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Molecular Mechanisms of Cardiovascular Toxicity of Targeted Cancer Therapeutics

Cardiovascular Disease: Hidden Killer in Adult Survivors of Childhood Cancer

Emerging Anticancer Strategies: Is There Cause for Concern? Yes!

The Vulnerability of the Heart As a Pluricellular System: Lessons From Unexpected Triggers of Heart Failure in Targeted Cancer Therapy

Thomas Force, Guest Editor

Molecular Mechanisms of Cardiovascular Toxicity of Targeted Cancer Therapeutics

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Abstract: In 2002, Hoshijima and Chien drew largely theoretical parallels between the dysregulation of the signaling pathways driving cancer and those driving cardiac hypertrophy (Hoshijima M, Chien KR. *J Clin Invest.* 2002;109:849–855). On the surface, this statement appeared to stretch the limits of reason, given the fact that cancer cells are known for their proliferative capacity, and adult cardiomyocytes are, except under unusual circumstances, terminally differentiated and incapable of re-entering the cell cycle. However, on closer examination, there are numerous parallels between signaling pathways that drive tumorigenesis and signaling pathways that regulate hypertrophic responses and survival in cardiomyocytes. Indeed, this issue appears to be at the core of the cardiotoxicity (often manifest as a dilated cardiomyopathy) that can result from treatment with agents typically referred to as “targeted therapeutics,” which target specific protein kinases that are dysregulated in cancer. Herein, we examine the cardiotoxicity of targeted therapeutics, focusing on the underlying molecular mechanisms, thereby allowing an understanding of the problem but also allowing the identification of novel, and sometimes surprising, roles played by protein kinases in the heart. (*Circ Res.* 2010;106:21-34.)

Key Words: cardiotoxicity ■ cancer ■ therapeutics ■ kinase inhibitors

Cancer therapeutics have evolved dramatically over the past few years. This has revolutionized the approach to treatment of some cancers.¹ These novel treatment strategies target specific factors that are causal (or strongly contributory) in the initiation and progression of disease. The greatest advances have come with the development of monoclonal antibodies and small molecule inhibitors of protein kinases, typically receptor tyrosine kinases. Herein, we focus on the small molecules. At present, 11 agents are FDA-approved, with several more seeking approval over the next 2 years (Table 1). Although these agents offer new hope to many

cancer patients, with some agents, treatment has been associated with cardiovascular complications including significant hypertension, left ventricular dysfunction, and/or heart failure.² This is not a so-called “class effect,” however, because risk of significant cardiotoxicity for most of the approved agents appears to be low. However, the caveat to this statement is that, because of the recent introduction of these drugs, long-term follow-up of patients is not available. Long-term follow-up is particularly important with kinase inhibitors because as opposed to traditional chemotherapeutics, kinase inhibitors are often taken for life because if they

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Non-standard Abbreviations and Acronyms

AMPK	AMP-activated protein kinase
CML	chronic myeloid leukaemia
DDR	discoidin domain receptor
ER	endoplasmic reticulum
ERK	extracellular signal-regulated kinase
GIST	gastrointestinal stromal tumor
JAK	Janus kinase
LV	left ventricular
MAPK	mitogen-activated protein kinase
mTOR	mammalian target of rapamycin
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PDK	phospholipid-dependent kinase
PI3K	phosphatidylinositol 3-kinase
PK	protein kinase
PLK	polo-like kinase
PTEN	phosphatase and tensin homolog deleted on chromosome 10
RSK	ribosomal S6 kinase
RTK	receptor tyrosine kinase
STAT	signal transducer and activator of transcription
T1D	type 1 diabetes
TK	tyrosine kinase
TKI	tyrosine kinase inhibitor
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor

are withdrawn, this can lead to reemergence of the malignancy. Furthermore, few prospective studies carefully examining the issue of cardiotoxicity have been performed.

Currently, small molecule kinase inhibitors account for $\approx 20\%$ of all moneys spent in drug development and the majority of that ($\approx 80\%$) is in cancer (with a small component in inflammatory and other diseases). This class is second only to drug development targeting G protein-coupled receptors. Based on the number of kinase inhibitors currently in phase 1 or later clinical trials (≈ 150),³ there appears to be no slowing down in drug development in this area. This, plus the fact that the area is increasingly lucrative, means that we will likely see a large increase in the number of agents on the market over the next several years. Therefore, we believe it is critical for the basic and clinical cardiovascular sciences communities to understand these agents, their mechanisms of action (including mechanisms by which they kill cancer cells), and how these actions can lead to cardiotoxicity. By defining mechanisms of toxicity we believe that we can importantly inform the pharmaceutical industry, regulatory agencies, and clinicians. Furthermore, we believe that by identifying specific targets of drugs, inhibition of which lead to the toxicity, we can inform drug design so that problematic targets can be avoided where possible. Finally, we believe that these agents can serve as valuable pharmacological tools with which to define the roles in the heart of many kinases of poorly understood function (ie, a chemical biology approach).

Ultimately, clinicians will need to develop approaches to patients that balance effective treatment of the cancer with risk of cardiotoxicity. For example, in a patient with metastatic disease, risk of cardiotoxicity becomes a very minor concern. However in a patient being offered adjuvant therapy who already has a good prognosis, risk of cardiotoxicity becomes more important.

Role of Protein Kinases in Cancer: Cellular Transformation, Tumor Growth, and Tumor Angiogenesis

Data from tumor sequencing projects have found remarkable mutation rates in protein kinases. One study found that mutations in as many as 120 kinases (or $\approx 20\%$ of the kinome) could be present in individual cancers.^{4,5} Furthermore, there was evidence that these mutations were not just bystanders but were so-called driver mutations (ie, playing a role in tumorigenesis). Given this complexity, it seems inconceivable that inhibiting individual kinases in cancer would be effective, except for the relatively rare malignancies that are truly “oncogene-addicted” to a specific kinase.⁶ Surprisingly, “targeted therapeutics,” a term that refers to drugs that inhibit the specific gene products that drive tumorigenesis, have radically transformed the treatment of some hematologic malignancies and solid tumors.

Although the gain of function mutations, gene amplifications, and/or overexpression that drive tumorigenesis can occur in a variety of different gene classes,⁶ in many cases (but not all; see below), the genes encode protein kinases, typically tyrosine kinases.¹ Approximately 90 of the 518 kinases in the human kinome are tyrosine kinases (TKs), and can be either receptor (RTKs, of which there are 58) or nonreceptor TKs of which there are 32 that can be divided into 9 subfamilies (eg, Src family). The identification of these mutated or amplified oncogenes has allowed the development of therapeutics specifically targeting the oncogenic kinases. Although oncogenic mutations commonly occur in other classes of proteins in addition to kinases (eg, cell cycle regulators, and pro- or antiapoptotic factors),⁷ kinases have become favorite targets of the pharmaceutical industry due not only to their importance in tumor initiation and progression but also to the relative ease with which inhibitors can be made (see below). This has led to an explosion in drug development targeting TKs (TK inhibitors [TKIs]) and, to a lesser but increasing extent, serine/threonine kinases.

The first TKI to reach market was imatinib (Gleevec, Novartis), and it was approved in 2001.⁸ It is still the most successful TKI with \$3.67 billion in sales in 2008. Imatinib revolutionized the treatment of chronic myeloid leukemia (CML). Before the introduction of imatinib, CML was uniformly fatal within 5 years, whereas now, $\approx 90\%$ of patients are alive 5 years after diagnosis. Indeed, this and other drugs have changed our thinking about cancer, which can now be viewed as a group of diseases that, even if not curable, can be managed effectively for years, similar to many other chronic diseases.

Imatinib was designed to target Bcr-Abl, which is causal in $>90\%$ of cases of CML and $\approx 20\%$ of acute lymphocytic leukemia (ALL).¹ The Bcr-Abl fusion protein is created by a

Table 1. Kinase Inhibitors in Cancer, Their Targets, and Representative Malignancies

Agent (Trade Name)	Targets		Representative Malignancies
	Primary	Other	
FDA-approved			
Imatinib (Gleevec)	Bcr-Abl	Abl, c-Kit, PDGFRs, DDR1, Cdc2, etc ^{9,13}	CML, Ph ⁺ ALL, CMML, HES, GIST
Nilotinib (Tasigna)	Bcr-Abl and most IRMs	Abl, c-Kit, PDGFRs	imatinib-resistant CML, ALL, GIST
Dasatinib (Sprycel)	Bcr-Abl and most IRMs	Abl, c-Kit, PDGFRs, DDR1, SFKs, EphB4, etc ^{9,13}	imatinib-resistant CML, ALL, GIST
Sunitinib (Sutent)	VEGFRs	PDGFRs, c-Kit, CSF-1R, FLT3, RET*	RCC, GIST
Sorafenib (Nexavar)	VEGFRs	PDGFRs, c-Kit, FLT3, Raf-1/B-Raft†	RCC, hepatocellular carcinoma
Lapatinib (Tykerb)	EGFR (ERBB1), HER2 (ERBB2)	NI	HER2 ⁺ breast cancer, ovarian cancer, gliomas, NSCLC
Gefitinib (Iressa)	EGFR	NI	NSCLC, gliomas
Erlotinib (Tarceva)	EGFR	NI	NSCLC, pancreatic cancer, gliomas
Temsirolimus (Torisel)	mTOR	NI	RCC
Everolimus (Afinitor)	mTOR	NI	RCC
Sirolimus (Rapamune)	mTOR	NI	RCC
Projected			
Bosutinib	Bcr-Abl and several IRMs	Abl, SFKs, EphB4, etc ^{9,13}	imatinib-resistant CML
Lestaurtinib	JAK2, FLT3	TrkA/B/C	MPD (PCV, ET, IMF), AML
Pazopanib	VEGFR, PDGFR, c-Kit	NK	RCC
Vandetanib	VEGFR, EGFR	NK	NSCLC
Cediranib	VEGFR	NK	Colorectal cancer
Alvocidib	CDK	NK	CLL
Enzastaurin	PKC β	NK	Diffuse large B-cell lymphoma
Deforolimus	mTOR	NK	RCC, metastatic sarcomas

* ≥ 50 targets; † ≥ 15 targets. AML indicates acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; EphB4, ephrin receptor tyrosine kinase B4; ET, essential thrombocytosis; FLT3, FMS-like tyrosine kinase 3; HES, hypereosinophilic syndrome; IMF, idiopathic myelofibrosis; IRM, imatinib-resistant Abl mutant; MPD, myeloproliferative disorder; NI indicates none identified; NK indicates not known; NSCLC, non-small cell lung cancer; PCV, polycythemia vera; Ph⁺ ALL, Philadelphia chromosome-positive acute lymphocytic leukemia; RCC, renal cell carcinoma; RET, rearranged during transfection; SFK, SRC family kinase.

balanced translocation in bone marrow progenitor cells that creates the Philadelphia chromosome. The protein contains part of the nonkinase domain of Bcr (a kinase of unclear function) and the kinase domain of the nonreceptor TK c-Abl. The Bcr-Abl fusion dimerizes leading to cross-phosphorylation and constitutive activation of the Abl kinase domain. This suppresses apoptosis by activating the Ras-Raf-ERK pathway (which increases antiapoptotic Bcl2 expression), the phosphatidylinositol 3-kinase (PI3K)-Akt pathway (which inhibits the proapoptotic factors Bad and FOXO3A), and STAT5 (signal transducer and activator of transcription 5) (which induces expression of antiapoptotic Bcl-x).² Imatinib blocks all Bcr-Abl-dependent signaling leading to apoptosis in CML cells: one example of oncogene addiction.

