REVIEW

Cardiotoxicity Associated with Targeting Kinase Pathways in Cancer

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Cardiotoxicity, also referred to as drug-induced cardiac injury, is an issue associated with the use of some small-molecule kinase inhibitors and antibody-based therapies targeting signaling pathways in cancer. Although these drugs have had a major impact on cancer patient survival, data have implicated kinase-targeting agents such as sunitinib, imatinib, trastuzumab, and sorafenib in adversely affecting cardiac function in a subset of treated individuals. In many cases, adverse cardiac events in the clinic were not anticipated based on preclinical safety evaluation of the molecule. In order to support the development of efficacious and safe kinase inhibitors for the treatment of cancer and other indications, new preclinical approaches and screens are required to predict clinical cardiotoxicity. Laboratory investigations into the underlying molecular mechanisms of heart toxicity induced by these molecules have identified potentially common themes including mitochondrial perturbation and modulation of adenosine monophosphate-activated protein kinase activity. Studies characterizing cardiac-specific kinase knockout mouse models have developed our understanding of the homeostatic role of some of these signaling mediators in the heart. Therefore, when considering kinases as potential future targets or when examining secondary pharmacological interactions of novel kinase inhibitors, these models may help to inform us of the potential adverse cardiac effects in the clinic.

Key Words: toxicology; cardiotoxicity; kinase; cancer; oncology.

The Issue—Cardiotoxicity

Cardiotoxicity is a term often used to describe a broad range of adverse effects on heart function induced by therapeutic molecules. This may emerge preclinically in animals during drug discovery and development phases. Alternatively, cardiotoxicity may only become apparent in clinical testing or when the drug has already been licensed for human use. It presents as a defect in cardiac function that may be either symptomatic or asymptomatic. The vital function of the heart is maintained by an integrated and highly coordinated cycle of molecular signaling events. In addition, cardiac muscle tissue is highly energetic, with a particular reliance on aerobic metabolism as a source of ATP. Over the last 10 years, considerable steps forward have been made in our understanding of the role of pharmacological inhibition of cardiac ion channels, such as the human Ether-a-go-go Related Gene (hERG) channel, in the development of cardiac arrhythmias. However, our understanding of how drug molecules can perturb metabolic and signaling pathways, which maintain cardiac energy homeostasis and contractile function, requires further development and prioritization. As a result, the majority of the molecular mechanisms underlying therapy-induced cardiotoxicity observed in preclinical drug development, or which emerge clinically, remain to be elucidated.

The use of certain chemotherapeutics for the treatment of cancer is associated with a risk of cardiovascular complications. The classic example of this concerns the use of anthracyclines such as doxorubicin, commonly prescribed to treat various hematological cancers and tumors of the ovary and breast (Elliott, 2006; Schimmel et al., 2004; Tokarska-Schlattnert et al., 2006). The magnitude of the cardiotoxicity evoked is often, but not always, dose dependent and cumulative, and this varies with the treatment (Ewer and Lippman, 2005). One of the most significant forms of cardiotoxicity is that which affects the myocardium and primarily presents as cardiomyopathy with a decrease in left ventricular (LV) ejection fraction (LVEF). This can progress to heart failure (HF) and in some cases death. In the case of anthracyclines, the patients most at-risk of cardiotoxicity have underlying cardiac dysfunction or a predisposition to cardiac complications (Barrett-Lee et al., 2009; Ryberg et al., 2008).

Where the cancer is incurable and treatment options are severely limited, the improvement in overall survival through treatment with a potentially cardiotoxic agent will frequently outweigh the safety risk. However, where other treatments are available and/or the drug is to be used as an adjuvant, the balance between risk and benefit remains a delicate one. The
emergence of targeted anticancer drugs, notably kinase inhibitors, in recent years has brought new opportunities coupled with new challenges with respect to our understanding and management of cardiotoxicity.

Most “cardiotoxic” kinase inhibitors, e.g., sunitinib (Motzer et al., 2007; Telli et al., 2008), trastuzumab (Seidman et al., 2002), dasatinib (Brave et al., 2008), and sorafenib (Escudier et al., 2009; Llovet et al., 2008), have been granted approval despite adverse cardiac events (e.g., LV dysfunction [LVD], HF, cardiac ischemia, and myocardial infarction) being reported in a subset of patients during clinical trials. This is because these drugs are highly effective in settings where treatment options are limited and reflects the fact that in oncology drug development, where the challenges of achieving anticancer efficacy are so great, cardiotoxicity risk is considered on balance and is not necessarily a regulatory barrier. To compound matters, many approved kinase inhibitors with cardiac liabilities are also associated with adverse effects on other organs, including the liver, kidneys, skin, and gastrointestinal tract.

From the perspective of primary pharmacological effects, a significant overlap is emerging between the signaling pathways being identified (and therapeutically targeted) as crucial regulators of tumor growth and survival and those which are important in maintaining cardiac functional homeostasis (see “Signaling in the heart—learning from kinase knockout (KO) mouse models” section and Table 2). This issue clearly makes target selection in drug discovery crucial if cardiac complications are to be avoided later. Other major challenges are predicting cardiotoxicity both preclinically and clinically and understanding when preclinical observations are likely to translate to the patient, a problem exacerbated by the time course of occurrence of cardiac side effects: some develop acutely, whereas others manifest over a period of several years (Zuppinger and Suter, 2010).

Kinases as Therapeutic Targets in Oncology

Kinases are enzymes that catalyze the transfer of phosphate from ATP, usually on to a serine, threonine, or tyrosine residue on a protein substrate. Through these phosphorylation reactions, kinases act as critical mediators of cellular signal transduction. In addition, via modulation of substrate activity, kinases regulate diverse cellular processes including cell cycle progression, metabolism, transcription, and apoptosis. In cancer cells, many kinases, particularly receptor tyrosine kinases (RTKs), are constitutively active, often through mutation or translocation, and are drivers of cancer cell proliferation and survival. For this reason, in the last 10 years, kinases have emerged as key therapeutic targets in the area of oncology (Gschwind et al., 2004) and there has been an intensive drive to develop both novel small- and large-molecule inhibitors of emerging kinase targets. To date, approximately 80 small-molecule kinase inhibitors have been progressed to the point of clinical evaluation (Zhang et al., 2009). There are over 500 members of the kinome, providing enormous scope for future drug development (Manning et al., 2002).

Kinases have an ATP-binding site, and most small-molecule inhibitors developed to date are ATP mimetics designed to interact with this well-conserved domain of the target protein (Zhang et al., 2009). This often negatively impacts on selectivity against individual kinase targets. For example, a screen of 20 structurally diverse kinase inhibitors against 113 kinases, using ATP site-dependent competition binding assays, revealed wide variation in molecular specificity. For most inhibitors, the tightest interaction was observed for the kinase intended as the target; however, the difference between on and off target affinities was highly variable. This variability in selectivity was independent of the particular chemical scaffold or the intended target. For example, two vascular endothelial growth factor receptors (VEGFR) 2 inhibitors, Vatalanib and SU11248, bound to 4 and 73 kinases, respectively, in addition to the intended target (Fabian et al., 2005). Highly selective kinase inhibitors that bind to an allosteric site outside of the ATP-binding domain tend to be the minority (Zhang et al., 2009). A clear safety concern relating to kinase inhibitor promiscuity is that alongside any primary pharmacology-associated cardiac risks, off target pharmacological activity might be associated with further effects on the heart, thereby creating significant potential for additive or synergistic toxicities.

