains highly challenging as toxicities in patients are 'personalized' as a result of a specific predisposition brought about by specific prior drug treatments or pre-existing disease(s). The use of young, healthy and drug-naïve animals for toxicity studies likely reflects poorly in terms of translatability to patients whereas animals carrying a background disease, are stressed by prior drug treatment, or are genetically modified, may reflect better the clinical condition. However, differentiating toxicity from natural worsening of disease can be a significant challenge in both preclinical and clinical settings. A key challenge is to know which 'compromised' animal models to apply and in which disease and therapeutic settings. Successful case studies and further research is required to inform use of these models and to better understand the comparative cardiovascular physiology/pathophysiology to help address preclinical species to human translatability. It is inappropriate to consider rodent models as truly predictive and they should be regarded as tools to detect putative liabilities; nevertheless, many compounds have been discarded because of rodent toxicity findings, which may or may not have translated to clinical adverse effects. Although standard preclinical safety studies have prevented any phase 1 catastrophes from occurring they have not yielded much insight into later stage toxicities.

(d). No predictive *in vitro* approaches to detect structural cardiovascular toxicity have been described, in part because of the challenge of recreating complex cardiovascular physiology in an isolated set-up, as well as an incomplete understanding of the mechanisms of toxicity to decide the best end points. Promising findings have been published recently, however, by Pointon *et al*. (2013) using hESC-CMs to phenotypically profile a panel of structural and non-structural cardiotoxins. There is a need, however, to generate cell models that reconstitute multicellular cardiac physiology more accurately and co-culture of cardiomyocytes, cardiac fibroblasts and cardiac endothelial cells may achieve this goal. A well-characterized set of structural cardiotoxins is now required by the scientific community to help compare and contrast the different *in vitro* and *in vivo* models (and species). An extensive and rigorous comparative study to determine if agents that are cardiotoxic after repeat dosing in rodents and dogs also elicit changes in human (as well as rodent/dog) isolated or co-cultured cardiomyocytes would help the identification of more predictive models and may lead to the development of a template for future screening strategies and a risk assessment plan.

(e). Pre-competitive data sharing on targets, which are intrinsically associated with toxicity, is considered a key goal in order to avoid potentially costly and wasted efforts by the industry. The publication by AstraZeneca scientists (Anderton *et al.*, 2011) showing that inhibiting a potential oncology target (ALK5) results in histopathological heart valve lesions in rodents benefited a significant number of companies who were already engaged in similar programmes or were considering starting chemical efforts on the target. We encourage industry to follow the lines taken by AstraZeneca and publish their findings on target toxicities at earliest possible opportunities to reduce needless animal usage and for broader scientific benefit.

(f). A strategy to mitigate the risks of cardiotoxicity in patients is the development of cardio-protective agents for co-administration. Currently, the only approved clinical cardio-protective drug is dexazoxane, targeting anthracycline-induced cardiotoxicity. New approaches based on different mechanisms (e.g. neuregulin-1 and cdk 4/6 inhibitors) are currently under early investigation. This area is ripe for further development of more effective agents and ultimately will allow maximally effective therapies to be administered with minimized risk of cardiotoxicity.

(g). Cardio-oncology patients represent a unique cohort as the timing of the insult is known and there is some understanding of the mechanism of insult induced by the drug. Much can be learned from this population, which may be applicable to heart disease from other causes. However, closer interactions between cardiologists and oncologists, along the lines of the newly established UK cardio-oncology services and networks are encouraged to better address the issue of early assessment/identification. Greater interaction is required also between preclinical and clinical safety scientists to ensure that any gaps occurring in development of new agents are quickly identified. A more iterative approach is developing within the industry such that any clinical findings trigger preclinical modelling to better understand the liability and its mechanism. Equally, if a cardiovascular safety signal is seen preclinically it is essential that this is followed with special attention in the clinic and a strategy is implemented for clinical monitoring.

Conflict of interest

Some authors of this paper are employed in the pharmaceutical industry or serve as consultants to the pharmaceutical industry. However, the subjects presented in the paper do not advocate or support purchase of any of the products offered by the respective organization.