Escape from imatinib suppression of CML occurs and often leads to blast crisis. In most cases, this is attributable to spontaneous point mutations within the ATP-binding pocket of Bcr-Abl. These mutations, which arise from the inherent genomic instability of malignant cells, typically reduce binding affinity of imatinib to the ATP pocket. The power of targeted therapeutics is that knowing this, more potent agents have been identified (eg, nilotinib and dasatinib; Table 1), which inhibit Bcr-Abl and all Bcr-Abl drug resistant mutants

except the missense mutation that creates Bcr-Abl (T315I). Indeed, “intelligently” designed inhibitors can be created based on the known structure of the pocket harboring the mutation.⁹

Although not originally designed to do so, imatinib was later found to inhibit 2 additional protein kinases that are involved in a variety of malignancies: (1) c-Kit, the receptor for stem cell factor, which is overexpressed in gastrointestinal stromal tumors (GISTs) (a rare tumor of the upper gastrointestinal tract derived from cells of neuroendocrine origin) and is mutated in systemic mastocytosis; and (2) platelet-derived growth factor receptors (PDGFRs) (Table 1). Fusion proteins involving PDGFRs such as FIP1L1-PDGFR α and ETV6-PDGFR β cause rare diseases including hypereosinophilic syndromes, dermatofibrosarcoma protuberans, and chronic myelomonocytic leukemia. Mutations and overexpression of PDGFRs play key roles in other cancers including GIST and glioblastoma.

Two other approved agents, sunitinib and sorafenib, are so-called “multitargeted” agents. Although the majority of kinase inhibitors (approved or in development) inhibit multiple kinases, this term has come to mean agents that not only inhibit the mutated/overexpressed kinases driving cancer cell

growth (eg, in the case of sorafenib, c-Kit, CSF1R, and Raf family members B-Raf and Raf-1) but also inhibit tumor angiogenesis, primarily via inhibition of vascular endothelial growth factor receptors (VEGFRs) and, to a lesser extent, PDGFRs. The essential role of angiogenesis in tumor progression was originally proposed by Folkman in 1971¹⁰ and was subsequently confirmed in numerous studies. VEGFA is a central proangiogenic protein that is expressed and secreted by up to 60% of human cancers.¹¹ It acts through its cognate tyrosine kinase receptor VEGFR2 (FLK1) on endothelial cells to activate signaling pathways that promote endothelial cell proliferation and survival, orchestrated by a range of angiogenic factors that facilitate endothelial cell migration and sprout formation, resulting in tumor expansion and metastasis. Currently, the majority of antiangiogenesis cancer agents (approved or in development) are targeted to VEGFA-VEGFR2 and have proved to be very effective in some cancers. However, a substantial fraction of tumors are resistant or eventually escape. This fact led to the identification of VEGFR1 (FLT1) and its 2 ligands VEGFB and placental growth factor as novel and complementary targets for treatment of cancer.¹² Sunitinib and sorafenib have demonstrated success against several difficult to treat solid tumors including GIST, renal cell carcinoma, and hepatocellular carcinoma. As is discussed below, however, anti-VEGF therapeutics are associated with a number of adverse effects including hypertension and left ventricular (LV) dysfunction.

Mechanisms of Action of the TKIs

Small molecule kinase inhibitors typically compete with ATP for binding to the ATP pocket. If ATP cannot bind, phosphotransferase activity is blocked and downstream substrates cannot be phosphorylated, even if the kinase is fully activated. In the cell, ATP is present in mM concentrations but TKIs will be present in nanomolar to very low micromolar concentrations. Thus the compounds must bind with very high affinity. Because the structure of the ATP pocket is highly conserved across the more than 500 kinases of the human genome, it is relatively easy to make an inhibitor that blocks a kinase of interest. However, it is not surprising that lack of selectivity is an issue with most kinase inhibitors that target the ATP pocket (termed Type I inhibitors).¹³ For example, based on an assay that determines the avidity with which a drug binds to a panel of kinases, it is predicted that sunitinib inhibits at least 50 kinases.¹⁴ This can (and does) lead to so-called "off-target" effects (ie, a kinase not intended to be inhibited by a drug is inhibited and this leads to toxicity). If one of these kinases plays a critical role in the heart, the off-target toxicity may include cardiotoxicity.

The relatively poor selectivity of Type I inhibitors can be addressed by targeting additional regions of the kinase.^{15–17} The so-called type II inhibitors (eg, imatinib and the related nilotinib) do just that: they bind the ATP pocket but in addition interact with a site adjacent to the pocket. This not only allows enhanced selectivity but also allows binding to the kinase when it is in the inactive conformation. Thus, these agents are typically (though not always) more potent. In contrast, type I inhibitors typically only bind to an active kinase (because the ATP pocket is only accessible when the

kinase is activated). Type III inhibitors (eg, the families of MEK inhibitors including PD98059 and UO126) bind to regions remote from the ATP pocket. These regions are typically not highly conserved, accounting for the excellent selectivity of the aforementioned MEK inhibitors.¹⁵ Although type III agents are more selective, they are a small minority of agents in development because they are more difficult to design and not as predictably effective. With that said, there is intense interest in these types of compounds, particularly for the treatment of imatinib-resistant CML because the mutations leading to resistance are, to date, all in the kinase domain and, therefore, the mutated kinases should be sensitive to type III agents.

Although it seems logical that more selective inhibitors would be as effective as nonselective ones at treating a specific cancer, and would have less off-target toxicity, the concept of equal efficacy of selective and nonselective kinase inhibitors has been challenged both in oncological and inflammatory diseases.^{14,18} The premise is that one or more of these additional targets may also play a role in disease progression and its inhibition will lead to better anticancer efficacy. Add to this the fact that a nonselective agent can be used in more cancers (and therefore may be more lucrative), it seems safe to say that for the near future, we will be forced to identify mechanisms of cardiotoxicity of relatively nonselective drugs. This will make it essential to know the full selectivity profile of any agent against the entire kinome to be able to define mechanisms of toxicity. At present, this can routinely be done with ≈ 250 kinases or approximately half of the kinome.

Molecular Mechanisms of Cardiotoxicity

Against the backdrop of success in cancer with the small molecule inhibitors, and the belief that these targeted therapeutics would be far less toxic than traditional chemotherapy, it was something of a surprise when cardiotoxicity was detected. The first report of cardiotoxicity with a small molecule TKI was a case series of 10 patients who developed congestive heart failure while receiving imatinib.¹⁹ Subsequently, much more serious toxicity was identified in the first study specifically focused on cardiotoxicity of a TKI. In this study, serial evaluations of LV ejection fraction and biomarker determinations (troponin I) were performed in patients with GIST receiving sunitinib.²⁰ In this study, fully 18% of patients developed either congestive heart failure or a decline in LV ejection fraction of $\geq 15\%$. Subsequently cardiotoxicity has been reported with sorafenib though overall risk is unclear.²¹ It is critical to note, however, that cardiotoxicity is not a class effect of kinase inhibitors because it is only those that target essential kinases in the heart and vasculature that will likely have associated cardiotoxicity.

On-Target Toxicity

What are the underlying molecular mechanisms of this toxicity? As noted above, there are 2 types of toxicity. The first is on-target toxicity (also known as mechanism-based or target related). An ideal target in cancer would be one that serves a discrete yet critical function in the cancer cell but is dispensable for the function of all other cells.¹⁴ Unfortunately

