Mechanisms of Cardiac Dysfunction Associated With Tyrosine Kinase Inhibitor Cancer Therapeutics

Ming Hui Chen, MD, MMSc; Risto Kerkelä, MD, PhD; Thomas Force, MD

Treatment of patients with cancer has changed radically over the last several years with the advent of "targeted therapeutics." Whereas traditional chemotherapy was directed at all rapidly dividing cells, whether cancerous or not, today's anticancer drugs are increasingly tailored to the specific genetics of each cancer. This targeted approach, predominantly via inhibition of tyrosine kinase activity, has markedly improved the management of cancers including chronic myeloid leukemia (CML), breast cancer, gastrointestinal stromal tumor (GIST), renal cell carcinoma (RCC), and colon carcinoma.[1–5]

Inhibitors of tyrosine kinases are of 2 classes: monoclonal antibodies (mAbs), typically targeting growth factor receptor tyrosine kinases, and small molecules, referred to as tyrosine kinase inhibitors (TKIs), targeting both receptor and nonreceptor tyrosine kinases. The goal of targeted therapy is to improve antitumor activity with fewer toxic side effects than traditional anticancer therapies; given the initial success of this approach, the number of targeted therapy drugs entering into development in the last 5 years has increased dramatically.[6,7] However, several recent studies have revealed unanticipated side effects of targeted therapy, including left ventricular (LV) dysfunction and heart failure, the primary manifestations of cardiotoxicity we will be examining here.[5,8,9]

Herein, we will examine the potential risk of LV dysfunction of targeted therapy and the molecular mechanisms that underlie that risk. We will review the importance of tyrosine kinase signaling pathways both for oncogenesis and for the survival of normal cardiomyocytes. To understand basic mechanisms of cardiomyopathy of TKIs, it is critical to understand 2 general classes of toxicity. The first is "on-target" toxicity, wherein the tyrosine kinase target regulating cancer cell survival and/or proliferation (and therefore a good target in cancer therapy) also serves an important role in normal cardiomyocyte survival, and thus inhibition leads to myocardial dysfunction. "Off-target" toxicity occurs when a TKI leads to toxicity via inhibition of a kinase not intended to be a target of the drug. This type of toxicity is intrinsically related to 2 issues: (1) the inherent nonselectivity of TKIs and (2) a trend toward "multitargeting" or purposefully designing drugs to inhibit a broad range of targets that include kinases regulating both tumorigenesis and tumor angiogenesis. Although multitargeting may broaden efficacy of an anticancer agent, likelihood of toxicity would also increase.

With the growing number of Food and Drug Administration (FDA)–approved agents and many more in development,[6,7] some of these will inhibit novel kinase targets for which little or no clinical data exist on risk of heart failure or LV dysfunction. Therefore, we will also review basic science studies that raise concerns over the potential risk of LV dysfunction in patients treated with drugs that inhibit these kinases. Finally, we will discuss cardiovascular considerations for development of future targeted therapy that may maximize antitumor effects while minimizing cardiac effects in patients being treated with these potentially life-saving medications.

Tyrosine Kinases in Signal Transduction

Response to extracellular and intracellular stimuli is vital for all complex living organisms. Activation of signal transduction cascades allows a relatively small stimulus to be amplified into a larger biological response, such as the reprogramming of gene expression.[10] Tyrosine kinases, of which there are ≈90 in the human genome,[11] play central roles in transducing extracellular signals (ie, growth factors and cytokines) into activation of signaling pathways that regulate cell growth, differentiation, metabolism, migration, and programmed cell death (apoptosis). Tyrosine kinases are families of enzymes that catalyze transfer of a phosphate residue from ATP to tyrosine residues in other proteins (substrates). Phosphorylation can change factors such as the activity, subcellular location, and stability of the phosphorylated substrate protein.

There are 2 major classes of tyrosine kinases. Receptor tyrosine kinases (RTKs) are embedded in the cell membrane with an extracellular ligand-binding domain and an intracellular kinase domain that signals to the interior of the cell. In contrast, nonreceptor tyrosine kinases (NRTKs) are located within the cell. By their location, tyrosine kinases can mediate

From the Cardiology Department, Children's Hospital Boston, Boston, Mass (M.H.C.); Department of Medicine, Divisions of Cardiology and Women's Health, Brigham and Women's Hospital, Harvard Medical School, Boston, Mass (M.H.C.); Center for Translational Medicine and Cardiology Division, Jefferson Medical College, Philadelphia, Pa (R.K., T.F.); and Program in Cell and Developmental Biology, Jefferson College of Graduate Studies, Philadelphia, Pa (T.F.).