KINASE INHIBITORS AND CARDIOTOXICITY—PRECLINICAL AND CLINICAL EXPERIENCE

Many approved RTK inhibitors have been found to evoke cardiac dysfunction in some cancer patients (see Table 1); yet, the full extent of the problem remains unclear and is still emerging. Furthermore, a systematic analysis of the literature identified a strong association between kinase pathway perturbation and cardiac liabilities as compared with other organ toxicities (S. Roberts and D. Cook, unpublished personal communication; AstraZeneca; De Keulenaer et al., 2010; Peng et al., 2010; Zuppinger and Suter, 2010).

Published data describing cardiotoxicity associated with kinase inhibitors in the preclinical setting remain somewhat limited; however, some information is available in regulatory approval documents, in particular Federal Drug Administration (FDA) New Drug Application (NDA) pharmacology reviews. In some cases, e.g., for imatinib and trastuzumab, adverse cardiac events in the clinic were not anticipated based on preclinical safety evaluation of the molecules (Speyer, 2002) (see “Imatinib” and “Trastuzumab” sections). This may result from (1) a lack of translation from preclinical species to man, with respect to the specific toxicological mechanism; (2)
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<td>Imatinib mesylate (Gleevec)</td>
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<td>Decreased LVEF, LVD, and HF Gleevec FDA Pharm Review, Gleevec Prescribing Information, and Kerkela et al. (2006)</td>
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<td>Nilotinib (Tasigna)</td>
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<td>AP effects in isolated purkinje fibers indicated potential for hERG K-current and Ca-inward current inhibition. No ECG, BP, or HR changes observed in 12-month dog study. Autolysis, degeneration, and inflammation in 3-month rat study. In the 12-month dog study, there was an increase in CK levels with hemorrhage and congestion of the heart being observed in one animal.</td>
<td>Cardiac ischemia, infarction, biochemical/ECG changes, and increased BP.</td>
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<td>Sunitinib</td>
<td>VEGFR1-3, PDGFR-α and β, colony-stimulating factor 1 receptor (RET kinase, c-kit, and FLT3 kinase)</td>
<td>RCC and GIST</td>
<td>Increased AP duration in canine purkinje fibers. Sunitinib and its primary metabolite are potent hERG channel blockers. 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.</td>
<td>QT interval prolongation, Decreased LVEF, LVD, HF, and increased BP. HF linked to cardiovascular comorbidities</td>
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*Note. ALL, acute lymphocytic leukemia; AP, action potential; ASM, aggressive systemic mastocytosis; CEL, chronic eosinophilic leukemia; HF, heart failure; CK, creatine kinase; DFSP, dermatofibrosarcoma protuberans; HES, hypereosinophilic syndrome; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorder.*
preclinical cardiac dysfunction being subtle and/or asymptom-
atic with no overt pathology; (3) the specific issue not being
assessed preclinically or not being deemed biologically
meaningful; (4) preexisting cardiac disease and dysfunction
in patients (potentially as a consequence of prior chemother-
apy) may be exacerbated by treatment; and (5) some clinical
adverse drug reactions may not be predicted from preclinical
studies in young, healthy, and drug-naive animals and there
may be other confounding factors such as gender (Nicolson
et al., 2010).

Some cardiovascular toxicities (e.g., myocardial ischemia,
HF, and coronary heart disorders) do not appear to be
highlighted during drug development, rather they are reported
at the postapproval stage, indicating that current preclinical
assays and clinical development studies are failing to capture
these liabilities. An important consideration in putting clinical
drug-induced cardiotoxicity into context is the background
incidence of cardiac dysfunction in these cancer patients.
However, this can be difficult to assess in the absence of
a placebo-controlled study. Drugs associated with a risk of
cardiotoxicity are often not prescribed to patients with a history
of significant cardiovascular disease, which therefore makes
unbiased comparison of untreated and treated difficult (Sukel
et al., 2008). In the following section, we review the studies
describing cardiotoxicity associated with some of most
commonly used RTK inhibitors in the clinical setting and
compare these findings to those in the preclinical data package.

**Sunitinib**

Sutent (sunitinib malate) is a broad-spectrum RTK inhibitor
(Table 1) that has shown considerable efficacy in the treatment
of various cancers including neuroendocrine, colorectal, non-
small cell lung, melanoma, chronic myeloid leukemia (CML),
and those of the kidney and breast (Chow and Eckhardt, 2007).
Sunitinib is currently approved for use in the United States
and European Union for the treatment of advanced renal cell
carcinoma (RCC) and gastrointestinal stromal tumors (GIST)
during disease progression on or intolerance to imatinib mesylate
(Sutent Prescribing Information). Molecular targets of sunitinib
include VEGFR1–3, platelet-derived growth factor receptors
(PDGFRA) α and β, colony-stimulating factor 1 receptor,
rearranged during transfection kinase, c-kit, and FMS-like
tyrosine kinase 3.

During preclinical evaluation of sunitinib, there was an
extensive profile of adverse cardiovascular effects. The drug
increased the action potential duration in canine purkinje fibers,
and both sunitinib and its primary metabolite were found to be
potent hERG channel blockers. In monkeys, QT interval
prolongation was observed along with reductions in heart rate
(HR) at doses equivalent to human clinical exposures.
Numerous echocardiography (ECHO) parameter changes were
also observed in primate studies. Changes included reductions
in the ratio of right atrial to aortic diameter, LV ejection time,
and LV area. Histopathological findings included capillary
proliferation, myocardial vacuolization, and pericardial inflam-
mation (Sutent FDA Pharm Review).

The adverse preclinical cardiac observations arising from
sunitinib exposure translated into clinical cardiotoxicity in
some patients. In a single arm, blinded study in patients with
advanced solid tumors, sunitinib was shown to induce a dose-
dependent QT interval prolongation (Bello et al., 2009). In
a study of sunitinib treatment in patients with GIST, 11% of
patients, compared with 3% on placebo, experienced a decline
in LVEF to below the “lower limit of normal” (Sutent
Prescribing Information). In a trial of sunitinib against
interferon alpha in 750 patients with previously untreated
metastatic RCC, the incidence of a reversible decline in
ejection fraction (EF) to below normal in the sunitinib group
was 10%. A grade 3 decline in LVEF was experienced in 2%
of sunitinib-treated patients compared with 1% for the
interferon alpha group (Motzer et al., 2007). In a retrospective
study involving 48 patients, 7 (15%) developed symptomatic
grade 3/4 LVD while receiving sunitinib. Significant risk
factors for LVD included a low body mass index (BMI) or
a prior history of HF and/or coronary artery disease (CAD)
(Telli et al., 2008). The BMI association could be related to
the fact that patients were all given a standard dose, and therefore
the serum levels and cardiomyocyte exposure may have been
greatest in those with a low BMI.