Table 2. Kinase Targets in Cancer and Their Roles in the Cardiovascular System

Kinase	Inhibitors	Role of Kinase in the Heart/Vasculature/Models Used
Abl	Imatinib/D/N bosutinib, etc ⁹	Inhibition of Abl by imatinib induced ER stress and cell death in cardiomyocytes ^{19,22}
Growth factor receptors		
EGFR (ERBB1)	Gefitinib, erlotinib, lapatinib, XL647, BIBW-2992 ⁷	Inhibition of EGFR by erlotinib led to reduced LV function under conditions of chronic catecholamine stimulation ⁷¹
HER2 (ERBB2)	Lapatinib, XL647, BIBW-2992 ⁷	Conditional deletion of ERBB2 led to DCM ⁷²
c-Kit	Imatinib/D/N sunitinib, sorafenib vatalanib	c-Kit deficiency blocked: (1) homing of CSC to sites of post-MI injury ⁶⁷ ; (2) CSC differentiation ⁶⁵ ; and (3) cardiomyocyte terminal differentiation. ⁶⁸ Imatinib reduced stenosis after arterial injury ⁶⁵
VEGFRs	Sunitinib, sorafenib pazopanib, vandetanib, cediranib, vatalanib	VEGF trap ³¹ or inhibition ³² caused cardiac dysfunction after PO
PDGFRs	Imatinib/D/N sunitinib, sorafenib pazopanib, vatalanib	Intramyocardial delivery of PDGF improved post-MI ventricular function ⁷³ ; deletion of PDGFR β led to LV dysfunction after PO ⁶⁴
PI3K pathway		
PI3K(p110 α)	SF-1126, XL765 ³⁴	Mediates physiological heart growth and provides protection from pathological stress ³⁶
PI3K(p110 γ)	SF-1126, XL765	Regulates contractility and pathological hypertrophy ^{74,75}
Akt	VQD-002, perifosine	Regulates cardiomyocyte growth, proliferation, survival and metabolism ⁷⁶⁻⁷⁸ ; promotes CSC proliferation and expansion ⁷⁹ ; inhibits cardiac sarcolemmal Na(+)/H(+) exchanger activity ⁸⁰
PDK1	UCN-01	A dual effector for cardiac cell survival and beta-adrenergic response ³⁹
Ras/Raf/MEK/ERK pathway		
Raf-1/B-Raf	Sorafenib RAF-265	Conditional deletion/DN led to LV dilatation and HF after PO ^{62,63} ; specific gain-of-function mutations cause an HCM phenocopy ⁶¹
MEK1/2	PD-0325901, AZD-6244, ARRY-162	Regulation of cardiac hypertrophy and cell survival ⁶¹
Cell cycle regulation		
CDK	Alvocidib, BI2526 ⁴⁵	Cardiomyocyte cell cycle control in normal development and in response to injury ^{44,82}
Aurora kinases	AZD-1152	
PLKs	BI2526	
Others		
mTOR	Temsirolimus, everolimus, sirolimus, deforolimus	Central regulator of cardiac cell growth/hypertrophy ^{41,42} ; integrates energy/metabolic status
JAK2	Lestaurtinib, CP-690550, TG101348	JAK2-STAT3 generally protective, especially in I/R injury, hypertrophy, and postpartum cardiomyopathy ^{56,57}
p38	GW856553	p38 inhibition attenuated biomarkers of atherosclerosis ⁴⁹ ; reduced inflammatory burden in subjects already on statin therapy and undergoing PCI ⁵⁰

CSC indicates bone marrow-derived cardiac stem cell; DCM, dilated cardiomyopathy; D/N, dasatinib/nilotinib; DN, dominant-negative; ESC, embryonic stem cells; HCM, hypertrophic cardiomyopathy; HF, heart failure; I/R, ischemia/reperfusion; MI, myocardial infarction; PCI, percutaneous coronary intervention; PO, pressure overload.

this appears to be a very small minority of targets. More commonly, targets mediating cancer progression play key roles in other organ systems including the heart and vasculature (Table 2), and inhibiting these kinases leads to on-target toxicity. This is a fairly straightforward problem to understand but is obviously difficult to address clinically.

To illustrate on-target toxicity, we use the example of imatinib. To identify mechanisms of imatinib cardiotoxicity, Kerkela et al used neonatal rat cardiomyocytes in culture to

demonstrate that incubation of cells with imatinib, in the absence of any other stressor, led to activation of the endoplasmic reticulum (ER) stress response.¹⁹ This included sustained activation of the IRE1 kinase arm of the response, culminating in activation of the ASK1/JNK pathway and cell death (Figure 1). To identify the specific target of imatinib, inhibition of which mediated cardiomyocyte death, there were several possibilities (Figure 2): c-Abl and the highly related ARG (Abl-related gene or Abl2), PDGFRs, c-Kit, the

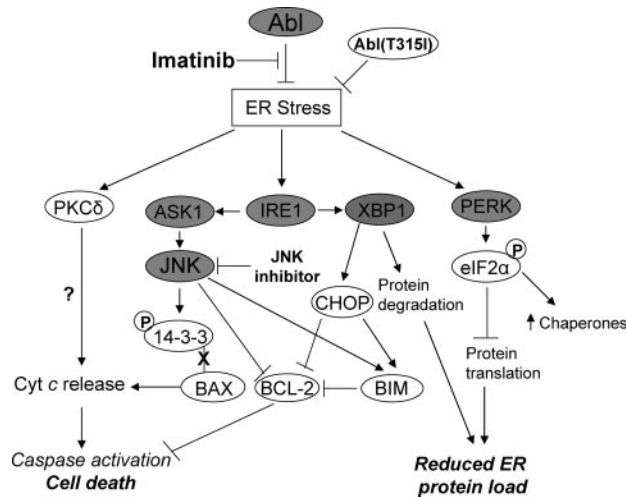


Figure 1. Proposed signaling events in the ER stress response that mediate imatinib-induced cardiomyocyte toxicity. In cardiomyocytes, Abl maintains ER homeostasis by mechanisms that are not yet clear. The Abl inhibitor imatinib induces ER stress, leading to activation of the PKR-like ER kinase (PERK) and IRE1 (a dual protein kinase and endoribonuclease) and to increased expression of PKC δ .¹⁹ PERK phosphorylates and inactivates the eIF2 α (eukaryotic translation initiation factor 2 α) as part of a protective response, shutting off general mRNA translation but selectively promoting the translation of certain mRNAs that encode ER chaperones, thereby reducing the unfolded protein load on the ER. The endoribonuclease activity of IRE1 splices the X-box-binding protein 1 (XBP1) mRNA, and the protein product induces expression of unfolded protein response (UPR) and ER-assisted degradation (ERAD) genes, leading to protein degradation and reduced ER protein load. On sustained ER stress, IRE1 activates apoptosis signal-regulating kinase 1 (ASK1), leading to c-Jun N-terminal kinase (JNK) activation, and: (1) phosphorylation of 14-3-3 proteins and release of Bax (BCL2-associated X protein), followed by cytochrome c release from mitochondria; and (2) inhibition of antiapoptotic Bcl-2 (B-cell leukemia/lymphoma 2) and activation of the proapoptotic family member Bim.⁶⁹ Also with sustained stress, the transcription factor, C/EBP homologous protein (CHOP), is induced which inhibits Bcl-2 expression and induces Bim expression. ASK1/JNK and CHOP activation contribute to caspase activation and cell death. In cultured cardiomyocytes, inhibiting JNK protects against imatinib-induced cardiomyocyte toxicity. Furthermore, when the imatinib-resistant Abl mutant T315I was expressed in cardiomyocytes, imatinib failed to induce cell death, demonstrating that imatinib-mediated inhibition of Abl was key to the toxicity. The role, if any, of increased expression of PKC δ in imatinib-induced cardiomyocyte toxicity is not known, although it is generally thought to mediate cellular injury in cardiomyocytes.

Src family member Lck, CSF1R, Cdc2, and discoidin domain receptor (DDR)1 are all inhibited by imatinib, although c-Kit and Lck are not expressed in adult cardiomyocytes. Kerkela et al used the Abl (T315I) point mutant that renders the kinase resistant to imatinib to demonstrate rescue of cardiomyocyte death following gene transfer of T315I (but not wild-type) Abl (Figure 1). Thus, the T315I mutant was used as a tool to determine that imatinib-mediated inhibition of c-Abl induced ER stress and that this played a key role in driving cell death. Supporting this as the mechanism, Fernandez et al redesigned imatinib to no longer inhibit Abl, and cardiotoxicity was not seen with this agent in mouse models.^{22,23} Although the redesigned drug was obviously ineffective in treating CML (driven by Bcr-Abl), it was equally effective to imatinib in

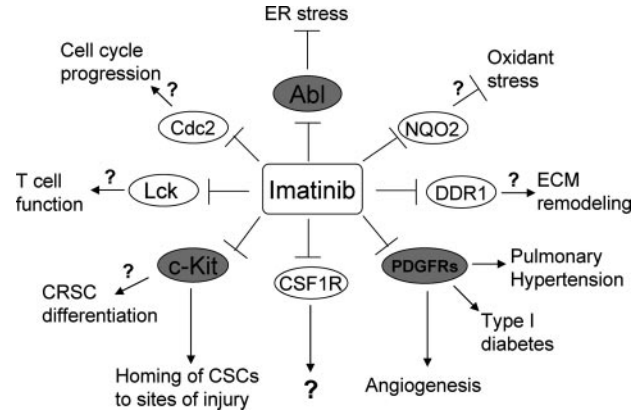


Figure 2. Real and predicted alterations in cellular functions attributable to imatinib-mediated inhibition of its various targets. Inhibition of the best known targets of imatinib (Abl, c-Kit, PDGFRs) account for the efficacy of the drug in various malignancies (see the text). However, inhibition of these kinases and others in normal cells can also have profound effects on cellular function. c-Kit has been reported to mediate cardiac resident stem cell (CRSC) differentiation⁶⁸ and homing of bone marrow-derived cardiac stem cells (CSC) to sites of endothelial injury.⁶⁷ Imatinib has been shown to block the latter, suggesting a possible protective role in post-percutaneous coronary intervention (PCI) restenosis.⁶⁵ Imatinib-mediated PDGFR inhibition (via unclear mechanisms) blocks monocrotaline-induced pulmonary hypertension⁵⁹ and appears to improve T1D in the nonobese diabetic mouse model.⁵⁸ Imatinib-mediated inhibition of other targets^{9,13} may alter T cell function (via Lck inhibition), and cell cycle progression (via Cdc2 inhibition). Inhibition of DDR1 (a kinase of unclear function) might alter extracellular matrix (ECM) remodeling, and inhibition of the oxidoreductase NCO2 might worsen oxidant stress injury. The more speculative effects of imatinib are denoted with a question mark. The role of CSF1R in the heart is not known at this point. CSF1R indicates colony-stimulating factor 1 receptor.

treating GIST models driven by c-Kit mutations. Thus, by knowing the mechanism of toxicity, and redesigning the drug accordingly, one could theoretically reduce cardiotoxicity in GIST patients. However, the on-target toxicity of imatinib-mediated inhibition of Abl appears to be unavoidable in CML patients. Fortunately, this is not a major problem clinically, but as we will discuss, with other inhibitors in development for other cancers, including inhibitors of the PI3K pathway, on-target toxicity could be very problematic. Of note, the studies with imatinib are one example of the use of TKIs to identify novel biological functions of kinases in the heart- in this case, Abl-mediated maintenance of ER homeostasis (Figure 1).