Correspondence to Thomas Force, MD, Center for Translational Medicine, College Building, Room 316, 1025 Walnut St, Philadelphia, PA 19107. E-mail thomas.force@jefferson.edu

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Table 1. Kinase Inhibitors in Cancer

Agent	Class	Target(s)	Malignancies	Cardiovascular Toxicity/(Rate)/Type
Imatinib (Gleevec)	TKI	Abl1/2, PDGFRα/β, Kit	CML, Ph$^+$ B-cell ALL, CMML, HES, GIST	Yes/(low)‡/HF
Dasatinib (Sprycel)	TKI	Abl1/2, PDGFRα/β, Kit, SRC family	CML	Yes/(low to moderate)‡/HF, generalized edema
Nilotinib (Tasigna)	TKI	Abl1/2, PDGFRα/β, Kit	CML	Yes/(low)‡/QT prolongation, rare sudden death
Sunitinib (Sutent)	TKI	VEGFR1/2/3, Kit, PDGFRα/β, RET, CSF-1R, FLT3	RCC, GIST	Yes/(moderate)/HF, hypertension
Lapatinib (Tykerb)	TKI	EGFR (ERBB1), ERBB2	HER2$^+$ breast cancer	No
Sorafenib (Nexavar)	TKI S/TKI	Raf-1/B-Raf, VEGFR2/3, PDGFRα/β, Kit, FLT3	RCC, melanoma	Yes/(low)‡/ACS, hypertension, HF
Gefitinib (Iressa)	TKI	EGFR (ERBB1)	NSCLC	No‡
Erlotinib (Tarceva)	TKI	EGFR (ERBB1)	NSCLC, pancreatic cancer	No‡
Temsirolimus (Torisel)	Novel	mTOR (indirect; binds to FKBP12 and complex inhibits mTOR)	RCC	No‡
Trastuzumab (Herceptin)	mAb	ERBB2	HER2$^+$ breast cancer	Yes/(moderate)/HF
Bevacizumab (Avastin)	mAb	VEGF-A	Colorectal cancer, NSCLC	Yes/(low to moderate)‡/arterial thrombosis, hypertension
Cetuximab (Erbitux)	mAb	EGFR (ERBB1)	Colorectal cancer, squamous cell carcinoma of head/neck	No‡
Panitumumab (Vectibix)	mAb	EGFR (ERBB1)	Colorectal	No‡
Rituximab (Rituxan)	mAb	CD20	B-cell lymphoma	Unknown
Alemtuzumab (Campath)	mAb	CD52	B-cell CLL	Yes (in patients with mycosis fungoides/Sézary syndrome[13])/HF
Lestaurtinib*	TKI	JAK2/FLT3	PCV, IMF	Unknown
Pazopanib*	TKI	VEGFRs; PDGFRs; Kit	RCC	Unknown
Vandetanib*	TKI	VEGFR/EGFR	NSCLC	Unknown
Cediranib†	TKI	VEGFR	NSCLC	Unknown
Alvocidib†	S/TKI	CDK	CLL	Unknown
Enzastaurin†	S/TKI	PKCβ	B-cell lymphoma	Unknown

mAb indicates humanized monoclonal antibody; S/TKI, serine/threonine kinase inhibitor; ALL, acute lymphocytic leukemia; CMML, chronic myelomonocytic leukemia; HES, hypereosinophilic syndrome; NSCLC, non–small-cell lung cancer; CLL, chronic lymphocytic leukemia; PCV, polycythemia vera; IMF, idiopathic myelofibrosis; mTOR, mammalian target of rapamycin; PKC, protein kinase C; HF, heart failure; CDK, cyclin-dependent kinase; and ACS, acute coronary syndrome. Please see text for additional abbreviations. For agents not yet FDA approved, efficacy in malignancies is projected.

*NDA expected 2008.

†NDA expected 2010.