Another study found that 11% of patients with imatinib-
resistant metastatic GIST treated with sunitinib for a median
duration of 33.6 weeks underwent an adverse cardiac event,
with HF predominating (occurring in 8% of individuals).
A steady decrease in LVEF was observed over the first four
cycles of therapy (24 weeks) (Chu et al., 2007). A history of
CAD was a significant predictor of HF occurring in response to
sunitinib treatment. Sunitinib-induced LVD often appears to be
a consequence of multiple insults that have an additive effect,
e.g., previous cardiovascular disease or anthracycline therapy
predisposes to LVD upon sunitinib treatment.

**Imatinib**

Imatinib is an orally available inhibitor of the breakpoint
carrier region-abelson (Bcr-Abl) fusion RTK, which is over-
expressed in CML (Deininger et al., 1997). However, imatinib
also inhibits PDGFRA-α and β (Carroll et al., 1997) and the
kinase c-kit (Heinrich et al., 2000), which forms the basis of its
use in the treatment of GIST.

Imatinib cardiotoxicity in the clinic is uncommon, and it was
not predicted based on the preclinical studies performed and
summarized in the FDA pharmacology review. There were no
significant adverse cardiac observations preclinically; hepatic,
renal, immunological, and teratogenic effects were highlighted
as the safety issues being relevant to human use (Gleevec FDA
Pharm Review). There were no abnormal pathological changes in rat hearts in a 26-week repeat dose study, although interestingly there was a reversible increase in the heart:body weight ratio. Similarly, no cardiac pathological changes were observed in a 13-week dog study or a 39-week study in monkeys. A dose-dependent, short-lasting decrease in arterial blood pressure (BP) was observed immediately after single dose iv administration in rats but no electrocardiogram (ECG) changes were observed. Imatinib had no effect on the rate of beating or force of contraction in the isolated atria of guinea pigs, and a single oral dose had no effect on BP, HR, or ECG in dogs.

Interestingly, a later postapproval research study in mice reported adverse cardiovascular changes in response to imatinib, including LVD (see “Mitochondrial Toxicity” section for molecular changes). In the same publication, Kerkela et al. (2006) reported 10 cases where patients receiving imatinib developed significant LVD. It is important to note that in this study, the total number of patients reviewed by the authors in identifying these cases was unclear. However, affected individuals had a normal LVEF (56 ± 7%) prior to therapy but presented with HF after an average of 7.2 ± 5.4 months of treatment. HF was associated with mild LV dilation, and the mean LVEF declined to 25 ± 8% (Kerkela et al., 2006). The majority of the affected patients in the Kerkela report had comorbidities including hypertension, CAD, or diabetes (Hatfield et al., 2007).

Atallah et al. (2007) performed a retrospective review of all reported adverse cardiac events in patients on clinical trials involving imatinib. Of the 1276 patients enrolled, 22 (1.7%) had symptoms consistent with HF. Interestingly, as observed in the study of Kerkela et al. (2006), the majority of the affected individuals (82%) had other conditions predisposing to HF, e.g., hypertension, CAD, or diabetes. All were over the age of 48, and 59% had previously received one or more cardiotoxic drugs. Another retrospective study examining the Novartis database, comprising of 2327 patients who received imatinib as a mono-therapy, identified 12 (0.5%) “incidence” cases of HF (where patients have no prior HF of LVD) with a possible or probable relationship to imatinib exposure (Hatfield et al., 2007).

Trastuzumab

The selectivity afforded by antibody-based therapeutics (in contrast to the promiscuity of many small-molecule drugs) can provide insight into adverse cardiac events associated with primary target inhibition. Trastuzumab (Herceptin), approved in 1998, is a humanized monoclonal antibody that inhibits the RTK human epidermal growth factor receptor-2 (ErbB2), which is overexpressed in approximately 20–25% of breast cancers (Slamon et al., 1987, 1989). In the absence of trastuzumab treatment, ErbB2 expression in tumors is associated with a poor disease-free patient survival (Ravdin and Chamness, 1995). A meta-analysis of five published randomized trials assessing the utility of trastuzumab in combination with standard chemotherapy demonstrated a significantly decreased risk of breast cancer mortality but a 2.5-fold increase in the likelihood of cardiotoxicity (Viani et al., 2007).

Cardiotoxicity was observed in the trastuzumab pivotal trials, appearing similar to the cardiomyopathy observed with anthracyclines. In the comparative chemotherapy pivotal trial, the risk of cardiac dysfunction associated with trastuzumab and concurrent anthracyclines was 27% (16% demonstrating class II/IV HF) compared with a cardiotoxicity risk of 8% with the anthracycline combination alone (Seidman et al., 2002). Cardiac dysfunction in this context was defined as a decline in LVEF to a level below 55%, which also represented at least a 5% drop from the pretreatment LVEF. The majority of the patients who developed cardiovascular dysfunction received treatment for HF, which led to an improvement in cardiovascular function in 79% of instances (Seidman et al., 2002).

The cardiotoxicity associated with trastuzumab in the clinic was an unexpected finding. Preclinical safety studies in the mouse were designed to support chronic administration, and there was no evidence of acute or multiple dose-related cardiotoxicity in studies up to 6 months (Herceptin MoH Approved Prescribing Information). When trastuzumab binding was evaluated against an in vitro panel of normal human and monkey tissues, the qualitative similarity and lack of nonspecific binding suggested that the monkey was an appropriate animal model for toxicity testing. Binding was predominantly to epithelial cells, known to constitutively express low levels of the ErbB2 receptor (Herceptin MoH Approved Prescribing Information).

Lapatinib

Lapatinib (Tykerb/Tyverb) is a tyrosine kinase inhibitor that inhibits both ErbB1 epidermal growth factor receptor (EGFR) and ErbB2. ErbB1/ErbB2 heterodimers are highly active inducers of tumor growth and survival (Cohen et al., 1996). In 2007, the FDA approved lapatinib in combination with capecitabine for the treatment of patients with advanced or metastatic breast cancer whose tumors overexpress ErbB2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab.

An analysis of the cardiac safety of lapatinib using prospective data collected from 44 clinical studies (3689 treated patients) revealed a low incidence of symptomatic HF (0.2%) or asymptomatic cardiac events (1.4%) (Perez et al., 2008). Asymptomatic events generally manifested as reversible decrease in LVEF, and no cardiac deaths were observed among the treated patients, although data on LVEF recovery were only available for 40 patients. There was no association between prior anthracycline therapy and cardiac events. The patients involved in this study were a heterogeneous group, but despite this, it appears that lapatinib is considerably less cardiotoxic
than trastuzumab, which also inhibits ErbB2 (Perez et al., 2008) (see “Trastuzumab” section). Interestingly, this discrepancy has been proposed to relate to off target cardioprotective effects of lapatinib that counteract potential cardiotoxicity associated with ErbB2 inhibition (Spector et al., 2007) (see “Inhibition of adenosine monophosphate (AMP)-Activated Protein Kinase” section). Alternatively, differences in drug pharmacokinetics and in the duration of ErbB2 blockade between lapatinib and trastuzumab, as a result of their distinct modalities, may also account for the difference in cardiotoxicities.

Lapatinib has been observed to induce QT prolongation in the clinic (Tykerb Prescribing Information) and was recently been shown to inhibit the hERG current in a concentration-dependent manner (Lee et al., 2010). However, this potential for QT prolongation was not indicated in rat and dog preclinical telemetry studies. There was a recommendation in the FDA NDA pharmacology review executive summary to specifically assess the activity of lapatinib against hERG, should QT prolongation events become more prevalent in the clinical setting (Tykerb FDA Pharm Review).