Approaches to deal with unavoidable on-target toxicity have been proposed and include (1) targeted delivery of drug specifically to the cancer, sparing normal tissue (an area of obvious intense interest); and (2) inhibiting cell death pathways in the heart that are activated by a compound but that are not necessary for tumor cell death. For the latter, JNK inhibition has been proposed as a strategy to limit imatinib-induced cardiomyocyte death without reducing antitumor efficacy (Figure 1). At present, both of the above strategies are much more theoretical than real.²²

Off-Target Toxicity

The second class of toxicity is off-target. In this case, a kinase not intended to be inhibited by the compound, plays

a key role in the heart. Often the kinase mediating the toxicity will not be known, and the complexity of identifying the key target, inhibition of which leads to cardiotoxicity, can be very difficult. This is made all the worse by multitargeting (as noted, sunitinib has ≥ 50 targets and sorafenib has ≥ 15).¹⁴ Furthermore, it is important to understand that not just kinases, but any enzyme that binds purines could also be inhibited. For example, imatinib has been shown to not only inhibit off-target kinases such as Cdc2 and DDR1 but also to inhibit the oxidoreductase NQO2 which plays a protective role in the cell's response to oxidant stress (Figure 2).¹³ We illustrate off-target toxicity using sunitinib as an example.

As noted above, sunitinib led to cardiotoxicity in a significant percentage of patients. Although the pronounced hypertension induced by this agent²⁰ almost certainly played a role in the LV dysfunction seen, this was likely an exacerbating factor rather than causal because some of the patients who developed LV dysfunction did not develop hypertension on sunitinib. Chu et al and Kerkela et al examined possible mechanisms of toxicity in cultured cardiomyocytes and found clear-cut evidence of energy compromise.^{20,24} Surprisingly, whereas this should have recruited AMP-activated protein kinase (AMPK), the master regulator of the response of the cardiomyocyte to energy stress, this did not occur in the sunitinib-treated cardiomyocytes. Kinase assays determined that sunitinib was a very potent direct inhibitor of AMPK with IC_{50} values for the catalytic (α) subunits in the very low nanomolar range, accounting for the failure to recruit the kinase.²⁴ Adenoviral-mediated gene transfer of a constitutively active α subunit of AMPK partially rescued sunitinib-induced cell death, suggesting off-target inhibition of AMPK accounted, at least in part, for the toxicity.

This example illustrates off-target kinase inhibition that may be dispensable for tumor cell killing but is important for metabolic homeostasis in the heart. As a caveat, however, AMPK has been proposed to be central to the adaptation (and survival) of not only hypoxic/energy-deprived cardiomyocytes but also energy-deprived cancer cells located in hypoxic regions of solid tumors.²⁵ Thus inhibition of AMPK might be key to effective treatment of cancers and could account, in part, for some of the success of sunitinib in solid tumors. More recently, Korennykh et al reported the surprising finding that several kinase inhibitors, including sunitinib, can directly activate the endoribonuclease activity of IRE1, leading to XBP-1 splicing and a reduction in ER stress.²⁶ This response can be expected to be cytoprotective for both stressed cancer cells and cardiomyocytes. Although the authors suggested that sunitinib be redesigned to no longer activate the endoribonuclease activity, thereby enhancing cancer cell death, this might enhance cardiotoxicity. These examples again highlight the similar critical roles played by many of these kinases in cancer progression and cardioprotection.

What Causes LV Dysfunction With TKIs and Is It Reversible?

Although it has been possible to implicate critical kinases, inhibition of which lead to cardiotoxicity, it remains unclear

whether LV dysfunction with TKIs is attributable to myocyte loss (and therefore largely irreversible) or myocyte dysfunction (potentially reversible). Neurons and cardiomyocytes appear to be quite resistant to apoptosis induced by cytochrome c release and caspase activation. Contributing to this is the decreased expression of Apaf1 that is directly linked to the tight regulation of caspase activation by endogenous XIAP.²⁷ Consistent with this, we did not see an increase in apoptosis in mice treated with sunitinib until we exposed the mice to a pressure load induced by infusion of phenylephrine via implanted minipump, and even then, the increase, although statistically significant, was modest.²⁰ In contrast, we saw clear evidence of opening of the mitochondrial permeability transition pore in the mice as evidenced by marked mitochondrial swelling and destruction of the normal mitochondrial architecture.²⁰ Strikingly, we saw a very similar picture in transmission electron micrographs of an endomyocardial biopsy obtained from a patient who presented with advanced heart failure while receiving sunitinib.^{20,24} This picture could be consistent with LV dysfunction being secondary to impaired energy generation, with or without cell death by so-called programmed necrosis. Finally, a recent study with adult rat ventricular myocytes showed that the inhibition of ErbB2 by PKI166, did not induce necrosis or apoptosis but caused myofibrillar structural damage and inhibited excitation-contraction coupling.²⁹ Although, to our knowledge this has not been described with any TKIs, if present, this could contribute to LV dysfunction.

It is clear that TKI-induced LV dysfunction can normalize after withdrawal of drug and/or institution of standard heart failure therapy,²⁰ but this is not a universal response.³⁰ Furthermore, whether any reversibility of LV dysfunction is accompanied by reversibility of injury at the myocyte level, and whether it will be long-lasting, remains unclear. With that said, in the patient noted above, there was very striking reversibility at the myocyte level with marked restoration of mitochondrial number and integrity after withdrawal of sunitinib.²⁴

One final note on mechanisms of cardiotoxicity of VEGFR inhibitors is that a number of studies in mouse models have suggested that angiogenesis is key to maintaining cardiac homeostasis in response to pressure overload^{31,32} and ischemia,³³ and this, taken together with the significant hypertension induced by VEGFR inhibitors, might explain the LV dysfunction seen with sunitinib. However, reported rates of LV dysfunction with sorafenib and bevacizumab (a monoclonal antibody that functions as a VEGF trap) are significantly lower than with sunitinib. The higher cardiotoxicity rates reported for sunitinib²⁰ could be attributable to a variety of reasons including more potent inhibition of VEGFRs, different patient selection (comorbidities, prior treatments), or better ascertainment of dysfunction resulting from closer follow-up, but, if true, more likely reflects an intrinsic difference between the agents attributable to off-target inhibition of other critical kinases by sunitinib (eg, AMPK).

Reading the Tea Leaves: Can We Predict Future Problematic Agents?

In this section, we take a target-based approach to highlight novel targets that are the present focus of drug development

in cancer and will use studies previously done in various models to raise concerns about kinase inhibitors targeting these kinases. Predicting problematic agents based on studies with gene-targeted mice can give valuable information, especially when combined with pathway analysis programs (eg, Jubilant [http://www.jubilantbiosys.com], ToxWiz [http://www.camcellnet.com], Ingenuity [http://www.ingenuity.com]). However, kinase inactive knock-ins probably would predict phenotypes better than knockouts because, for example, any scaffolding functions of the kinase would be preserved (as they likely would with drug treatment), unlike in the knockout. In addition, because drugs never inhibit their targets 100%, both the knockout and kinase-dead knock-in would likely have more severe phenotypes than any drug targeting the kinase of interest. With those caveats noted, we will proceed to examine a few key cancer targets that raise significant concerns for the heart.

The PI3K Pathway: Roles in Cancer and the Heart

Probably the most active drug development in cancer is directed at the many components that make up the PI3K pathway, and therefore we discuss this pathway, the potential implications for the heart of targeting it in cancer, and where drug development in this area stands. The PI3K pathway is very notable in that all of the major factors in the pathway have been found to be mutated or amplified in a wide range of cancers, making this pathway an ideal target in cancer therapy.³⁴ Furthermore, Yuan and Cantley, in an elegant review of theories of cancer treatment,¹⁸ proposed that redundant activation of the PI3K pathway by multiple mutations or amplifications of pathway components (ie, redundancy), combined with activation of multiple nonoverlapping pathways will, with the exception of the rare cancer that is truly oncogene-addicted to a single mutated kinase, require combination therapy. For example, one could simultaneously target PI3K, Akt, and mTOR (mammalian target of rapamycin), in addition to mutated RTKs (Figure 3). Although this is undoubtedly true, with the central role played by the PI3K pathway in cardiomyocyte survival in the setting of stress, in insulin sensitivity, and in physiological growth (driven by p110 α), concerns over aggressively targeting this pathway are obvious.

The PI3K pathway is immediately downstream (and is a critical mediator) of cellular responses initiated by mutated or amplified RTKs in cancer. These include insulin-like growth factor receptor, epidermal growth factor receptor, human epidermal growth factor receptor 2, Kit, PDGFRs, and Met, which are dysregulated in a host of cancers (Table 2). The p110 α isoform of PI3K is also commonly mutated in breast (27%), endometrial (23%), and other cancers. Three missense mutations, which are in the kinase and helical domains of p110 α , lead to constitutive activation of the kinase. p110 α is also amplified in a variety of solid tumors.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a tumor suppressor phosphatase that dephosphorylates the 3' residue of PIP3 (phosphatidylinositol 3,4,5-trisphosphate), counteracting p110 α (and other PI3K isoforms), thereby blocking activation of Akt.³⁵ Germline