‡Effect on left ventricular ejection fraction has not been determined, and therefore these represent best guesses.

transduction of both extracellular and intracellular signals. Because of their critical role in normal cellular communication and maintenance of homeostasis, tyrosine kinase activity is tightly regulated.[10] Tyrosine kinases are normally quiescent until activated by extracellular stimuli or ligands, such as growth factors (eg, vascular endothelial growth factor [VEGF] and platelet-derived growth factor [PDGF]) or intracellular stimuli (such as oxidant stress, activating NRTKs). An exquisite balance between activity of tyrosine kinases and of tyrosine phosphatases, which mediate dephosphorylation of tyrosine residues and therefore act in opposition to kinases, controls the timing and duration of cell signaling.

Abnormal Tyrosine Kinase Activity and Cancer: Malignant Transformation and Tumor Angiogenesis

Tyrosine kinase signaling is central to both the malignant transformation of cells and tumor angiogenesis.[12] Malignant transformation often results from dysregulation of tyrosine kinase signaling. Constitutive activation (ie, ongoing, even in the absence of an activating signal) of tyrosine kinases has been implicated in ≈70% of cancers (Table 1).[12] In leukemias and solid cancers, the gene encoding the causal (or contributory) kinase is either amplified or mutated; the former leads to overexpression of the kinase and the latter to a constitutively activated state. Both mechanisms drive proliferation of the cancerous clonal cells and/or prevent them from undergoing apoptosis.

CML is a classic example of a cancer that results from a genetic mutation that creates a constitutively active tyrosine kinase. Four decades ago, Peter Nowell first described the association of CML with the Philadelphia chromosome, which is created by a balanced translocation in myeloid precursors. The Philadelphia chromosome encodes a fusion protein of the Bcr (breakpoint cluster region) protein kinase and the NRTK Abl (named after Herbert Abelson, who first

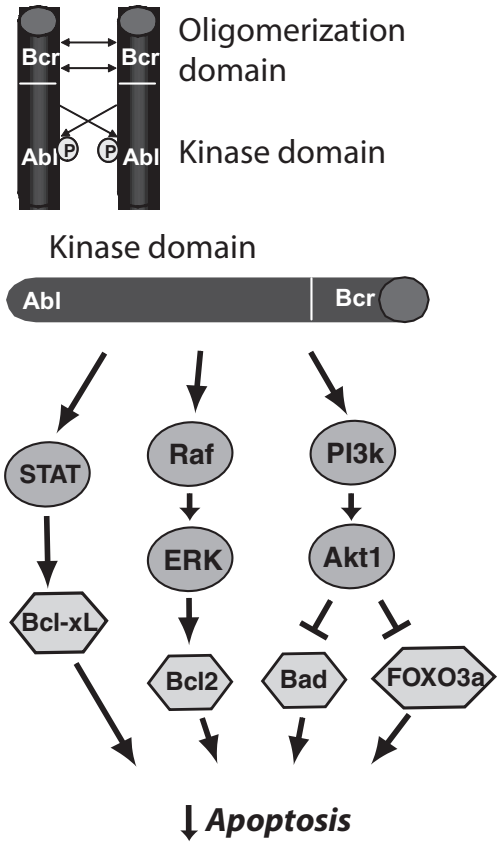

Figure 1. Mechanisms of carcinogenesis of Bcr-Abl in CML. Oligomerization and cross-phosphorylation (P) of Bcr-Abl fusion proteins (top) lead to constitutive activation of the Abl kinase domain. This leads (bottom) to activation of 3 key prosurvival pathways: STAT5, which leads to increased expression of antiapoptotic Bcl-X_L; the Raf→ERK (or mitogen-activated protein kinase) pathway, which increases expression of antiapoptotic Bcl2; and the phosphoinositide-3 kinase→Akt pathway, a major antiapoptotic pathway in cancer cells and cardiomyocytes, that inhibits proapoptotic factors FOXO3a and Bad. This culminates in potent inhibition of apoptosis in CML cells.[12]