References

Aguirre SA, Heyen JR, Collette W, Bobrowski W, Blasi ER (2010). Cardiovascular effects in rats following exposure to a receptor tyrosine kinase inhibitor. Toxicol Pathol 38: 416–428.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, McGrath JC *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: Overview. Br J Pharmacol 170: 1449–1458.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013c). The Concise Guide to PHARMACOLOGY 2013/14: Ion channels. Br J Pharmacol 170: 1607–1651.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013d). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic receptors. Br J Pharmacol 170: 1676–1705.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013e). The Concise Guide to PHARMACOLOGY 2013/14: Enzymes. Br J Pharmacol 170: 1797–1867.

Anderton MJ, Mellor HR, Bell A, Sadler C, Pass M, Powell S *et al.* (2011). Induction of heart valve lesions by small-molecule ALK5 inhibitors. Toxicol Pathol 39: 916–924.

Bello CL, Mulay M, Huang X, Patyna S, Dinolfo M, Levine S *et al.* (2009). Electrocardiographic characterization of the QTc interval in patients with advanced solid tumors: pharmacokinetic–pharmacodynamic evaluation of sunitinib. Clin Cancer Res 15: 7045–7052.

Berardi R, Caramanti M, Savini A, Chiorrini S, Pierantoni C, Onofri A *et al.* (2013). State of the art for cardiotoxicity due to chemotherapy and to targeted therapies: a literature review. Crit Rev Oncol Hematol 88: 75–86.

Billingham ME, Mason JW, Bristow MR, Daniels JR (1978). Anthracycline cardiomyopathy monitored by morphologic changes. Cancer Treat Rep 62: 865–872.

Boivin F, Hutchison GB, Lubin JH, Mauch P (1992). Coronary artery disease mortality in patients treated for Hodgkin's disease. Cancer 69: 1241–1247.

Brave M, Goodman V, Kaminskas E, Farrell A, Timmer W, Pope S *et al.* (2008). Sprycel for chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia resistant to or intolerant of imatinib mesylate. Clin Cancer Res 14: 352–359.

Braz JC, Bueno OF, Liang Q, Wilkins BJ, Dai YS, Parsons S (2003). Targeted inhibition of p38 MAPK promotes hypertrophic cardiomyopathy through upregulation of calcineurin-NFAT signaling. J Clin Invest 111: 1475–1486.

Broekman F, Giovannetti E, Peters GJ (2011). Tyrosine kinase inhibitors: multi-targeted or single-targeted? World J Clin Oncol 2: 80–93.

Brutsaert DL (2003). Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. Physiol Rev 83: 59–115.

Cardinale D, Sandri MT, Colombo A, Colombo N, Boeri M, Lamantia G *et al.* (2004). Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation 109: 2749–2754.

Cardinale D, Colombo A, Lamantia G, Colombo N, Civelli M, De Giacomi G *et al.* (2010). Anthracycline-induced cardiomyopathy – clinical relevance and response to pharmacologic therapy. J Am Coll Cardiol 55: 213–220.

Casartelli A, Lanzoni A, Comelli R, Crivellente F, Defazio R, Dorigatti R *et al.* (2011). A novel and integrated approach for the identification and characterization of drug-induced cardiac toxicity in the dog. Toxicol Pathol 39: 361–371.

Chen XL, Lei YH, Liu CF, Yang QF, Zuo PY, Liu CY *et al.* (2013). Angiogenesis inhibitor bevacizumab increases the risk of ischemic heart disease associated with chemotherapy: a meta-analysis. PLoS ONE 8: e66721.

Cheng H, Kari G, Dicker AP, Rodeck U, Koch WJ, Force T (2011). A novel preclinical strategy for identifying cardiotoxic kinase inhibitors and mechanisms of cardiotoxicity. Circ Res 109: 1401–1409.

Chintalgattu V, Rees ML, Culver JC, Goel A, Jiffar T, Zhang J *et al.* (2013). Coronary microvascular pericytes are the cellular target of sunitinib malate-induced cardiotoxicity. Sci Transl Med 5: 187ra69.

Chiusa M, Hool SL, Truetsch P, Djafarzadeh S, Jakob SM, Seifriz F *et al.* (2012). Cancer therapy modulates VEGF signaling and viability in adult rat cardiac microvascular endothelial cells and cardiomyocytes. J Mol Cell Cardiol 52: 1164–1175.