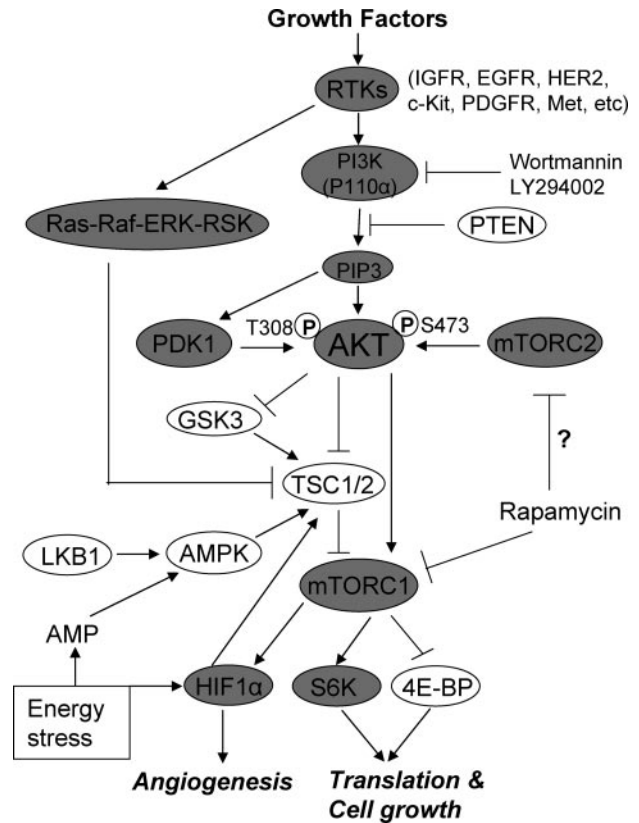


Figure 3. The extended RTK/PI3K/mTOR pathway in cancer and the heart. The pathways are essentially identical in the two systems, and primarily differ in magnitude and persistence of activation (greater in cancer cells). In cardiomyocytes, the PI3K pathway is activated by growth factors acting through RTKs. However, the pathway can also be activated in cancer by mutations or overexpression of RTKs, by mutations in p110 α or Akt, or by deletion of PTEN (see the text for details). In either system, Akt is phosphorylated at 2 key sites, T308 by PDK1 (which is constitutively active), and S473 by the mTORC2 complex. These growth signals converge on inhibition of TSC1/2, an endogenous mTORC1 inhibitor. Activation of mTORC1 promotes angiogenesis and cell growth, the latter via increased protein translation (mTORC1 has also been shown to interact with hypoxia-inducible factor 1 α [HIF1 α] and regulate its transcriptional activity, leading to angiogenesis⁷⁰). Thus, the pro-growth (and antiapoptotic) effects of activation of this pathway are conserved in both cellular systems. Disrupting the signaling pathways with kinase inhibitors leads to cancer cell death, and it is presumed, although certainly not proven, that the same may hold true for cardiomyocytes. Endogenous inhibitors of the pathway are shown with no shading and activators are shown with shading. See the text for further details. EGFR indicates epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IGFR, insulin-like growth factor; PIP3, phosphatidylinositol 3,4,5-trisphosphate.

mutations in PTEN cause hamartoma tumor syndromes, and sporadic somatic missense mutations are common in a wide variety of tumors. Loss of one allele of PTEN significantly contributes to tumor growth in the setting of other mutations. The critical role played by PTEN versus the PI3K/Akt pathway in tumorigenesis is highlighted by the fact that PTEN is second only to p53 as the most frequently affected tumor suppressor in cancers and this is particularly true in solid tumors. Of note, the effects of PTEN are not solely attributable to inhibition of the PI3K/Akt pathway because

PTEN has additional critical roles that include regulation of p53 stability and maintenance of genome stability via effects at the centromere.³⁵

Amplifications in all 3 of the Akt isoforms, as well as a common missense (E17K) mutation in the PH domain of Akt1 that leads to constitutive association with the cell membrane, and constitutive activation, have been identified in a variety of cancers. In mouse models, this mutation leads to leukemia. Of equal, if not greater, importance, Akt is potently activated by all of the mutations noted above, and is also strongly activated by mutations of Ras (particularly prevalent in colorectal cancer). Akt is key for the survival of many cancer cells via inhibitory effects on the intrinsic apoptosis pathway (Bad and FOXO3).

Not surprisingly, there has been a great deal of activity in the pharmaceutical industry to identify agents that target the PI3K pathway, and several agents are currently in clinical trials.^{18,34} Indeed the scaffolds of the well-known PI3K inhibitors, wortmannin (an irreversible inhibitor that forms a covalent bond with a key residue of the kinase) and LY294002 (a typical small molecule ATP-competitive inhibitor) were used as starting points to identify agents with superior pharmacokinetic properties. These newer agents typically inhibit all 4 class I PI3K isoforms including p110 α (the relevant target in cancer), - β , - γ (a regulator of pathological hypertrophy), and - δ . Many of these agents (eg, SF-1126, which is currently in phase I trials, and XL765) also inhibit other members of the extended PI3K family including DNA-PK (a key regulator of repair of DNA double strand breaks, especially occurring after ionizing radiation) and mTOR. Obvious concerns over cardiotoxicity exist for this class of compounds,³⁶ and it is the hope that p110 α -mutation-selective inhibitors can be made which would not inhibit wild-type p110 α . Although possible, these mutations are not near the ATP pocket and, as noted, this presents a major challenge for drug design.³⁴ Ultimately, however, we believe this may be necessary to avoid significant cardiotoxicity.

Akt has also been a major target of drug development. One compound, GSK690693, an ATP-competitive inhibitor, entered clinical trials, but one trial was withdrawn and another was terminated, although the reasons were not stated. This inhibitor also inhibited other members of the AGC kinase family (protein kinase [PK]A, PKG, and PKC), as well as the CAMK [calmodulin-dependent protein kinase] family. Allosteric inhibitors would be more selective (and likely less toxic), but, unfortunately, they appear to be less potent against the constitutively active E17K mutation identified in breast and other cancers.³⁴ As with p110 α inhibition, the critical role of Akt in cardiomyocyte survival in the setting of stress raises serious concerns over the use of these agents in patients with cardiovascular comorbidities.

Phospholipid-dependent kinase (PDK)1 is another potential target in this pathway. It is particularly attractive from an oncological perspective because it is upstream of a number of pro-growth kinases in addition to Akt, including S6K, SGK [serum/glucocorticoid regulated kinase], PKCs, and ribosomal S6 kinase (RSKs), and, unlike many other kinases in this pathway, there is only one isoform. This, and the fact that

inhibition of PDK1 prevents tamoxifen resistance in breast cancer cells, make it a kinase of significant interest. Furthermore, it is constitutively active, making it both important to inhibit and straightforward to do so with ATP-competitive inhibitors. Although mutations in PDK1 promoting cancer have not been described, because PDK1 is necessary for phosphorylation of the key T308 residue of Akt, inhibition could reduce Akt activity even in the E17K PH domain mutant. Compounds targeting PDK1 are in development and one, UCN-01 (a compound based on the structure of staurosporine which, not surprisingly, has poor selectivity), has been used in clinical trials. Of note, the compound induced insulin resistance, which could be attributable to downstream inhibition of Akt. Another drug targeting PDK1 that has been used in clinical trials (phase 2 and 3) is the cyclooxygenase-2 inhibitor celecoxib. It competes for binding to the ATP pocket, and although a relatively weak inhibitor, more active compounds have been derived.

The PDK1 KO mouse dies prematurely as a result of heart failure with thinned ventricular walls, smaller myocytes, and depressed LV function.³⁷ Lawlor et al studied a germline hypomorphic PDK1 KO (10% of normal levels of PDK1) and reported that the mice were 40% to 50% smaller than controls, including the heart, and this was attributable to inability of myocytes to undergo physiological hypertrophy.³⁸ More recently, conditional deletion of PDK1 in the heart identified key roles in coordinating both cardiomyocyte survival and responsiveness to β -adrenergic stimulation. PDK1 deletion led to profound heart failure.³⁹

The PI3K/Akt pathway converges on mTOR, which is a central regulator of cell growth, including cardiomyocyte growth, acting by regulating the protein translation machinery (mTOR complex 1 or mTORC1) and Akt phosphorylation at Ser473 (mTORC2; Figure 3).⁴⁰ Indeed, mTOR is a convergence point for pro-growth pathways (including not only PI3K/Akt but also the Ras-Raf-ERK-RSK pathway, which activate mTOR), and anti-growth pathways including AMPK and GSK-3, as well as hypoxia, energy deprivation, and reduced abundance of amino acids (all of which inhibit mTORC1 via activation of the tuberous sclerosis [Tsc1/2] complex; Figure 3). Thus, mTOR is activated in response to pro-growth stimuli and is inhibited in response to withdrawal of growth factors or energy depletion. Of note, cancer cells in which PTEN is inactivated (and, therefore, Akt and mTOR are activated) are sensitized to cell death following rapamycin-mediated inhibition of mTORC1, suggesting that the survival of cancer cells harboring PTEN mutations is dependent on intact mTOR signaling. Furthermore, deletion of PTEN in the hematopoietic system resulted in acute myeloid leukemia, and rapamycin led to a marked reduction in leukemic burden.

Although mutations in mTOR do not appear to drive transformation, and inhibitors of mTOR will typically be used in combination with inhibitors of other kinases in the pathway, the position of mTOR as a central clearinghouse that integrates pro- versus anti-growth signals makes it a very attractive target. Consistent with this, the mTOR inhibitors, temsirolimus (Torisel), everolimus (Afinitor), and sirolimus (Rapamune) are being evaluated for anticancer efficacy in

hundreds of clinical trials and are approved for treatment of advanced renal cell carcinoma (<http://www.cancer.gov/cancertopics/druginfo/fda-temsirolimus>). These agents complex with FKBP-12, thereby inhibiting mTORC1 activity. The mTOR inhibitors are now being tested in combination with sunitinib and sorafenib in renal cell carcinoma.

This PI3K/PDK1/Akt/mTOR/S6K system, more than any other, epitomizes the similarity between cancer signaling and hypertrophy/survival signaling in cardiomyocytes. Although mTOR inhibitors have been relatively well-tolerated long-term in transplant patients and have shown beneficial effects in experimental models of hypertrophy,^{41,42} there are concerns that when used in combination with therapeutics targeting other components of the pathway, the inability of mTOR to respond appropriately to the energy status of the cardiomyocyte will enhance toxicity. We believe that nowhere will the careful selection of patients and close follow-up be more important than with agents targeting the PI3K pathway.

Cell Cycle Regulators: CDKs, Polo-Like Kinase, and Aurora Kinases

Targeting cell cycle regulators is a major focus in cancer. From a cardiac perspective, it seems that inhibiting kinases involved in cell cycle regulation might be well tolerated because cardiomyocytes are generally viewed as terminally differentiated. However, data challenging this concept are well known, including a recent study which used C¹⁴ dating to conclude that 1% of cardiomyocytes turnover annually in people at the age of 25 years, dropping to 0.45% at the age of 75 years.⁴³ Despite this low annual turnover rate, it was estimated that fully 55% of myocytes in the adult heart were from new myocyte formation that occurred after birth. Furthermore, proliferation of any cardiac-resident stem cell populations or bone marrow-derived cardiac stem cells, which has been reported to increase significantly in the setting of stress, would almost certainly be inhibited by inhibitors targeting the critical Cdk: Cdk2, -4, -6 (the interphase Cdk), and CDK1 (Cdc2, the mitotic Cdk). Although a recent study shed some light on a cardioprotective role for Cdk2 inhibition *in vivo* during ischemia/reperfusion injury,⁴⁴ in general, the consequences of Cdk inhibition in the adult heart are simply not clear.