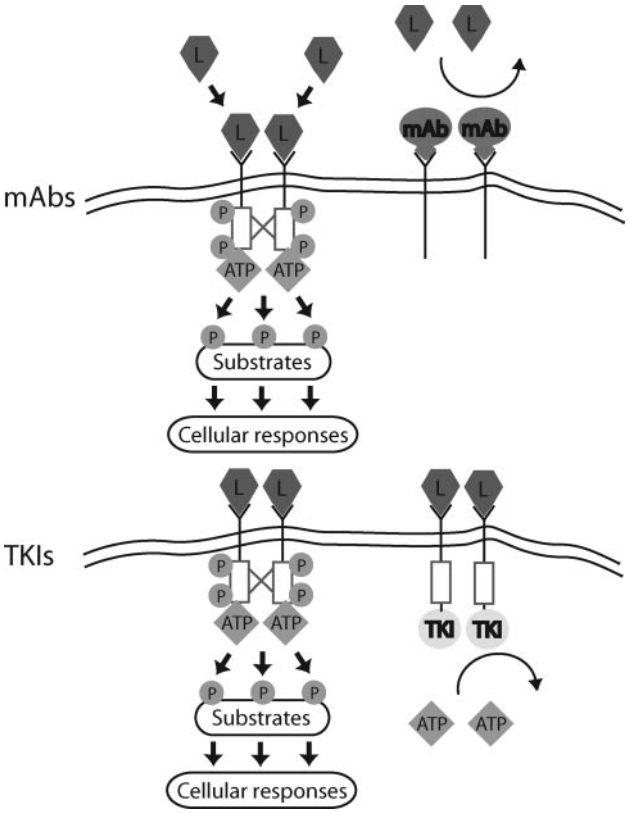

Figure 2. Mechanisms of action of mAbs vs small-molecule TKIs. Ligand (L) binding to RTKs leads to receptor dimerization, cross-phosphorylation (red lines and P), and activation of the intracellular tyrosine kinase domain (red boxes). Substrates are then phosphorylated, leading to cellular responses. mAbs (top) interfere with ligand binding to receptor and/or receptor dimerization and cross-phosphorylation, blocking activation of the RTKs.[17] TKIs (bottom) do not prevent ligand binding or dimerization, but by preventing ATP from binding to the kinase domain, they block cross-phosphorylation of receptors and phosphorylation of substrates.

identified v-Abl, a viral oncogene in mice encoding the kinase).[12] The Bcr-Abl fusion protein spontaneously forms homodimers consisting of 2 Bcr-Abl proteins that interact via the Bcr domains (Figure 1). This leads to constitutive activation of the Abl kinase portion of Bcr-Abl. Dimers of Bcr-Abl then activate multiple downstream signaling pathways that result primarily in inhibition of apoptosis in CML cells (Figure 1).[12,14]

In addition to driving tumorigenesis, tyrosine kinase signaling also plays a central role in mediating tumor angiogenesis. The vital role of tumor angiogenesis was proposed >3 decades ago by the pioneer Judah Folkman.[15] Cancers more than a few millimeters in size are dependent on formation of new microvessels for their continued growth and ability to metastasize.[15] Almost all of the proangiogenic growth factors that were subsequently identified (ie, VEGF, placental growth factor [PlGF], PDGF, transforming growth factor-α, and fibroblast growth factor) are ligands of RTKs.

Inhibitors of Tyrosine Kinases as Anticancer Agents

The dependency of certain cancers on 1 or just a few genes for maintenance of the malignant phenotype, termed *oncogene addiction*, provides a rationale for molecular targeting in cancer therapy.[16] Inhibition of aberrant tyrosine kinase activity has become a central and exciting focus of anticancer therapy. Two classes of targeted tyrosine kinase therapeutics have been developed: humanized mAbs and small-molecule TKIs[17] (Table 1; Figure 2). mAbs are antibodies produced from a single parent clonal cell. For anticancer therapy, mAbs are designed to bind the cancer cell–specific antigens, commonly to the extracellular portion of RTKs, thereby inhibiting tyrosine kinase activation (Figure 2). The binding of mAbs to the extracellular domain of the RTKs can block ligand binding to the receptor, inhibit subsequent dimerization and activation of the tyrosine kinase domain, and/or induce downregulation of expression of the receptor.[17] Furthermore, mAbs also may induce an immune response against the targeted tumor cell. An example of a mAb that binds to receptors is trastuzumab (Herceptin; Genentech), which binds to the ERBB2 receptor (also known as HER2; Table 1). Other mAbs do not bind to the kinase receptors themselves but instead bind the growth factor ligands that activate the receptors. For example, bevacizumab (Avastin; Genentech) targets VEGF-A, thereby preventing it from interacting with

the VEGF receptor and leading to inhibition of tumor angiogenesis.