Dose-responsive increases in mean systolic, diastolic, and arterial BP were observed in the preclinical dog telemetry studies after a single oral dose. It remains a possibility that these changes observed in dogs could be linked to the reversible decreases in LVEF observed occasionally in the clinic. Low-frequency histopathological changes, including focal fibrosis and myocyte degeneration in the heart, were noted in rat and dog toxicology studies (Tykerb FDA Pharm Review). However, these pathological changes were not considered indicators of a strong potential for clinical cardiotoxicity, and this judgment appears to have been correct.

**Sorafenib**

Sorafenib (Nexavar) is an inhibitor of multiple kinases including the Raf kinases Raf-1 (c-Raf) and b-Raf, VEGFR1, 2, and 3, PDGFR family members, and c-kit (Petrelli and Valabrega, 2009), and it is a highly effective treatment for advanced hepatocellular carcinoma (HCC) and RCC. In a phase 3 randomized, double-blind, placebo-controlled trial of sorafenib in patients with advanced clear cell RCC, cardiac ischemia or infarction occurred in 12 patients (3%) in the sorafenib group compared with 2 (<1%) in the placebo group. Patients with unstable CAD or who had suffered a recent MI were excluded from the study (Escudier et al., 2009). In concordance with the RCC trial, in a multicenter phase III study in patients with advanced HCC, the relative incidence of cardiac ischemia/infarction between the sorafenib or placebo groups was also 3:1 (Llovet et al., 2008).

In another study involving 74 patients with metastatic RCC treated with either sorafenib or sunitinib, patients were assessed for symptoms of cardiac disease, ECGs were performed, and cardiac biomarkers were measured (Schmidinger et al., 2008). One-third (33.8%) experienced adverse “cardiac events” of varying degrees (11 sunitinib and 14 sorafenib) and 16.2% were asymptomatic, having biochemical and ECG changes only, whereas 17.6% had mild to life-threatening cardiac symptoms. Therapy was interrupted for all symptomatic patients, and cardiovascular treatment was applied prior to re-initiation of treatment. The cardiotoxicity was reversible, and no further episodes were observed upon restoration of therapy in combination with cardiovascular medication, highlighting the importance of cardiac monitoring and intervention (Schmidinger et al., 2008). It has been suggested that for patients sequentially treated with sunitinib and sorafenib, overt cardiotoxicity may be a consequence of the additive effects of these drugs and that it may be exacerbated if the interval between these treatments is short (Mego et al., 2007).

During preclinical safety assessment of sorafenib, the in vitro hERG inhibition assay showed potential for K-channel blockade. Action potential effects in isolated purkinje fibers indicated the potential for hERG K-current and Ca-inward current inhibition. Despite this, no ECG, BP, or HR changes were observed in the 12-month dog toxicology study, although a decrease in HR of 15% was observed after a single intraduodenal injection in an earlier experiment.

Interestingly, in a 3-month rat study, some signs of autolysis, degeneration, and inflammation were seen in the heart. In a 12-month dog study, there was an increase in creatine kinase levels with hemorrhage and congestion of the heart being observed in one animal. Based on these preclinical findings and prior knowledge of the cardiovascular effects of other RTK inhibitors, the pharmacology review of the preclinical package summated that sorafenib carried a high potential for cardiovascular toxicity (Nexavar FDA Pharm Review).

**Dasatinib**

Dasatinib inhibits multiple tyrosine kinases including Bcr-Abl, c-kit, EPHA2, PDGF-β, and multiple members of the Src family. It is approved for the treatment of CML or Ph+ acute lymphocytic leukemia with resistance or intolerance to prior therapy including imatinib.

In the preclinical setting, dasatinib was associated with a significant profile of cardiac toxicities including QT prolongation and increased systolic, diastolic, and arterial BP. Other toxicities observed were vascular and cardiac fibrosis, cardiac hypertrophy, myocardial necrosis, hemorrhage of the valves, ventricle, and atrium, and cardiac inflammation (Brave et al., 2008).

A pooled analysis of four clinical trials, including 445 patients to support the safety and efficacy of dasatinib prior to FDA approval, revealed that the drug prolonged QT interval by an average of 3–6 ms, and tachycardia was experienced by two patients. Twenty patients (4%) developed HF or LVD, with 12 (60%) having prior histories of cardiovascular disease (Brave et al., 2008).
MECHANISMS OF CARDIOTOXICITY ASSOCIATED WITH KINASE INHIBITORS

A considerable effort is being directed at identifying signaling proteins and pathways that are drivers of tumor development and/or progression and therefore represent potential efficacious targets for selective inhibition. However, the association between kinase inhibitors and cardiotoxicity has prompted the need to develop a better understanding of the molecular mechanisms underlying kinase-inhibitor-induced cardiotoxicity. An associated aim is to develop a clearer picture of the signaling pathways that can be perturbed without inducing subsequent cardiotoxicity. To inform target selection and secondary pharmacological screening, there is a strong drive among drug discoverers and developers to identify whether members of the kinome have potential cardiac risk associations. There is a traditional view associated with chemotherapeutic development that some target risk is acceptable for agents developed for the treatment of life threatening, late-stage cancers. However, the aim is always to achieve maximal anticancer efficacy without any associated cardiovascular risk. Improving the safety profile of a candidate drug through greater mechanistic insight could lead to increased dosing, enhanced exposure, and ultimately greater efficacy.

An important question to be answered is—are there common mechanisms of toxicity associated with kinase inhibitors that lead to cardiac dysfunction? A major problem for toxicologists and safety pharmacologists is that kinases regulate the majority of cellular processes and act through interconnected networks rather than via distinct linear pathways. Therefore, the range of potential adverse cellular effects that could arise through inhibition of either the intended kinase targets or off-target kinases is enormous. However, despite this, some common themes are beginning to emerge.

Mitochondrial Toxicity

Mitochondria play a crucial role in the cell, providing the majority of the cell’s energy, in the form of ATP, through electron transport and oxidative phosphorylation. Cardiac muscle is highly reliant upon aerobic metabolism and therefore is susceptible to drugs that perturb mitochondrial metabolism or homeostasis. Drug-induced mitochondrial toxicity can be evoked via inhibition of numerous mitochondrial processes including biogenesis, substrate oxidation, and oxidative phosphorylation (Wallace, 2008). Also, a number of drugs with FDA “Black Box Warnings” for hepatotoxicity and cardiotoxicity have known mitochondrial liabilities (Dykens and Will, 2007). Several kinases localize to and regulate mitochondrial function including c-Jun N-terminal kinase (JNK), pyruvate dehydrogenase kinase (PDK), branched-chain α-ketoacid dehydrogenase kinase, and protein kinase A (PKA) (Thomson, 2002). Of these kinases, JNK appears to be a frequent off target of small-molecule kinase inhibitors (Fabian, et al., 2005). To date, no causative link has emerged between JNK inhibition and cardiotoxicity. However, there is one reported example of JNK blockade being cardioprotecive, which is described in detail later in this section (Kerkela et al., 2006).