Choueiri TK, Schutz FAB, Je Y, Rosenberg JE, Bellmunt J (2010). Risk of arterial thromboembolic events with sunitinib and sorafenib: a systematic review and meta-analysis of clinical trials. J Clin Oncol 28: 2280–2285.

Choueiri TK, Mayer EL, Je Y, Rosenberg JE, Rosenberg JE, Nguyen PL *et al.* (2011). Congestive heart failure risk in patients with breast cancer treated with bevacizumab. J Clin Oncol 29: 632–638.

Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L *et al.* (2007). Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet 370: 2011–2019.

Cove-Smith L, Woodhouse N, Hargreaves A, Kirk J, Smith S, Price SA *et al.* (2014). An integrated characterisation of serological, pathological and functional events in doxorubicin-induced cardiotoxicity. Toxicol Sci 140: 3–15.

Crackower MA, Oudit GY, Kozieradzki I, Sarao R, Sun H, Sasaki T *et al.* (2002). Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. Cell 110: 737–749.

Crone SA, Zhao YY, Fan L, Gu Y, Minamisawa S, Liu Y *et al.* (2002). ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med 8: 459–465.

Dagres N, Hindricks G (2013). Risk stratification after myocardial infarction: is left ventricular ejection fraction enough to prevent sudden cardiac death. Eur Heart J 34: 1964–1971.

De Rosa S, Curcio A, Indolfi C (2014). Emerging role of microRNAs in cardiovascular diseases. Circ J 78: 567–575.

Dolci A, Dominici R, Cardinale D, Sandri MT, Panteghini M (2008). Biochemical markers for prediction of chemotherapy-induced cardiotoxicity. Am J Clin Pathol 130: 688–695.

Dzeja PP, Bast P, Pucar D, Wieringa B, Terzic A (2007). Defective metabolic signaling in adenylate kinase AK1 gene knock-out hearts compromises post-ischemic coronary reflow. J Biol Chem 282: 31366–31372.

Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M *et al.* (2009). Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J Clin Oncol 27: 3312–3318.

Ewer MS, Ewer SM (2010). Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. Nat Rev Cardiol 7: 564–575.

Ewer MS, Lippman SM (2005). Type II chemotherapy-related cardiac dysfunction: time to recognize a new entity. J Clin Oncol 23: 2900–2902.

Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N *et al.* (2006). Safety, pharmacokinetic and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol 24: 25–35.

Farolfi A, Melegari E, Aquilina M, Scarpi E, Ibrahim T, Maltoni R *et al.* (2013). Trastuzumab-induced cardiotoxicity in early breast cancer patients: a retrospective study of possible risk and protective factors. Heart 99: 634–639.

Force T, Kolaja KL (2011). Cardiotoxicity of kinase inhibitors: the prediction and translation of preclinical models to clinical outcomes. Nat Rev Drug Discov 10: 111–126.

Force T, Wang Y (2013). Mechanism-based engineering against anthracycline cardiotoxicity. Circulation 128: 98–100.

Fukazawa R, Miller TA, Kuramochi Y, Frantza S, Kima Y-D, Marchionnic MA *et al.* (2003). Neuregulin-1 protects venricular myocytes from anthracycline-induced apoptosis via erbB4-dependent activation of PI3-kinase/Akt. J Mol Cell Cardiol 35: 1473–1479.

Harmer AR, Abi-Gerges N, Morton MJ, Pullen GF, Valentin JP, Pollard CE (2012). Validation of an *in vitro* contractility assay using canine ventricular myocytes. Toxicol Appl Pharmacol 260: 162–172.

Hooning MJ, Botma A, Aleman BMP, Baaijens MHA, Bartelink H, Klijn JGM *et al.* (2007). Long-term risk of cardiovascular disease in 10-year survivors of breast cancer. J Natl Cancer Inst 99: 365–375.

Hu W, Hirakawa B, Jessen B, Lee M, Aguirre S (2012). A tyrosine kinase inhibitor-induced myocardial degeneration in rats through off-target phosphodiesterase inhibition. J Appl Toxicol 32: 1008–1020.

Ito K, Akazawa H, Tamagawa M, Furukawa K, Ogawa W, Yasuda N *et al.* (2009). PDK1 coordinates survival pathways and beta-adrenergic response in the heart. Proc Natl Acad Sci U S A 106: 8689–8694.