The vast majority of small-molecule inhibitors used in cancer therapy are directed at tyrosine kinases, although some target serine/threonine kinases, the other superfamily of kinases involved in intracellular signaling (see below). Small-molecule inhibitors have been designed to target both classes of tyrosine kinases: RTKs and NRTKs. Inhibitors of RTKs block activity of the intracellular kinase domain. Normally, ligand binding to a RTK initiates dimerization and cross-phosphorylation of one kinase domain by the other, thereby activating the kinase (Figure 2). The activated kinase dimer then phosphorylates downstream substrates in a signaling cascade that ultimately results in changes such as altered gene expression and cell proliferation. TKIs can directly inhibit the cross-phosphorylation of the kinase domains and also inhibit phosphorylation of downstream substrates, thereby terminating the signaling cascade. TKIs that block signaling by NRTKs (eg, Abl) target intracellular kinases and work in a fashion similar to those that target RTKs.

TKIs can block substrate phosphorylation in 3 ways (Okram et al[18] and references therein). Substrate phosphorylation is dependent on the binding of both ATP and the substrate to an activated kinase. Type I inhibitors (eg, sunitinib) compete with ATP for binding to the ATP pocket of a fully activated kinase and are by far the dominant type in use today. However, they generally lack selectivity because of the highly conserved structure of the ATP pocket across the >500 kinases in the human genome and thus typically inhibit several kinases. Type II inhibitors (eg, imatinib and nilotinib) bind 2 different regions on the kinase: the ATP pocket and an adjacent region that is accessible only when the kinase is inactive. Type II inhibitors thus bind and lock kinases in an inactive state. Type II TKIs generally are more potent and more selective than type I. However, type II agents still typically inhibit ≥3 kinases (Table 1). Type III inhibitors (eg, the archetypal extracellular signal-regulated kinase [ERK] pathway inhibitors PD98059 and U0126) bind to sites remote from the ATP pocket, such as the substrate recognition region (blocking binding of substrate to kinase), or other regions of kinases that are much more divergent across the genome. Consequently, type III inhibitors promise to be the most selective. Despite their potential for greater selectivity, however, type III inhibitors represent a small minority of TKIs in development because they are more difficult to design and not as predictably effective. Overall, TKIs are inherently less selective than mAbs and typically inhibit several kinases, some known and others not.

One particular subgroup of TKIs is the so-called multitargeted agents. Theoretically, agents that inhibit growth factors or their receptors involved in angiogenesis, as well as kinases involved in tumor cell proliferation, could have very broad anticancer activity arising from this dual pharmacological effect.[19] This concept led to the development of the multitargeted agents sorafenib (Nexavar, Onyx-Bayer) and sunitinib (Sutent, Pfizer). This strategy, and its real and potential problems, will be discussed in detail below.

Success With Targeted Therapeutics

Monoclonal antibodies and small-molecule TKIs have validated the "oncogene addiction theory" and greatly improved the management of certain cancers. Trastuzumab was the first FDA-approved targeted anticancer agent, and it was approved for the treatment of women with metastatic ERBB2-positive breast cancer. Overexpression of the ERBB2 receptor was associated with more poorly differentiated tumors, higher rates of metastases, and poorer patient survival.[20] Trastuzumab, a recombinant, humanized, IgG mAb that binds to the extracellular domain of ERBB2, causes growth inhibition and apoptosis of tumor cells expressing ERBB2.[12] In women with metastatic breast cancer, addition of trastuzumab to standard chemotherapy resulted in a 20% decrease in risk of death among patients at 1 year.[5] Furthermore, in 5 phase III randomized trials of women with early stage ERBB2-positive breast cancer, trastuzumab used in the adjuvant setting after standard chemotherapy reduced the risk of disease recurrence at 3 years.[20]

At about the same time, imatinib (Gleevec, Novartis), a drug that inhibits Bcr-Abl, the causal factor in 90% of the cases of CML, dramatically improved the survival of patients with this disease,[4] leading to its approval by the FDA in 2001. Ninety percent of patients with CML treated with imatinib are alive 5 years after diagnosis of a disease that was uniformly fatal before this targeted therapy.[4]

On the basis of the success of trastuzumab and imatinib, the development of targeted therapeutics has exploded in the last few years. Currently, there are 29 FDA-approved agents that inhibit kinase activity, 21 mAbs and 8 TKIs (Table 1).[6,7] Three New Drug Application (NDA) filings for TKIs are expected in 2008 and an additional 3 in 2010 (Table 1). However, this number is truly the tip of the iceberg because there are ≈175 mAbs and 150 TKIs in clinical trials, with many more in preclinical development.[6,7] Currently, there are ≈600 agents somewhere between discovery and market, with 80% being developed as anticancer agents. Although sales of TKIs were only ≈$4 billion in 2005–2006, with imatinib accounting for $2.5 billion, sales of TKIs are projected to grow substantially in the next few years.[6]