With respect to specific kinase inhibitors, direct mitochondrial effects of sorafenib have been reported as a potential mechanism of cardiotoxicity (Will et al., 2008). Cultured cells are generally exposed to high media concentrations of glucose and have adapted to generate ATP predominantly via glycolysis rather than through oxidative phosphorylation. Mitochondria-dependent cytotoxic effects of drugs can be revealed in cultured cells supplemented with galactose rather than glucose. Galactose cannot easily be directed into glycolysis, so this approach ensures cells generate ATP through oxidative phosphorylation via the mitochondria (Marroquin et al., 2007). H9c2 cells cultured in galactose are fourfold more sensitive to sorafenib than glucose-cultured cells, as measured by ATP depletion. In addition, sorafenib inhibits ADP-stimulated respiration, as measured by oxygen consumption, in isolated rat heart mitochondria. Sorafenib is also an inhibitor of complex V (5.1 μM half maximal inhibitory concentration [IC50]) and complex II + III (IC50 3μM) of the electron transport chain (Will et al., 2008). The mean peak plasma concentration of sorafenib in cancer patients is in the range of 9.7 ± 4.7μM (Blanchet et al., 2009), so it remains questionable as to whether a sufficient complex inhibitory concentration can be reached in the cardiomyocyte mitochondrial compartment in the clinic.

Histopathological examination of endomyocardial biopsies from sunitinib-treated patients who developed HF revealed cardiomyocyte hypertrophy and mitochondrial abnormalities including swelling, membrane whorls, and effaced cristae (Chu et al., 2007). The same mitochondrial abnormalities were observed in cardiomyocytes of mice treated with a human-equivalent dose of sunitinib for 12 days. In cultured cardiomyocytes, sunitinib-induced apoptosis was associated with mitochondrial cytochrome c release and caspase-9 activation (Chu et al., 2007).

Electron microscopic (EM) analysis of monocytes derived from imatinib-treated patient heart biopsies revealed prominent “membrane whorls” in dilated sarcoplasmic reticulum (SR) structures and “pleomorphic mitochondria with effaced cristae.” Cytosolic lipid droplets, glycogen accumulation, and vacuoles were also observed (Kerkela et al., 2006). Imatinib treatment of mice led to LVD and dilation. EM analysis of myocytes from these mice also revealed membrane whorls in dilated SR and increased numbers of pleomorphic mitochondria. Mitochondria isolated from these hearts exhibited enhanced Ca2+ -induced swelling. Treatment of isolated cardiomyocytes with imatinib led to a dose-dependent collapse in mitochondrial membrane potential followed by cytochrome c release and a decline in cellular ATP levels. Features of both apoptosis and necrosis were observed. The authors found that imatinib induced the endoplasmic reticulum stress response
followed by activation of JNK in the hearts of treated mice and in cardiomyocytes in vitro. JNK inhibition attenuated the mitochondrial effects and reduced cell death. Interestingly, these events seem to be a consequence of c-Abl inhibition. Cellular expression of an imatinib-insensitive mutant of c-Abl (T315I) prevented imatinib-induced cytochrome c release and cell death (Kerkela et al., 2006).

The work of Fernandez et al. (2007) supported the findings of Kerkela et al. (2006). The authors rationaly reengineered imatinib through the addition of a methyl group, which prevented the new compound (WBZ_4) from inhibiting Ber-Abl and promoted a new inhibitory activity against JNK. A comparison of the heart effects of imatinib and WBZ_4 in mice revealed an increase in the cardiotoxicity biomarker brain Abl and promoted a new inhibitory activity against JNK. Assessment of cardiac function by magnetic resonance imaging revealed a significant drop in LVEF in imatinib-treated mice, but not in WBZ_4-treated or control animals (Fernandez et al., 2007).

A study by Grazette et al. (2004) showed that ErbB2 inhibition resulted in mitochondrial apoptotic signaling in rat cardiomyocytes. Treatment with an antibody directed against the Tyr882 phosphorylation site involved in ErbB2 activation resulted in a decline in receptor phosphorylation, an increase in pro-apoptotic Bcl-xS expression and a concurrent decrease in anti-apoptotic Bcl-xL expression. There was an increase in both the expression and homoooligomerization of pro-apoptotic Bak (in the mitochondrial fraction). Release of cytochrome c into the cytosol and caspase-3 cleavage was also observed. ErbB2 inhibition also resulted in decreased mitochondrial membrane potential and a decrease in cellular ATP. The apoptotic changes observed could be inhibited with a cell-permeable Bcl-xL peptide, demonstrating the significance of the Bcl-xL down-regulation in this process (Grazette et al., 2004).

A similar, recent study by Gordon et al. (2009) implicated the generation of reactive oxygen species as being a possible upstream trigger for the induction of mitochondrial apoptosis by an ErbB2-blocking antibody. Again the process involved Bak/Bax activation, mitochondrial permeability transition pore opening, and cytochrome c release from the mitochondria into the cytosol (Gordon et al., 2009).

It is important to consider that mitochondria are a central component of the intrinsic apoptotic process. Therefore, mitochondrial functional and structural changes will be a common outcome of adverse interactions between drug molecules and cells. Mitochondrial morphological changes such as aggregation and remodeling of the cristae are common in apoptotic cells (Haga et al., 2003; Karbowski and Youle, 2003). Therefore, caution should be taken not to overinterpret any observed “mitochondrial effects” occurring as a consequence of drug treatment as representing direct drug-induced mitochondrial toxicity. In order to uncouple apoptosis from mitochondrial toxicity, sensitive assays to assess mitochondrial function versus apoptosis should be employed during safety screening of drug candidates. For example, common features of mitochondrial toxicity, which precede apoptosis, can be screened for. These could include decreased oxygen consumption, ATP depletion, and lactic acidosis (Dykens and Will, 2007; Wallace, 2008).

Another point of note is that in patients with inherited mitochondrial defects, disease presents clinically in multiple organs (Wallace, 1999). The overrepresentation of cardiac toxicity observed with kinase inhibitors may therefore be indicative of nonmitochondrial, molecular mechanisms underlying these events.

### Inhibition of AMP-Activated Protein Kinase

It has been hypothesized that sunitinib cardiotoxicity could be mediated through inhibition of AMP-activated protein kinase (AMPK) (Force et al., 2007). It is an important regulator of cellular energy metabolism (Lage et al., 2008) and is required to maintain normal cardiac contractility (Zhang et al., 2008b). ATP reserves in cardiac tissue are small, and as AMPK is a major regulator of cellular ATP homeostasis, myocytes could be particularly sensitive to AMPK inhibition. Phosphorylation of the α and β isoforms of the AMPK target acetyl-coenzyme A carboxylase was reduced by sunitinib in a dose-dependent manner after 2-h exposure. However, this required an in vitro sunitinib concentration of 5μM, 25-fold greater than the therapeutic plasma level (Hasinoff et al., 2008). Sunitinib induced caspase activation at submicromolar concentrations in cardiomyocytes after only 4 h. Pretreatment with the AMPK activator metformin failed to protect monocytes from sunitinib-induced toxicity. Taken together, these data suggest that AMPK inhibition (Force et al., 2007) may not be the primary mechanism of sunitinib-induced cardiotoxicity (Hasinoff et al., 2008).