Jay SM, Murthy AC, Hawkins JF, Wortzel JR, Steinhauser ML, Alvarez LM *et al.* (2013). An engineered bivalent neuregulin protects against doxorubicin-induced cardiotoxicity with reduced proneoplastic potential. Circulation 128: 152–161.

Jones RL (2008). Utility of dexrazoxane for the reduction of anthracycline-induced cardiotoxicity. Expert Rev Cardiovasc Ther 6: 1311–1317.

Jonsson MKB, van Veen TAB, Goumans MJ, Vos MA, Duker G, Sartipy P *et al.* (2009). Improvement of cardiac efficacy and safety models in drug discovery by the use of stem cell-derived cardiomyocytes. Expert Opin Drug Discov 4: 357–372.

Kantarjian HM, Giles F, Gattermann N, Bhalla K, Alimena G, Palandri F *et al.* (2007). Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood 110: 3540–3546.

Kerkelä R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C *et al.* (2006). Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. Nat Med 12: 908–916.

Kimura TE, Jin J, Zi M, Prehar S, Liu W, Oceandy D *et al.* (2010). Targeted deletion of the extracellular signal-regulated protein kinase 5 attenuates hypertrophic response and promotes pressure overload-induced apoptosis in the heart. Circ Res 106: 961–970.

Kontaridis MI, Yang W, Bence KK, Cullen D, Wang B, Bodyak N *et al.* (2008). Deletion of Ptpn11 (Shp2) in cardiomyocytes causes dilated cardiomyopathy via effects on the extracellular signal-regulated kinase/mitogen-activated protein kinase and RhoA signaling pathways. Circulation 117: 1423–1435.

Ky B, Vejpongsa P, Yeh ET, Force T, Moslehi JJ (2013). Emerging paradigms in cardiomyopathies associated with cancer therapies. Circ Res 113: 754–764.

Larson JL, Pino MV, Geiger LE, Simeone CR (1996). The toxicity of repeated exposures to rolipram, a type IV phosphodiesterase inhibitor in rats. Pharmacol Toxicol 78: 44–49.

Laverty HG, Benson C, Cartwright E, Cross M, Garland C, Hammond T *et al.* (2011). How can we improve our understanding of cardiovascular safety liabilities to develop safer medicines? Br J Pharmacol 163: 675–693.

Lipshultz SE, Rifai N, Dalton VM, Levy DE, Silverman LB, Lipsitz SR *et al.* (2004). The effect of dexrazoxane on myocardial injury in doxorubicin-treated children with acute lymphoblastic leukemia. N Engl J Med 351: 145–153.

Lyu YL, Kerrigan JE, Lin CP, Azarova AM, Tsai YC, Ban Y *et al.* (2007). Topoisomerase IIbeta mediated DNA double-strand breaks: implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. Cancer Res 67: 8839–8846.

McClendon AK, Dean JL, Rivadeneira DB, Yu JE, Reed CA, Gao E *et al.* (2012). CDK4/6 inhibition antagonizes the cytotoxic response to anthracycline therapy. Cell Cycle 11: 2747–2755.

Mellor HR, Bell AR, Valentin JP, Roberts RRA (2011). Cardiotoxicity associated with targeting kinase pathways in cancer. Toxicol Sci 120: 14–32.

Mikaelian I, Buness A, de Vera-Mudry MC, Kanwal C, Coluccio D, Rasmussen E *et al.* (2010). Primary endothelial damage is the mechanism of cardiotoxicity of tubulin-binding drugs. Toxicol Sci 117: 144–151.

Milliken P, Aylott M, Clements A, Edmunds N, Engle S, Ewart L *et al.* (2012). A cross-company initiative assessing relationships between cardiovascular functional measurements and repeat-dose cardiovascular toxicity. Toxicologist 126: 491. 51st Annual Meeting of the Society of Toxicology. Poster #2275.

Montani D, Bergot E, Günther S, Savale L, Bergeron A, Bourdin A *et al.* (2012). Pulmonary arterial hypertension in patients treated by dasatinib. Circulation 125: 2128–2137.