Cardiac Side Effects

Targeted anticancer drugs were initially thought to affect tumors but not normal tissue in which kinases were not constitutively active. Thus, the hope of targeted therapy was high efficacy with minimal side effects. As with many drugs, clinical trials have revealed unanticipated side effects of targeted therapies involving the heart and other organs.[21] Cardiovascular side effects have, in general, been able to be managed medically and typically have not prevented their use. Still, the need to use TKIs on a long-term basis emphasizes the importance of knowing which agents are associated with cardiac effects and understanding mechanisms that underlie that toxicity.

Cardiovascular side effects of TKIs are varied and have included heart failure, LV dysfunction, conduction abnormalities, QT prolongation, acute coronary syndromes, myocardial injury, arterial thromboses, and hypertension[8,9,14,22] (see Yeh[23] for additional toxicities with cancer therapeutics in

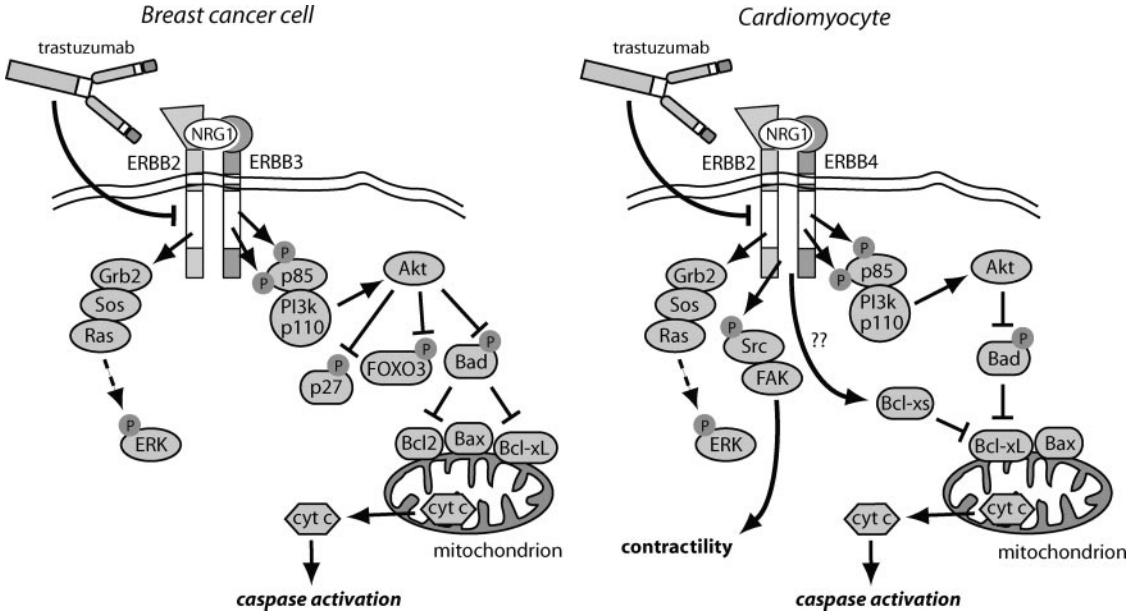

Figure 3. Comparison of ERBB2 signaling and its inhibition by trastuzumab in breast cancer cells vs cardiomyocytes.[14] In breast cancer cells overexpressing ERBB2, ERBB2 homodimers or ERBB2/ERBB3 heterodimers form, leading to constitutive activation of the ERK, PI3K/Akt, and STAT5 pathways (latter not shown). Akt blocks apoptosis by phosphorylating and inhibiting 2 key proapoptotic factors, Bad and FOXO3A, and also inactivates the cyclin-dependent kinase inhibitor p27, thereby enhancing cell proliferation. Trastuzumab blocks all downstream signaling, but particularly important may be reversing the inhibition of Bad, leading to activation of Bax, cyctochrome *c* (cyt c) release, and apoptosis. In cardiomyocytes exposed to Nrg1, ERBB2/ERBB4 heterodimers form, again activating ERK and Akt. Trastuzumab blocks this activation and, via multiple mechanisms including alterations in levels of Bcl-X family members,[48] leads to mitochondrial dysfunction, energy compromise, and cytochrome *c* release. Trastuzumab also blocks Nrg1-mediated activation of Src and Fak, and this appears to worsen left ventricular dysfunction.[49]