Interestingly, Spector et al. (2007) showed that dual small-molecule inhibitors of ErbB1 and ErbB2 such as GW2974 and lapatinib, but not trastuzumab, activate AMPK in an ErbB2 and calcium-dependent manner, which protects human cardiomyocytes from apoptosis-inducing stimuli such as tumor necrosis factor α. The protection afforded to myocytes by AMPK activation was linked to increased ATP production and could be prevented by inhibiting AMPK. Therefore, cardioprotective AMPK activation could explain the discrepancy between the ErbB2 inhibitors trastuzumab and lapatinib with respect to cardiotoxicity (See “Trastuzumab” and “Lapatinib” sections).

### PDGFR Inhibition

Imatinib, sunitinib, sorafenib, dasatinib, and nilotinib all inhibit PDGFR (see “Kinase Inhibitors and Cardiotoxicity—Preclinical and Clinical Experience” section and Table 1). Although the full picture is not yet clear, data indicate that for some malignancies, the anticancer effects of these drugs
are dependent upon PDGFR inhibition (Krause and Van Etten, 2005; McDermott et al., 2009; Sawyers, 2002). It remains a possibility that inhibition of PDGFR in the heart may be a contributory factor in the cardiotoxicity associated with the use of some of these small-molecule inhibitors (Cheng and Force, 2010).

PDGF-β signaling is known to be important in aspects of heart development (Van den Akker et al., 2008), and recently, some cardioprotective effects of PDGF have been demonstrated. For example, intramyocardial delivery of PDGF was shown to enhance cardiac performance after infarction (Hsieh et al., 2006). Cardiomyocyte PDGFR-β expression and activation increases in response to load-induced stress, and inducible cardiac-specific PDGFR-β KO mice develop severe HF after aortic constrict (Chintalgattu et al., 2010). These data support the design of future studies aimed at clarifying the role of PDGFR signaling inhibition in kinase inhibitor–induced cardiac injury.

**Effects of Kinase Inhibitors on the Vasculature**

Some of the major signaling pathways being targeted for cancer therapy also have an important role in normal vascular function and homeostasis. VEGF induces a dose-dependent decrease in BP (Yang et al., 2003) by stimulating the secretion of vasodilators including nitric oxide (NO) and prostacyclin from vascular endothelial cells (Verheul and Pinedo, 2007). Consistent with this, the anti-VEGF antibody bevacizumab (Yang et al., 2003) and inhibitors of VEGFR family RTKs including sunitinib and sorafenib are associated with the development of hypertension (Bhargava, 2009; Sica, 2006).

In one clinical study of sorafenib-treated patients, 75% developed an increase in systolic BP of greater than 10 mm Hg and 60% exhibited an increase of over 20 mm Hg after 3 weeks of therapy. Elevated BP persisted in patients for at least 18 weeks, and this effect was independent of VEGF itself, levels of which were unaffected by treatment. Interestingly, BP changes were not observed in the dog 12-month preclinical studies (Table 1). In both man and dog, no significant changes in heart rate have been observed (Veronese et al., 2006).

In another study, hypertension was observed in 5 from 28 treated patients after 3–4 weeks of sunitinib therapy. Antihypertensive treatment and cardiac monitoring was instigated, and no cardiac adverse events were later reported in this group (Faiivre et al., 2006). In a randomized study comparing sunitinib and interferon alpha for the treatment of RCC, the incidence of hypertension in the groups was 8 and 1%, respectively (Motzer et al., 2007). In the clinic, there are conflicting data regarding a direct link between hypertension and a subsequent LVEF decline (Chu et al., 2007; Telli et al., 2008). Interestingly, there is no record of BP changes being observed in the preclinical package for sunitinib (Table 1).

The phosphatidylinositol 3-kinase (PI3K) cascade has an important role in the vascular system. PI3K operates downstream of VEGF to positively regulate NO synthase and the subsequent production of NO (for a review of PI3K signaling in the vasculature, see Morello et al., 2009). Conversely, Rho kinase (ROK) participates in the maintenance of vascular resistance by inhibiting AKT and therefore suppressing NO production. Treatment of dogs with the ROK inhibitor Y-27632 elicited a decrease in peripheral vascular resistance and a subsequent increase in cardiac output (Takahara et al., 2003). Infusion of human subjects with the ROK inhibitor fasudil resulted in a dose-dependent increase in forearm blood flow, which was dependent upon NO production (Bussemaker et al., 2007).

In general, the extent to which kinase inhibitor–induced vascular effects, including hypertension, contribute to cardiac dysfunction remains to be clarified. However, the possibility exists that a decline in EF may occur as a secondary consequence of drug-induced modulation of vascular physiology rather than via a direct drug effect on the heart tissue (Verheul and Pinedo, 2007).

**Ion Channel Inhibition**

The QT interval represents the period of ventricular depolarization and repolarization from the beginning of the QRS interval to the end of the T wave on an ECG recording. Because of the association between QT prolongation and the development of cardiac arrhythmias, over the past decade, considerable progress has been made in the development of strategies to reduce the risk of pharmacological QT interval prolongation emerging in later drug development stages or in the clinic. Approaches include the screening of early drug candidates for cardiac ion channel inhibition (e.g., the hERG potassium channel) and using telemetry to monitor heart function in preclinical in vivo studies (Pollard et al., 2008).

For cancer indications, drug candidates exhibiting adverse electrophysiological effects are sometimes developed, in the absence of a “safer” compound, on the basis of a “risk versus patient benefit” assessment. Preclinical observations of QT interval prolongation frequently translate into the clinical setting (e.g., sunitinib, dasatinib, and nilotinib, see “Kinase Inhibitors and Cardiotoxicity—Preclinical and Clinical Experience” section and Table 1). Interestingly, lapatinib prolongs QT interval in the clinic, but this was not observed in preclinical rat and dog studies (Table 1). Dasatinib and nilotinib both inhibit hERG currents in vitro with IC50s of 14.3 and 0.66µM, which represent 150- and 0.1-fold the expected human Cmax, respectively (Freebern et al., 2007). This suggests that the mechanism underlying QT interval prolongation for dasatinib may be independent of hERG inhibition.

Recently, evidence has emerged that some RTKs directly modulate ion channel activity. In whole-cell patch clamp experiments, the EGFR inhibitor AG556, the Src family inhibitor PP2, and the broad-spectrum RTK inhibitor genistein all inhibited tyrosine phosphorylation of hERG and reduced...
channel activity. The pharmacological effects on hERG currents were antagonized by the phosphatase inhibitor orthovanadate, indicating that inhibition of tyrosine phosphorylation was the underlying mechanism rather than direct drug blockade of the channel (Zhang et al., 2008a). Other studies have also identified a regulatory role for EGFR on voltage-gated cardiac sodium current in guinea pig ventricular myocytes (Liu et al., 2007) and a dual modulatory role of EGFR and Src on the volume-sensitive chloride current in human atrial myocytes (Du et al., 2004).

Interactions with Adenosine Receptors

Adenosine acts as a local modulator with a predominantly cytoprotective role in the body, mediated via four broad categories of activity: increasing the ratio of oxygen supply to demand, protecting against ischemic damage by cell conditioning, triggering anti-inflammatory responses, and the promotion of angiogenesis (Jacobson and Gao, 2006).