Possibly of more (at least theoretical) concern is inhibition of the mitotic regulators of the Aurora and polo-like kinase (PLK) families. Aurora kinases A and B control chromosomal alignment and segregation during mitosis and are required to preserve genomic stability. Both regulate the spindle assembly checkpoint.⁴⁵ Inhibition of Aurora B abrogates the spindle assembly checkpoint, resulting in inappropriate M phase progression, polyploidy, and eventually apoptosis or senescence. PLK1 (by far the best understood member of the family) activates the CDK1–cyclin B complex at the G₂-to-M phase transition and is necessary for spindle formation, chromosome segregation, and cytokinesis. Inhibition of PLK1 induces prolonged mitotic arrest and subsequent apoptosis. Numerous Aurora and PLK inhibitors are in development with several in clinical trials.

Why should inhibition of Aurora and PLK be of concern in the heart? Whereas the importance of cardiomyocyte and cardiac stem cell proliferation continues to be debated, DNA duplication in cardiomyocytes, followed by karyokinesis with or without cytokinesis, is a well-described phenomenon that, at least in animal models, is increased in the setting of stress. Both karyokinesis and cytokinesis will be dysregulated by agents targeting Aurora and PLK. Furthermore, when M phase is disrupted, the cell typically undergoes apoptosis. With this backdrop, there is some cause for concern about even these agents that target kinases that would seem to be dispensable in the heart.

Kinase Inhibitors for Other Disease States

Small molecule kinase inhibitors are also in development for diseases in addition to cancer. In this section, we will briefly review these agents, focusing on their real or potential uses for the treatment of cardiovascular diseases.

Inflammatory Diseases

p38 Mitogen-Activated Protein Kinase Inhibition

Initial efforts to develop kinase inhibitors to treat inflammatory diseases focused on the p38 mitogen-activated protein kinase (MAPK) after it was discovered to be a key enzyme in the biosynthesis of the proinflammatory cytokines tumor necrosis factor- α and interleukin-1 β .⁴⁶ Although p38 MAPK was expected to be an ideal target for the treatment of chronic inflammatory diseases such as rheumatoid arthritis,⁴⁷ several well-established pyridinyl-imidazole inhibitors of p38 showed liver toxicity in animal studies. Nevertheless, attempts have been made to use p38 MAPK inhibitors for cardiovascular disease because chronic inflammation is believed to contribute to the pathogenesis.⁴⁸ Recent preclinical studies demonstrated that a specific p38 MAPK inhibitor, SB-239063, prevented endothelial dysfunction, blunted angiotensin II-induced hypertension and cardiac hypertrophy, and attenuated atherosclerotic plaque inflammation.⁴⁹ More recently, data were presented showing that when a p38 MAPK inhibitor was given at low doses over 3 months to patients with cardiovascular disease who were already receiving statin therapy, C-reactive protein declined and vascular reactivity improved.⁵⁰ Another p38 MAPK inhibitor, GW856553, has entered a phase 2 clinical trial in patients with acute coronary syndrome (<http://clinicaltrials.gov/ct2/show/NCT00910962?term=solstice&rank=1>). These studies support further investigation into the potential role of p38 MAPK inhibitors as a therapeutic strategy for atherosclerotic disease.

JAK/STAT Inhibition

Janus kinase (JAK) inhibitors, a new class of immunosuppressive drugs, are being extensively studied in various disease states. There are four JAKs (Jak1, Jak2, Jak3, and Tyk2), which respond to numerous cytokines and transmit signals from cytokine receptors to the downstream STAT proteins. Selective Jak3 inhibitors have been proposed to be superior to cyclosporine and corticosteroids in the treatment of autoimmune diseases and transplant rejection.¹⁴ A novel Jak3 inhibitor, CP-690550, is in clinical trials for rheumatoid

arthritis, psoriasis, Crohn's disease and renal transplant rejection.⁵¹

In the cancer field, interest in Jak2 has been driven by the findings that human myeloproliferative disorders such as polycythemia vera, essential thrombocytosis, and idiopathic myelofibrosis are associated with activating mutations in Jak2. Most myeloproliferative disorder patients carry an activating point mutation (V617F), which appears to act at least in part by activating STAT3 and STAT5 in leukemic cells, leading to cytokine hypersensitivity and enhanced proliferation.⁵² Among various Jak2 inhibitors that are in clinical trials for myeloproliferative disorders, lestaurtinib and dasatinib have been reported to have activity against mutated Jak2 and are under evaluation for treatment of polycythemia vera. Recently, selective Jak2 inhibitors TG101348,⁵³ and CP-690550,⁵⁴ which have relatively little activity against wild type Jak2 but show stronger activity in cells with mutated Jak2, were tested in preclinical models for V617F-induced polycythemia vera and demonstrated efficacy. These agents will likely advance to clinical trials.

Despite the potential of JAK inhibition for the treatment of immunologic diseases and myeloproliferative disorders, JAK/STAT signaling is generally believed to be protective in the heart, where 3 JAKs (JAK1, JAK2, and Tyk2), but not JAK3, and all 7 STAT proteins are present.⁵⁵ The protective role of JAK-STAT signaling in the heart has been extensively reviewed,^{56,57} especially the role of Stat3 in ischemia/reperfusion injury, hypertrophy, and postpartum cardiomyopathy. These pleiotropic roles of JAK/STAT signaling in the heart raise some concerns and, going forward, these agents will need to be watched.⁵⁷

Diabetes: Type 1 Autoimmune Diabetes Mellitus

In addition to its antitumor effects, imatinib has been shown to have antiinflammatory effects in various mouse models.^{14,58} Indeed, case reports and phase 1 studies in humans have demonstrated a positive effect of imatinib on several autoimmune diseases such as rheumatoid arthritis and psoriasis. Moreover, imatinib was recently reported to improve type 1 autoimmune diabetes mellitus (T1D) in nonobese diabetic mice, apparently by inhibiting PDGFR because specific neutralization of PDGF reversed T1D (Figure 2).⁵⁸ Similar results were observed with sunitinib (which also inhibits PDGFRs), suggesting that selective kinase inhibitors may prove to be a valid therapeutic for treatment of T1D.

Pulmonary Hypertension/Cardiac Hypertrophy

Studies with animal models have suggested that imatinib and related agents could have important applications in pulmonary hypertension by means of effects attributed to the inhibition of PDGFR signaling (Figure 2). Specifically, imatinib reversed monocrotaline-induced pulmonary hypertension.⁵⁹ Furthermore, sorafenib, which in addition to PDGFRs also inhibits the Raf/MEK/ERK pathway, reduced pulmonary artery remodeling and right ventricular hypertrophy more than imatinib in the monocrotaline model.⁶⁰ The authors concluded that sorafenib exerts additional direct myocardial antihypertrophic effects, which appeared to be mediated via inhibition of the Raf/MEK/ERK pathway. The data suggest

that the combined inhibition of PDGFRs and Raf may provide an option to treat pulmonary hypertension and the associated right heart remodeling. Raf pathway inhibition could also find a use in the therapy of certain cases of Noonan or Leopard syndromes, in which specific Raf pathway mutations that increase extracellular signal-regulated kinase (ERK) activation result in hypertrophic cardiomyopathy.⁶¹ However, caution is clearly indicated based on studies showing that deletion or inhibition of Raf-1^{62,63} or deletion of PDGFR β ⁶⁴ in the heart leads to left heart failure in the setting of pressure overload.

Post-Arterial Injury Restenosis

Several studies suggest that TKIs might also have therapeutic potential for restenosis after vascular injury/percutaneous coronary intervention (Figure 2). One such study showed that imatinib administered after femoral artery injury reduced intimal hyperplasia, and this was attributed to the inhibition of c-Kit in progenitor cells and consequent prevention of their homing to the injured vascular wall and differentiation to smooth muscle cells.⁶⁵ Additionally, in a balloon injury model of the rat carotid artery, imatinib inhibited intimal hyperplasia and arterial stenosis in a dose-dependent manner without blocking endothelial cell repair, an adverse effect of drug eluting stents.⁶⁶ This effect of imatinib was presumed, but not proven, to be mediated by inhibition of PDGFR signaling. On the other hand, c-Kit-deficient mice demonstrated worsened post-myocardial infarction remodeling and reduced cardiac function.⁶⁷ Furthermore, c-Kit is required *in vivo* for cardiac stem cell and cardiomyocyte differentiation.⁶⁸ Therefore, although agents targeting c-Kit and PDGFRs might be used to reduce restenosis after arterial injury, they might have detrimental effects in the context of post-myocardial infarction remodeling or pressure stress.

Use of Agents to Identify Novel Functions of Kinases in the Heart: Chemical Genetics

The biological functions of many kinases in the heart are entirely unclear. The availability of TKIs with excellent pharmacokinetic and pharmacodynamic properties sets up the possibility of defining these roles, with the major limitation again being poor selectivity. However, by using combinations of inhibitors that all inhibit the kinase of interest, but that have different off-target selectivity profiles, one can identify specific roles played by the kinase of interest. Applications could be in anything from the adult heart, to the developing heart, to studying the biology of various stem cell populations. We believe that this will become an important use of these agents as we try to divine the role of the ≈ 250 kinases for which biological functions are unknown.

In conclusion, drug development in the cancer arena has produced an explosion of new agents. At this point in time, preclinical testing is unable to accurately identify all (or maybe even most) agents that may have versus will not have associated cardiotoxicity. With a thorough knowledge of the spectrum of kinases inhibited by a specific inhibitor, and where possible the role the targeted kinases play in the heart, one can identify agents that raise concerns. The limitations remain: (1) lack of a full selectivity profile (coverage of

available commercial platforms is approximately half of the kinome), but this is a deficit that should be corrected shortly; (2) lack of knowledge concerning the role played by many kinases in the heart (a deficit that will not be corrected rapidly); and (3) lack of an understanding of what role prior or concurrent therapy with traditional chemotherapeutics plays in cardiotoxicity. Thus, there will continue to be surprises, but presumably over time, the number of surprises will shrink to manageable numbers. With (1) advances in drug design, 2) greater awareness of the problem, (3) better cooperation among clinical and basic cardiologists and oncologists and the pharmaceutical industry in the early phases of drug development, and (4) better preclinical screening approaches, we believe cardiotoxicity of cancer therapeutics will become a very manageable problem. There will, however, continue to be unavoidable instances of cardiotoxicity because of the similar prosurvival roles the kinases play in both cancer cells and cardiomyocytes. It will be essential to identify effective strategies to address this central issue of on-target toxicity.