general). Overall, systolic dysfunction with resultant heart failure is one of the most common important side effects. This often occurs because pathways that induce the pathological survival and abnormal proliferation of cancer cells may also regulate the survival of normal cells, including cardiomyocytes. Targeting these pathways in cancer cells may inherently lead to on-target cardiotoxicity, manifest as cardiomyopathy, because of inhibition of these same prosurvival kinases in normal cardiomyocytes. We will introduce examples of on-target cardiotoxicity of the widely used drugs trastuzumab and imatinib and probable off-target toxicity of another popular agent, sunitinib, to illustrate molecular mechanisms of cardiotoxicity.

Trastuzumab

The classic example of on-target cardiotoxicity of tyrosine kinase inhibition may be the cardiac effects of trastuzumab. As noted above, trastuzumab is directed at the ERBB2 RTK.[5,24] Activation of ERBB2 leads to activation of intracellular signaling pathways in breast cancer cells similar to those seen with Bcr-Abl including ERK, PI3K/Akt, and signal transducer and activator of transcription 5 (STAT5), which drive tumor cell growth and prevent apoptosis (Figure 3).[12,14] Trastuzumab is very effective in blocking activation of these signaling pathways in ERBB2$^+$ breast cancer cells.

In a pivotal phase III clinical trial of trastuzumab efficacy, the addition of trastuzumab to anthracycline improved survival in women with metastatic breast cancer.[5] However, cardiotoxicity was an unanticipated finding, with 27% of patients treated with the regimen of anthracycline, cyclophosphamide, and trastuzumab developing heart failure. Several large studies subsequently confirmed the importance of trastuzumab in increasing disease-free survival from cancer but also confirmed the association with heart failure (References 20 and 25 and references therein). When anthracyclines were not administered concurrently with trastuzumab, the incidence of LV dysfunction decreased to 13% in patients previously treated with anthracycline and then treated with paclitaxel and trastuzumab. In the adjuvant trials, 1.7% to 4.1% of trastuzumab-treated patients developed congestive heart failure.[25] The incidence of cardiotoxicity of trastuzumab in nontrial settings is beginning to be examined. Recently, McArthur and Chia[26] reported a 20% risk of left ventricular dysfunction in patients being treated with trastuzumab after anthracycline therapy when treated off trial.

The finding of trastuzumab-induced heart failure in breast cancer patients led to a search for the molecular mechanisms of this effect. There was abundant evidence in mice that ERBB2 and its activating ligand, neuregulin-1 (Nrg1), play important roles during cardiac development. Germline deletion of ERBB2[27] or Nrg1[28] in mice is lethal in mid gestation with failure of the ventricles to form properly, suggesting that ERBB2 signaling is required for cardiomyocyte proliferation during development. Mice with cardiac-specific deletion of ERBB2, after cardiac development was complete, were viable. However, these mice developed dilated cardiomyopathy as they aged and had decreased survival when subjected to pressure overload induced by aortic banding (Table 2).[29,30] Cardiomyocytes from these mice also exhibited enhanced sensitivity to anthracyclines, explaining in part the enhanced toxicity of the combination in patients.[29]

Table 2. Evidence From Experimental Models Suggesting Cardiotoxicity of TKIs by Tyrosine Kinase Target