Inhibition of adenosine receptors represents a potential, but largely underexplored, mechanism of kinase inhibitor–induced cardiotoxicity. In response to insults such as cardiac ischemia, the extracellular adenosine concentration rises, leading to enhanced activation of the adenosine receptors A_1 and A_3 in cardiomyocytes. The resulting downstream activation of phospholipases C and D has a cardioprotective effect (Parsons et al., 2000). Activation of the A_3 receptor has also been shown to attenuate doxorubicin-induced cardiotoxicity (Shneyveys et al., 2001). In the rabbit heart, adenosine has been shown to activate the PI3K/Akt signaling pathway (Krieg et al., 2002).

Signaling in the Heart—Learning from Kinase KO Mouse Models

It is becoming clear that many kinases have essential developmental and homeostatic roles in the heart. Although the electromechanical differences that exist between rodent and human hearts may limit electrophysiological interspecies translation (Gray, 2005), studies of cardiac function in mice where kinases have been genetically disrupted have helped to identify some of these critical signaling mediators. The ability to generate cardiac-specific conditional kinase KO mice and inducible knockdown models has allowed researchers to focus on the role of kinases in the heart, with less risk of issues arising from developmental complications or adverse effects on other organ systems. As safety considerations are of paramount importance in drug development, these studies have been, and will continue to be, a very useful tool in predicting cardiotoxicity associated with the primary pharmacology of small-molecule inhibitors of kinase targets. The advantage of knowing early in discovery that a drug carries a cardiac liability associated with primary pharmacology is that refinements to compound chemistry will be unlikely to circumvent the cardiotoxicity.

**Erβ2**

Cardiac-restricted deletion of ErbB2 in mice resulted in normal cardiac morphogenesis and survival into adulthood. However, the KOs exhibited enlargement of both the left and the right ventricles consistent with dilated cardiomyopathy, reduced contractility, and an increase in the heart:body weight ratio (Crone et al., 2002; Ozcelik et al., 2002), demonstrating that ErbB2 is essential for adult heart function. Decreased septal and posterior wall thickness and lower LV end-diastolic and end-systolic dimensions were also observed in KOs compared with wild-type mice. Myocytes displayed increases in vacuolization and mitochondrial number, and ventricles showed an increased number of apoptotic cells.

Interestingly, overexpression of anti-apoptotic Bcl-xL resulted in restoration of the chamber dilation and contractility to control animal levels, suggesting that apoptosis significantly contributes to the cardiomyopathy observed in ErbB2 conditional KO mice (Crone et al., 2002). Transmission EM analysis revealed an increased number of mitochondria and vacuoles in cardiomyocytes relative to control animals. Cardiomyocytes were also more sensitive to doxorubicin toxicity, consistent with the clinical experience with trastuzumab (Seidman et al., 2002) (see “Trastuzumab” section). The findings in the ErbB2 conditional KO mice support the hypothesis that trastuzumab-induced cardiotoxicity is a function of ErbB2 inhibition in the heart rather than a secondary effect.

**Raf-1**

The Raf kinase family represents important oncogenic signaling proteins and therapeutic targets (Gysin et al., 2005; Sridhar et al., 2005). Heart-specific knockdown of the sorafenib target Raf-1 in mice resulted in significant heart enlargement and reduced contractility. The LV end-diastolic and end-systolic diameters were increased, and the septal wall thickness was decreased compared with control mice (Yamaguchi et al., 2004). Raf-1 conditional KOs also displayed an increase in cardiomyocyte apoptosis and an associated increase in the (anti-apoptotic) Bax:Bcl-2 ratio. There was also increased phosphorylation of JNK and p38 in KO hearts, indicating a possible role for these in the cardiac dysfunction (Yamaguchi et al., 2004). faf-1 promotes cell survival by antagonizing apoptosis signal-regulating kinase (ASK1), an activator of p38 and JNK (Ichijo et al., 1997). ASK1 was found to be highly active inraf-1 KO hearts, and ASK1/raf-1 double KO mice had restored cardiac function. This implicates ASK1 activation as a key determinant of the cardiac dysfunction observed in the conditional raf-1 KO mice (Yamaguchi et al., 2004).

**Kinases and Phosphatases Required for Cardiac Function**

A number of studies have reported cardiac dysfunction as a consequence of deletion (often cardiac specific) of genes encoding proteins involved in signal transduction, including
kinases and phosphatases. Components of the PI3K and Mitogen-activated protein kinase (MAPK)/extracellular-signal regulated kinase (ERK) pathways are commonly represented within this group (Fig. 1). In most cases, small-molecule inhibitors of these proteins have not been assessed for cardiac effects in man (or reported in animals); therefore, the predictive power of mouse models in this regard remains to be fully tested (Table 2).

The cytoplasmic tyrosine phosphatase Shp2 has been implicated in the development of acute myeloid leukemia (Wang et al., 2009) and breast cancer (Zhou and Agazie, 2009). A striated muscle–specific KO of Shp2 resulted in mice with severe dilated cardiomyopathy, leading to HF and premature death (Princen et al., 2009). Mice were viable at 1 week after birth with no phenotypic differences from controls but died from 2 weeks onward (mean 13.4 weeks). Heart:body weight ratio was increased by 66% relative to controls. Mice had enlarged left ventricles and thinning of the interventricular septal walls, consistent with dilated cardiomyopathy. There was a significant decrease in LV fractional shortening. There were no significant differences in proliferation or apoptosis compared with control hearts, but there was increased Akt phosphorylation in the KO hearts (Princen et al., 2009).

Cardiac-specific KO of the lipid phosphatase and tensin homolog (PTEN) results in cardiac hypertrophy, decreased cardiac contractility, and an increase in anterior wall thickness and LV mass. Interestingly, despite a marked reduction in contractility in mice at 10 weeks of age, there was no further decrease in function when assessed at 12 months (Crackower et al., 2002). The contractility changes observed seemed to be associated with the decreased cyclic AMP (cAMP) levels observed in the PTEN-deficient cardiomyocytes. cyclic AMP (cAMP) is required to activate PKA, which phosphorylates phospholamban (PLN) leading to its dissociation from the SR Ca\(^2+\) ATPase, enhancing Ca\(^2+\) uptake and cardiac contractility. Therefore, the cardiac dysfunction associated with PTEN deletion is likely to result from altered calcium homeostasis in cardiomyocytes.

Two other examples also highlight the importance of kinases in regulating calcium homeostasis; these are PDK1 and Pim-1. Inhibition of PDK1 suppresses cancer cell proliferation and survival (Choi et al., 2008). A genetically engineered mouse model exhibiting tamoxifen-inducible heart-specific disruption of Pdk1 allowed a clear assessment of the role of the kinase, specifically in adult mouse hearts (Ito et al., 2009). Knockout of Pdk1 at 10 weeks of age resulted in progressive contractile dysfunction (1 week onward), global chamber dilation, wall thinning, and interstitial fibrosis by 4 weeks. All animals died of HF between 5 and 15 weeks after Pdk1 inactivation. The
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*Note. pptase, phosphatase; LVDP, LV diastolic pressure; LVEDP, LV end-diastolic pressure; wt, wild type I/R. ischemia/reperfusion.*
hearts of the Pdk1 cardiac knockout mice exhibited increased apoptosis and a decrease in cAMP and PLN phosphorylation, again implicating disruption of SR calcium homeostasis as the underlying reason for contractile dysfunction (Ito et al., 2009). Using this model, it would be interesting to examine the reversibility of the cardiovascular effects upon reestablishment of PDK1 expression via tamoxifen withdrawal.