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References

- Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. *N Engl J Med*. 2005;353:172–187.
- Force T, Krause DS, Van Etten RA. Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition. *Nat Rev Cancer*. 2007;7:332–344.
- Norman P. Kinase therapeutic pipelines: an assessment of targets and agents in development. *Insight Pharma Reports*. 2007. Available at http://www.insightpharmareports.com/reports/2007/90_Kinase_Inhibitors/overview.asp.
- Schwab M, Klemmner KH, Alitalo K, Varmus H, Bishop M. Rearrangement at the 5' end of amplified c-myc in human COLO 320 cells is associated with abnormal transcription. *Mol Cell Biol*. 1986;6:2752–2755.
- Haber DA, Settleman J. Cancer: drivers and passengers. *Nature*. 2007;446:145–146.
- Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell*. 2009;136:823–837.
- Ma WW, Adjei AA. Novel agents on the horizon for cancer therapy. *CA Cancer J Clin*. 2009;59:111–137.
- Sherbenou DW, Druker BJ. Applying the discovery of the Philadelphia chromosome. *J Clin Invest*. 2007;117:2067–2074.
- Quintas-Cardama A, Kantarjian H, Cortes J. Flying under the radar: the new wave of BCR-ABL inhibitors. *Nat Rev Drug Discov*. 2007;6:834–848.
- Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med*. 1971;285:1182–1186.
- Folkman J. Angiogenesis: an organizing principle for drug discovery? *Nat Rev Drug Discov*. 2007;6:273–286.
- Loges S, Schmidt T, Carmeliet P. “Antimyoangiogenic” therapy for cancer by inhibiting PlGF. *Clin Cancer Res*. 2009;15:3648–3653.
- Bantscheff M, Eberhard D, Abraham Y, Bastuck S, Boesche M, Hobson S, Mathieson T, Perrin J, Rida M, Rau C, Reader V, Sweetman G, Bauer A, Bouwmeester T, Hopf C, Kruse U, Neubauer G, Ramsden N, Rick J, Kuster B, Drewes G. Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat Biotechnol*. 2007;25:1035–1044.
- Ghoreschi K, Laurence A, O’Shea JJ. Selectivity and therapeutic inhibition of kinases: to be or not to be? *Nat Immunol*. 2009;10:356–360.
- Ohren JF, Chen H, Pavlovsky A, Whitehead C, Zhang E, Kuffa P, Yan C, McConnell P, Spessard C, Banotai C, Mueller WT, Delaney A, Omer C, Sebolt-Leopold J, Dudley DT, Leung IK, Flamme C, Warmus J, Kaufman M, Barrett S, Teclé H, Hasemann CA. Structures of human MAP kinase kinase 1 (MEK1) and MEK2 describe novel noncompetitive kinase inhibition. *Nat Struct Mol Biol*. 2004;11:1192–1197.
- Okram B, Nagle A, Adrian FJ, Lee C, Ren P, Wang X, Sim T, Xie Y, Wang X, Xia G, Spraggon G, Warmuth M, Liu Y, Gray NS. A general strategy for creating “inactive-conformation” abl inhibitors. *Chem Biol*. 2006;13:779–786.
- Sebolt-Leopold JS, Dudley DT, Herrera R, Van Becelaere K, Wiland A, Gowan RC, Teclé H, Barrett SD, Bridges A, Przybranowski S, Leopold WR, Saltiel AR. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. *Nat Med*. 1999;5:810–816.
- Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene*. 2008;27:5497–5510.
- Kerkela R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, Van Etten RA, Alroy J, Durand JB, Force T. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med*. 2006;12:908–916.
- Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L, Woulfe K, Pravda E, Cassiola F, Desai J, George S, Morgan JA, Harris DM, Ismail NS, Chen JH, Schoen FJ, Van den Abbeele AD, Demetri GD, Force T, Chen MH. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. *Lancet*. 2007;370:2011–2019.
- Schmidinger M, Zielinski CC, Vogl UM, Bojic A, Bojic M, Schukro C, Ruhsam M, Hejna M, Schmidinger H. Cardiac toxicity of sunitinib and sorafenib in patients with metastatic renal cell carcinoma. *J Clin Oncol*. 2008;26:5204–5212.
- Fernández A, Sanguino A, Peng Z, Ozturk E, Chen J, Crespo A, Wulf S, Shavrin A, Qin C, Ma J, Trent J, Lin Y, Han HD, Mangala LS, Bankson JA, Gelovani J, Samarel A, Bornmann W, Sood AK, Lopez-Berestein G. An anticancer c-Kit kinase inhibitor is reengineered to make it more active and less cardiotoxic. *J Clin Invest*. 2007;117:4044–4054.
- Demetri GD. Structural reengineering of imatinib to decrease cardiac risk in cancer therapy. *J Clin Invest*. 2007;117:3650–3653.
- Kerkela R, Woulfe KC, Durand JB, Vagnozzi R, Kramer D, Chu TF, Beahm C, Chen MH, Force T. Sunitinib-induced cardiotoxicity is mediated by off-target inhibition of AMP-activated protein kinase. *Clin Transl Sci*. 2009;1.
- Liu L, Cash TP, Jones RG, Keith B, Thompson CB, Simon MC. Hypoxia-induced energy stress regulates mRNA translation and cell growth. *Mol Cell*. 2006;21:521–531.
- Korenykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, Shokat KM, Stroud RM, Walter P. The unfolded protein response signals through high-order assembly of Ire1. *Nature*. 2009;457:687–693.
- Potts MB, Vaughn AE, McDonough H, Patterson C, Deshmukh M. Reduced Apaf-1 levels in cardiomyocytes engage a strict regulation of apoptosis by endogenous XIAP. *J Cell Biol*. 2005;171:925–930.
- Deleted in proof.
- Pentassuglia L, Graf M, Lane H, Kuramochi Y, Cote G, Timolati F, Sawyer DB, Zuppinger C, Suter TM. Inhibition of ErbB2 by receptor tyrosine kinase inhibitors causes myofibrillar structural damage without cell death in adult rat cardiomyocytes. *Exp Cell Res*. 2009;315:1302–1312.
- Khakoo AY, Kassiotis CM, Tannir N, Plana JC, Halushka M, Bickford C, Trent J II, Champion JC, Durand JB, Lenihan DJ. Heart failure associated with sunitinib malate: a multitargeted receptor tyrosine kinase inhibitor. *Cancer*. 2008;112:2500–2508.
- Izumiya Y, Shiojima I, Sato K, Sawyer DB, Colucci WS, Walsh K. Vascular endothelial growth factor blockade promotes the transition from compensatory cardiac hypertrophy to failure in response to pressure overload. *Hypertension*. 2006;47:887–893.
- Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, Akazawa H, Tateno K, Kayama Y, Harada M, Shimizu I, Asahara T, Hamada H, Tomita S, Molkenin JD, Zou Y, Komuro I. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature*. 2007;446:444–448.
- May D, Gilon D, Djonov V, Itin A, Lazarus A, Gordon O, Rosenberger C, Keshet E. Transgenic system for conditional induction and rescue of chronic myocardial hibernation provides insights into genomic programs of hibernation. *Proc Natl Acad Sci U S A*. 2008;105:282–287.
- García-Echeverría C, Sellers WR. Drug discovery approaches targeting the PI3K/Akt pathway in cancer. *Oncogene*. 2008;27:5511–5526.