Mora A, Davies AM, Bertrand L, Sharif I, Budas GR, Jovanović S *et al.* (2003). Deficiency of PDK1 in cardiac muscle results in heart failure and increased sensitivity to hypoxia. EMBO J 22: 4666–4676.

Motzer RJ, Hutson TE, Cella D, Reeves J, Hawkins R, Guo J *et al.* (2013). Pazopanib versus sunitinib in metastatic renal-cell carcinoma. N Engl J Med 369: 722–731.

Muraski JA, Fischer KM, Wu W, Cottage CT, Quijada P, Mason M *et al.* (2008). Pim-1 kinase antagonizes aspects of myocardial hypertrophy and compensation to pathological pressure overload. Proc Natl Acad Sci U S A 105: 13889–13894.

Naib T, Steingart RM, Chen CL (2011). Sorafenib-associated multivessel coronary artery vasospasm. Herz 36: 348–351.

Oudit GY, Kassiri Z, Zhou J, Liu QC, Liu PP, Backx PH *et al.* (2008). Loss of PTEN attenuates the development of pathological hypertrophy and heart failure in response to biomechanical stress. Cardiovasc Res 78: 505–514.

Ozcelik C, Erdmann B, Pilz B, Wettschureck N, Britsch S, Hübner N *et al.* (2002). Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. Proc Natl Acad Sci U S A 99: 8880–8885.

Patnaik JL, Byers T, DiGuiseppi C, Dabelea D, Denberg TD (2011). Cardiovascular disease competes with breast cancer as the leading cause of death for older females diagnosed with breast cancer: a retrospective cohort study. Breast Cancer Res 13: R64.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.*; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl Acids Res 42 (Database Issue): D1098–D1106.

Perez EA, Koehler M, Byrne J, Preston AJ, Rappold E, Ewer MS (2008). Cardiac safety of lapatinib: pooled analysis of 3689 patients enrolled in clinical trials. Mayo Clin Proc 83: 679–686.

Perik PJ, de Korte MA, van Veldhuisen DJ, Gietema JA, Sleijfer DT, de Vries EGE (2007). Cardiotoxicity associated with the use of trastuzumab in breast cancer patients. Expert Rev Anticancer Ther 7: 1763–1771.

Pointon A, Abi-Gerges N, Cross MJ, Sidaway JE (2013). Phenotypic profiling of structural cardiotoxins *in vitro* reveals dependency on multiple mechanisms of toxicity. Toxicol Sci 132: 317–326.

Pollard CE, Abi-Gerges N, Bridgland-Taylor MH, Easter A, Hammond TG, Valentin JP (2010). An introduction to QT interval prolongation and non-clinical approaches to assessing and reducing risk. Br J Pharmacol 159: 12–21.

Princen F, Bard E, Sheikh F, Zhang SS, Wang J, Zago WM *et al.* (2009). Deletion of Shp2 tyrosine phosphatase in muscle leads to dilated cardiomyopathy, insulin resistance, and premature death. Mol Cell Biol 29: 378–388.

Regan CP, Li W, Boucher DM, Spatz S, Su MS, Kuida K (2002). Erk5 null mice display multiple extraembryonic vascular and embryonic cardiovascular defects. Proc Natl Acad Sci U S A 99: 9248–9253.

Roberts LD, Souza AL, Gerszten RE, Clish CB (2012). Targeted metabolomics. Curr Protoc Mol Biol 98: 30.2.1–30.2.24. Chapter 30: Unit 30.2.

Sawaya H, Sebag IA, Plana JC, Januzzi JL, Ky B, Tan TC (2012). Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. Circ Cardiovasc Imaging 5: 596–603.

Scheffel RS, Dora JM, Siqueira DR, Burttet LM, Cerski MR, Maia AL (2013). Toxic cardiomyopathy leading to fatal acute cardiac failure related to vandetanib: a case report with histopathological analysis. Eur J Endocrinol 168: K51–K54.

Schmidinger M, Zielinski CC, Vogl UM, Bojic A, Bojic M, Schukro C *et al.* (2008). Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. J Clin Oncol 26: 5204–5212.

Seidman AJ, Hudis C, Pierri MK, Shak S, Paton V, Ashby M *et al.* (2002). Cardiac dysfunction in the trastuzumab clinical trials experience. J Clin Oncol 20: 1215–1221.