Although not strictly a KO model, expression of a dominant-negative (DN) form of Pim-1 kinase in mouse hearts resulted in reduced cardiac contractile function (Muraski et al., 2008). This was revealed by ECHO as a progressive dilation from 17 weeks of age and as a depression of fractional shortening and ejection fraction by 27 weeks of age. Mice exhibited increased LV end-diastolic pressure and decreased LV developed pressure. Results from isolated myocytes suggest that the reduced cardiac contractility observed in Pim-1 DN mice is mediated by a decline in Ca\(^{2+}\) release from the SR together with slower Ca\(^{2+}\) reuptake (Muraski et al., 2008).

So far, we have predominantly focused on those examples where the KO of a signaling protein in the mouse heart results in basal cardiac dysfunction. However, for KOs with no resulting alteration in basal heart activity, a protective role of the kinase (e.g., PDGFR-β, Chintalgattu et al., 2010; PI3K \[p110\]x], McMullen et al., 2003; AKT1, DeBosch et al., 2006b; ERK1/2, Purcell et al., 2007; AKT2, DeBosch et al., 2006a) or alternatively a pathological function (CaM kinase II, Backs et al., 2009 and polycystic kidney disease 1 (PKD1), Fielitz et al., 2008) is often revealed in response to adverse cardiac stimuli. Therefore, when assessing potential kinase oncology targets such as AKT (Steelman et al., 2008), CaM Kinase II (Franklin and McCubrey, 2009; Takai et al., 2009), and PKD1 (Eiseler et al., 2009), the protective role of the kinase under cardiac stress conditions should also be a safety consideration.

The observations derived from the KO mouse models described are informative from the drug discovery safety perspective as the approach can be used to discriminate between essential and nonessential pathways in the developed heart (see Fig. 1). Importantly, these models can provide useful information regarding which kinases/phosphatases may be therapeutically targeted with a reduced risk of associated cardiotoxicity.

In contrast to pharmacological inhibition, genetic ablation clearly represents a permanent signaling blockade, albeit with an increased likelihood of compensatory mechanisms being active. Therefore, when an oncology target is also a cardiac-active kinase, an important question remains—is it possible to achieve anticancer efficacy prior to the onset of (and without predisposing to) cardiotoxicity? This clearly needs to be addressed on a case-by-case basis. In addition, inducible KO mouse models provide the added advantage of allowing assessment of reversibility, as mentioned previously.

As the efficiency and technology associated with generating KO animals, particularly conditional ones, improve further, it appears likely that in the near future, the generation of such models will become an increasingly common component of kinase target safety validation in drug discovery. However, it is important to be aware that there are proven examples where the phenotype of a KO mouse is inconsistent with the equivalent human phenotype. The generation of some mouse models of human metabolic diseases has highlighted this issue particularly well. The genetic ablation of mouse genes encoding enzymes including HexA (β-hexosaminidase A), Galt (galactose-1-phosphate uridylytransferase), Gla (α-galactosidase), and Ocril (human Lowe syndrome gene) results in no clinical phenotype in mouse, in stark contrast to the human diseases associated with deficiencies in these enzymes (Elsea and Lucas, 2002). The reason for these interspecies differences is frequently unclear but may represent redundancy of the enzyme in the rodent, a compensatory pathway being active in mouse or interspecies pathophysiological variation.

Genetic KO clearly represents the most extreme form of loss of protein function. In contrast, small molecules often exhibit graded target inhibition, which may impact on the observed phenotype (Knight and Shokat, 2007). In addition, chemical

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Box 1 Summary of safety recommendations for targeted cancer therapy discovery and development

- The use of conditional KO mouse models to (a) assess the potential impact of pharmacological inhibition of primary target and (b) support definition of secondary pharmacology associated with candidate drug molecule assessment.
- Assessment of primary and secondary pharmacological potency utilizing protein panels representing both human and preclinical species.
- The application of animal models with ‘‘preexisting disease’’ in safety studies to more accurately replicate the impact of primary disease and comorbidities on cardiac functional response to experimental drug candidates.
- Chemotherapeutic ‘‘combination’’ or ‘‘preexposure’’ in vivo studies employing drugs relevant to the intended patient population (e.g., with anthracyclines) that may reveal potential additive or synergistic cardiotoxicities.
- The development and application of combined in silico, in vitro, and in vivo models and cascades.
- The identification of relevant, reliable, and robust translational biomarkers of drug-induced cardiac injury or dysfunction for preclinical risk assessment and application in the clinic in the context of risk management.
- Consideration of potential cardiotoxic drug metabolites and preferential cardiac accumulation of drug.
- To be successful, the approaches applied need to be driven by clearer understanding of the pathways that maintain cardiac homeostasis and an ability to screen for signals indicative of perturbations in these pathways.

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CONCLUDING REMARKS

It is clear that some small molecules and biologicals targeting kinase pathways provide enormous benefit to cancer patients but carry an associated risk of cardiotoxicity. The application of “diseased” or “aging” animal models in toxicological studies, to more accurately replicate underlying health problems in the human population, has been proposed (Dixit and Boelsterli, 2007; Gray, 2005) and is being explored. This is particularly important, as the majority of patients who experienced HF in response to imatinib had other conditions predisposing to HF, e.g., hypertension, CAD, or diabetes (Atallah et al., 2007; Hattfield et al., 2007). In addition, as anthracyclines are an early component of many treatments, e.g., early breast cancer, an evaluation of the cardiac response to novel kinase inhibitors in animals preexposed to anthracyclines may reveal potential additive or synergistic cardiotoxicities, as is the case of trastuzumab for example (“Trastuzumab” section).

One of the challenges for drug developers and clinicians is to discriminate reversible and manageable drug-induced changes in cardiac function from those that can result in fatal outcomes. In order to avoid life-threatening cardiotoxicity emerging at the late stages of drug development or in the clinic, the challenge for drug safety scientists in the pharmaceutical industry is to predict or identify potential drug-induced cardiotoxic effects as early as possible in drug discovery. This can be achieved through the development and application of in vitro, in vivo, and in silico models and through the identification of relevant, reliable, and robust biomarkers. Biomarkers are also key tools that can be translated to the clinical setting and applied in the context of risk management.

It is clearly a huge challenge to put in place early in vitro screens that are reliable predictors of adverse cardiac events in the clinic. Screening for hERG channel blockers currently represents a shining example (Pollard et al., 2008). However, the question remains as to whether other molecular-drug interactions or cellular readouts can provide as clear a toxicological “line of sight” from the in vitro setting to the in vivo setting and ultimately through to the clinic. If human proteins are used in early in vitro safety screens, a lack of sequence conservation between animal and human enzymes could confound the translation of any observations. Therefore, the use of protein panels representing both human and preclinical species should be considered.

Components of preclinical assessment should clearly also include accurate identification of nonintended secondary targets of kinase inhibitors. It is also important to address other factors that may play a role in adverse cardiac events, e.g., toxic drug metabolites and preferential cardiac drug accumulation. To be successful, the approaches applied need to be driven by a clearer understanding of the pathways that maintain cardiac homeostasis and an ability to screen for signals indicative of perturbations in these pathways.

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