35. Liu W, Zhou Y, Reske SN, Shen C. PTEN mutation: many birds with one stone in tumorigenesis. *Anticancer Res.* 2008;28:3613–3619.
36. McMullen JR, Jay PY. PI3K(p110alpha) inhibitors as anti-cancer agents: minding the heart. *Cell Cycle.* 2007;6:910–913.
37. Mora A, Davies AM, Bertrand L, Sharif I, Budas GR, Jovanovic S, Mouton V, Kahn CR, Lucocq JM, Gray GA, Jovanovic A, Alessi DR. Deficiency of PDK1 in cardiac muscle results in heart failure and increased sensitivity to hypoxia. *EMBO J.* 2003;22:4666–4676.
38. Lawlor MA, Mora A, Ashby PR, Williams MR, Murray-Tait V, Malone L, Prescott AR, Lucocq JM, Alessi DR. Essential role of PDK1 in regulating cell size and development in mice. *EMBO J.* 2002;21:3728–3738.
39. Ito K, Akazawa H, Tamagawa M, Furukawa K, Ogawa W, Yasuda N, Kudo Y, Liao CH, Yamamoto R, Sato T, Molkenin JD, Kasuga M, Noda T, Nakaya H, Komuro I. PDK1 coordinates survival pathways and beta-adrenergic response in the heart. *Proc Natl Acad Sci U S A.* 2009;106:8689–8694.
40. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J.* 2008;412:179–190.
41. Shioi T, McMullen JR, Tarnavski O, Converso K, Sherwood MC, Manning WJ, Izumo S. Rapamycin attenuates load-induced hypertrophy in mice. *Circulation.* 2003;107:1664–1670.
42. Siedlecki AM, Jin X, Muslin AJ. Uremic cardiac hypertrophy is reversed by rapamycin but not by lowering of blood pressure. *Kidney Int.* 2009;75:800–808.
43. Parmacek MS, Epstein JA. Cardiomyocyte renewal. *N Engl J Med.* 2009;361:86–88.
44. Liem DA, Zhao P, Angelis E, Chan SS, Zhang J, Wang G, Berthet C, Kaldis P, Ping P, MacLellan WR. Cyclin-dependent kinase 2 signaling regulates myocardial ischemia/reperfusion injury. *J Mol Cell Cardiol.* 2008;45:610–616.
45. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. *Nat Rev Drug Discov.* 2009;8:547–566.
46. Lee JC, Laydon JT, McDonnell PC, Gallagher TF, Kumar S, Green D, McNulty D, Blumenthal MJ, Keys JR, Land vatter SW, Strickler JE, McLaughlin MM, Siemens IR, Fisher SM, Livi GP, White JR, Adams JL, Young PR. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature.* 1994;372:739–746.
47. Karcher SC, Laufer SA. Aza-analogue dibenzepinone scaffolds as p38 mitogen-activated protein kinase inhibitors: design, synthesis, and biological data of inhibitors with improved physicochemical properties. *J Med Chem.* 2009;52:1778–1782.
48. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 2005;352:1685–1695.
49. Morris JB, Olzinski AR, Bernard RE, Aravindhan K, Mirabile RC, Boyce R, Willette RN, Jucker BM. p38 MAPK inhibition reduces aortic ultrasmall superparamagnetic iron oxide uptake in a mouse model of atherosclerosis: MRI assessment. *Arterioscler Thromb Vasc Biol.* 2008;28:265–271.
50. Sarov-Blat L, Morgan J, Fernandez P, James R, Lepore JJ, Fang Z, Sprecher DL. Inhibition of p38MAPK reduces inflammatory burden in subjects undergoing elective PCI. *J Am Coll Cardiol.* 2009;53:A419–A458.
51. West K. CP-690550, a JAK3 inhibitor as an immunosuppressant for the treatment of rheumatoid arthritis, transplant rejection, psoriasis and other immune-mediated disorders. *Curr Opin Investig Drugs.* 2009;10:491–504.
52. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med.* 2005;352:1779–1790.
53. Wernig G, Kharas MG, Okabe R, Moore SA, Leeman DS, Cullen DE, Gozo M, McDowell EP, Levine RL, Doukas J, Mak CC, Noronha G, Martin M, Ko YD, Lee BH, Soll RM, Tefferi A, Hood JD, Gilliland DG. Efficacy of TG101348, a selective JAK2 inhibitor, in treatment of a murine model of JAK2mu617F-induced polycythemia vera. *Cancer Cell.* 2008;13:311–320.
54. Manshour T, Quintas-Cardama A, Nussenzveig RH, Gaikwad A, Estrov Z, Prchal J, Cortes JE, Kantarjian HM, Verstovsek S. The JAK kinase inhibitor CP-690,550 suppresses the growth of human polycythemia vera cells carrying the JAK2V617F mutation. *Cancer Sci.* 2008;99:1265–1273.
55. Xuan YT, Guo Y, Han H, Zhu Y, Bolli R. An essential role of the JAK-STAT pathway in ischemic preconditioning. *Proc Natl Acad Sci U S A.* 2001;98:9050–9055.
56. Boengler K, Hilfiker-Kleiner D, Drexler H, Heusch G, Schulz R. The myocardial JAK/STAT pathway: from protection to failure. *Pharmacol Ther.* 2008;120:172–185.
57. Kurd M, Booz GW. Can the protective actions of JAK-STAT in the heart be exploited therapeutically? Parsing the regulation of interleukin-6-type cytokine signaling. *J Cardiovasc Pharmacol.* 2007;50:126–141.
58. Louvet C, Szot GL, Lang J, Lee MR, Martinier N, Bollag G, Zhu S, Weiss A, Bluestone JA. Tyrosine kinase inhibitors reverse type 1 diabetes in nonobese diabetic mice. *Proc Natl Acad Sci U S A.* 2008;105:18895–18900.
59. Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, Sydykov A, Lai YJ, Weissmann N, Seeger W, Grimminger F. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest.* 2005;115:2811–2821.
60. Klein M, Schermuly RT, Ellinghaus P, Milting H, Riedel B, Nikolova S, Pullamsetti SS, Weissmann N, Dony E, Savai R, Ghofrani HA, Grimminger F, Busch AE, Schafer S. Combined tyrosine and serine/threonine kinase inhibition by sorafenib prevents progression of experimental pulmonary hypertension and myocardial remodeling. *Circulation.* 2008;118:2081–2090.
61. Pandit B, Sarkozy A, Pennacchio LA, Carta C, Oishi K, Martinelli S, Pogna EA, Schackwitz W, Ustaszewska A, Landstrom A, Bos JM, Ommen SR, Esposito G, Lepri F, Faul C, Mundel P, Lopez Siguero JP, Tenconi R, Selicorni A, Rossi C, Mazzanti L, Torrente I, Marino B, Digilio MC, Zampino G, Ackerman MJ, Dallapiccola B, Tartaglia M, Gelb BD. Gain-of-function RAF1 mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy. *Nat Genet.* 2007;39:1007–1012.
62. Yamaguchi O, Watanabe T, Nishida K, Kashiwase K, Higuchi Y, Takeda T, Hikoso S, Hirota S, Asahi M, Taniike M, Nakai A, Tsujimoto I, Matsumura Y, Miyazaki J, Chien KR, Matsuzawa A, Sadamitsu C, Ichijo H, Baccarani M, Hori M, Otsu K. Cardiac-specific disruption of the c-raf-1 gene induces cardiac dysfunction and apoptosis. *J Clin Invest.* 2004;114:937–943.
63. Harris IS, Zhang S, Treskov I, Kovacs A, Weinheimer C, Muslin AJ. Raf-1 kinase is required for cardiac hypertrophy and cardiomyocyte survival in response to pressure overload. *Circulation.* 2004;110:718–723.
64. Khakoo AY. Regulation of hypertrophic responses by PDGFRβ. American Heart Association, Basic Cardiovascular Sciences Annual Meeting, Lake Las Vegas, Nev, July 20–23, 2009.
65. Wang CH, Anderson N, Li SH, Szmitko PE, Cherng WJ, Fedak PW, Fazel S, Li RK, Yau TM, Weisel RD, Stanford WL, Verma S. Stem cell factor deficiency is vasculoprotective: unraveling a new therapeutic potential of imatinib mesylate. *Circ Res.* 2006;99:617–625.
66. Makiyama Y, Toba K, Hirono S, Ozawa T, Saigawa T, Minagawa S, Isoda M, Asami F, Ikarashi N, Oda M, Moriyama M, Higashimura M, Kitajima T, Otaki K, Aizawa Y. Imatinib mesilate inhibits neointimal hyperplasia via growth inhibition of vascular smooth muscle cells in a rat model of balloon injury. *Tohoku J Exp Med.* 2008;215:299–306.
67. Ayach BB, Yoshimitsu M, Dawood F, Sun M, Arab S, Chen M, Higuchi K, Siatskas C, Lee P, Lim H, Zhang J, Cukerman E, Stanford WL, Medin JA, Liu PP. Stem cell factor receptor induces progenitor and natural killer cell-mediated cardiac survival and repair after myocardial infarction. *Proc Natl Acad Sci U S A.* 2006;103:2304–2309.
68. Li M, Naqvi N, Yahiro E, Liu K, Powell PC, Bradley WE, Martin DI, Graham RM, Dell'Italia LJ, Husain A. c-kit is required for cardiomyocyte terminal differentiation. *Circ Res.* 2008;102:677–685.
69. Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nat Rev Drug Discov.* 2008;7:1013–1030.
70. Land SC, Tee AR. Hypoxia-inducible factor 1alpha is regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif. *J Biol Chem.* 2007;282:20534–20543.
71. Noma T, Lemaire A, Naga Prasad SV, Barki-Harrington L, Tilley DG, Chen J, Le Corvoisier P, Violin JD, Wei H, Lefkowitz RJ, Rockman HA. Beta-arrestin-mediated beta1-adrenergic receptor transactivation of the EGFR confers cardioprotection. *J Clin Invest.* 2007;117:2445–2458.
72. Crone SA, Zhao YY, Fan L, Gu Y, Minamisawa S, Liu Y, Peterson KL, Chen J, Kahn R, Condorelli G, Ross J Jr, Chien KR, Lee KF. ErbB2 is essential in the prevention of dilated cardiomyopathy. *Nat Med.* 2002;8:459–465.

73. Hsieh PC, MacGillivray C, Gannon J, Cruz FU, Lee RT. Local controlled intramyocardial delivery of platelet-derived growth factor improves postinfarction ventricular function without pulmonary toxicity. *Circulation*. 2006;114:637–644.
74. Crackower MA, Oudit GY, Koziaradzki I, Sarao R, Sun H, Sasaki T, Hirsch E, Suzuki A, Shioi T, Irie-Sasaki J, Sah R, Cheng H-YM, Rybin VO, Lembo G, Fratta L, Oliveira-dos-Santos AJ, Benovic JL, Kahn CR, Izumo S, Steinberg SF, Wyman MP, Backx PH, Penninger JM. Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. *Cell*. 2002;110:737–749.
75. Patrucco E, Notte A, Barberis L, Selvetella G, Maffei A, Brancaccio M, Marengo S, Russo G, Azzolino O, Rybalkin SD, Silengo L, Altruda F, Wetzker R, Wyman MP, Lembo G, Hirsch E. PI3K γ modulates the cardiac response to chronic pressure overload by distinct kinase-dependent and -independent effects. *Cell*. 2004;118:375–387.
76. Muslin AJ, DeBosch B. Role of Akt in cardiac growth and metabolism. *Novartis Found Symp*. 2006;274:118–126.
77. Shiojima I, Yefremashvili M, Luo Z, Kureishi Y, Takahashi A, Tao J, Rosenzweig A, Kahn CR, Abel ED, Walsh K. Akt signaling mediates postnatal heart growth in response to insulin and nutritional status. *J Biol Chem*. 2002;277:37670–37677.
78. Matsui T, Nagoshi T, Hong EG, Luptak I, Hartil K, Li L, Gorovits N, Charron MJ, Kim JK, Tian R, Rosenzweig A. Effects of chronic Akt activation on glucose uptake in the heart. *Am J Physiol Endocrinol Metab*. 2006;290:E789–E797.
79. Gude N, Muraski J, Rubio M, Kajstura J, Schaefer E, Anversa P, Sussman MA. Akt promotes increased cardiomyocyte cycling and expansion of the cardiac progenitor cell population. *Circ Res*. 2006;99:381–388.
80. Snabaitis AK, Cuello F, Avkiran M. Protein kinase B/Akt phosphorylates and inhibits the cardiac Na⁺/H⁺ exchanger NHE1. *Circ Res*. 2008;103:881–890.
81. Bueno OF, Molkentin JD. Involvement of extracellular signal-regulated kinases 1/2 in cardiac hypertrophy and cell death. *Circ Res*. 2002;91:776–781.
82. Ahuja P, Sdek P, MacLellan WR. Cardiac myocyte cell cycle control in development, disease, and regeneration. *Physiol Rev*. 2007;87:521–544.