Shakir DK, Rasul KI (2009). Chemotherapy-induced cardiomyopathy: pathogenesis, monitoring and management. J Clin Med Res 1: 8–12.

Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M *et al.* (2011). Adjuvant trastuzumab in HER2-positive breast cancer. N Engl J Med 365: 1273–1283.

Sridurongrit S, Larsson J, Schwartz R, Ruiz-Lozano P, Kaartinen V (2008). Signaling via the TGF-beta type I receptor Alk5 in heart development. Dev Biol 322: 208–218.

Sterba M, Popelova O, Vavrova A, Jirkovsky E, Kovarikova P, Gersl V *et al.* (2013). Oxidative stress, redox signalling and metal chelation in anthracycline cardiotoxicity and pharmacological cardioprotection. Antioxid Redox Signal 18: 899–929.

Stortecky S, Suter TM (2010). Insights into cardiovascular side-effects of modern anticancer therapeutics. Curr Opin Oncol 22: 312–317.

Tandri H, Saranathan M, Rodriguez ER, Martinez C, Bomma C, Nasir K *et al.* (2005). Noninvasive detection of myocardial fibrosis in arrhythmogenic right ventricular cardiomyopathy using delayed-enhancement magnetic resonance imaging. J Am Coll Cardiol 45: 98–103.

Tefferi A (2013). Nilotinib treatment-associated accelerated atherosclerosis: when is the risk justified? Leukemia 27: 1939–1940.

Telli ML, Witteles RM, Fisher GA, Srinivas S (2008). Cardiotoxicity associated with the cancer therapeutic agent sunitinib malate. Ann Oncol 19: 1613–1618.

Thavandiran N, Dubois N, Mikryukov A, Masse S, Beca B, Simmons CA *et al.* (2013). Design and formulation of functional pluripotent stem-cell-derived cardiac microtissues. Proc Natl Acad Sci U S A 110: E4698–E4707.

Tirziu D, Giordano FJ, Simons M (2010). Cell communications in the heart. Circulation 122: 928–937.

Uraizee I, Cheng S, Moslehi J (2011). Reversible cardiomyopathy associated with sunitinib and sorafenib. N Engl J Med 365: 1649–1650.

Valentin J-P, Bialecki R, Ewart L, Hammond T, Leishmann D, Lindgren S *et al.* (2009). A framework to assess the translation of safety pharmacology data to humans. J Pharmacol Toxicol Methods 60: 152–158.

Veronese ML, Mosenkis A, Flaherty KT, Gallagher M, Stevenson JP, Townsend RR *et al.* (2006). Mechanisms of hypertension associated with BAY 43-9006. J Clin Oncol 24: 1363–1369.

Von Hoff DD, Lavard MW, Basa P, Davis HL, Von Hoff AL, Rozencweig M *et al.* (1979). Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91: 710–717.

Weisberg E, Manley PW, Breitenstein W, Brüggen J, Cowan-Jacob SW, Ray A *et al.* (2005). Characterization of AMN107, a selective inhibitor of native and mutant Bcr-Abl. Cancer Cell 7: 129–141.

White DE, Coutu P, Shi YF, Tardif J-C, Nattel S, St Arnaud R *et al.* (2006). Targeted ablation of ILK from the murine heart results in dilated cardiomyopathy and spontaneous heart failure. Genes Dev 20: 2355–2360.

Yamaguchi O, Watanabe T, Nishida K, Kashiwase K, Higuchi Y, Takeda T *et al.* (2004). Cardiac-specific disruption of the c-raf-1 gene induces cardiac dysfunction and apoptosis. J Clin Invest 114: 937–943.

Yoon GJ, Telli ML, Kao DP, Matsuda KY, Carlson RW, Witteles RM (2010). Left ventricular dysfunction in patients receiving cardiotoxic cancer therapies: are clinicians responding optimally? J Am Coll Cardiol 56: 1644–1650.

Zhang DY, Wang Y, Lau CP, Tse HF, Li GR (2008). Both EGFR kinase and Src-related tyrosine kinases regulate human ether-a-go-go-related gene potassium channels. Cell Signal 20: 1815–1821.

Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF *et al*. (2012). Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med 18: 1639–1642.