Tyrosine Kinase Target(s)	TKIs	Model	Cardiac Phenotype of Model	References
ERBB2	Trastuzumab, lapatinib	ERBB2 KO: ±TAC	Spontaneous dilated cardiomyopathy; worsened heart failure with pressure load; enhanced anthracycline sensitivity	29, 30
VEGF, VEGFRs	Sunitinib, sorafenib, bevacizumab	WT: VEGF trap+TAC	Pathological remodeling in response to pressure load	31–33
KIT	Imatinib/dasatinib, nilotinib, sunitinib, sorafenib	(1) W/W^v mouse (Kit-deficient)+MI; (2) WT: arterial injury+imatinib	(1) Adverse remodeling after MI due to reduced homing of bone marrow stem cells to sites of injury; (2) reduced stenosis after arterial injury	34–36
Raf-1/B-Raf	Sorafenib	Raf-1 KO and dominant negative+TAC	LV dilatation and HF with pressure load	37, 38
PDGFRs	Imatinib/dasatinib, nilotinib, sunitinib, sorafenib	WT: MI+administration of PDGF	Reduced injury (ischemic protection)	39–41
JAK2	Lestaurtinib	STAT3 KO: MI; aging; anthracycline administration; pregnancy	Increased ischemic injury; reduced capillary density with aging; increased anthracycline toxicity; peripartum cardiomyopathy	42, 43
Abl/Arg	Iimatinib/dasatinib, nilotinib	WT: imatinib	Decline in LV function; induction of ER stress	9, 44
Met (HGF receptor)	N/A	WT: MI or cardiomyopathy models+administration of HGF	Reduced fibrosis in MI and cardiomyopathy models; neoangiogenesis with HGF	45 and references therein
FGFR1/3	N/A	Cell culture models: administration of FGF	Enhanced proliferation of cardiomyocytes and cardiac-resident stem cells	46 and references therein

N/A indicates not available; WT, wild-type; KO, knockout, gene deleted; HGF, hepatocyte growth factor (ligand for Met); FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; MI, myocardial infarction; LV, left ventricular; HF, heart failure; and TAC, thoracic aortic constriction. See text for other abbreviations.

As in cancer cells, Nrg1-induced activation of ERBB2 in cardiomyocytes activates the ERK and PI3K/Akt pathways that promote cardiomyocyte proliferation during development and cardiomyocyte survival during adulthood (Figure 3).[47] The Src/focal adhesion kinase (Fak) pathway, which enhances cardiac contractility, is also activated by Nrg1.[49] Expression of the antiapoptotic protein Bcl-X_L in hearts of newborn mice by adenoviral gene transfer partially prevented the heart chamber dilation and the impaired contractility seen in the adult.[29] Thus inhibition of ERBB2 signaling appears to lead to dysfunction and death both in breast cancer cells overexpressing the ERBB2 receptor and in normal cardiomyocytes (ie, on-target toxicity).

Surprisingly, unlike trastuzumab, lapatinib (Tykerb, GlaxoSmithKline), the small-molecule dual inhibitor of ERBB2 and epidermal growth factor receptor (EGFR) (also known as ERBB1; Table 1), shows limited depression of cardiac function.[50,51] Whereas future trials will be important to confirm this apparent absence of cardiac dysfunction, inherent differences in mechanism of action between mAbs and TKIs may also contribute to the different cardiotoxicity profiles reported between trastuzumab and lapatinib. mAbs, as opposed to small-molecule inhibitors, initiate antibody-dependent cell cytotoxicity and complement-dependent cytotoxicity that could augment cardiotoxicity.[17] Furthermore, differential inhibition/activation by lapatinib versus trastuzumab of downstream signaling pathways may also contribute to the difference in observed rates of heart failure and LV dysfunction. For example, lapatinib activates the cytoprotective AMP-activated protein kinase in cardiomyocytes, whereas trastuzumab does not.[52] It is obviously critical to identify mechanisms of the apparent difference in cardiotoxicity with the 2 agents because it could significantly affect approaches to treatment of patients with ERBB2-positive breast cancer.

Imatinib

Imatinib is indicated for the treatment of Philadelphia chromosome–positive CML and acute lymphocytic leukemia; GIST, driven by activating mutations in either c-Kit (the receptor for stem cell factor) or PDGF receptor-α (PDGFRα); and chronic myelomonocytic or eosinophilic leukemias due to PDGFR mutations (Table 1).[12,14] Although not common,[53] imatinib-associated heart failure does occur.[9,54,55] In mice, imatinib causes modest, but consistent, declines in left ventricular function, but more striking was a loss of myocardial mass, consistent with cell loss. In cardiomyocytes in culture, imatinib led to cell death with features of both apoptosis and necrosis.[9]

The pathway mediating cell death appeared to be induction of the endoplasmic reticulum (ER) stress response,[56] in which a buildup of misfolded proteins in the ER induces cellular apoptosis (Figure 4).[9,57] Imatinib also induces ER stress in CML cells and in GIST cells, and this is believed to play a role in inducing their death.[58] The c-Jun N-terminal kinase (JNK) family of stress-activated mitogen-activated protein kinases functions as a key downstream pathway activated by

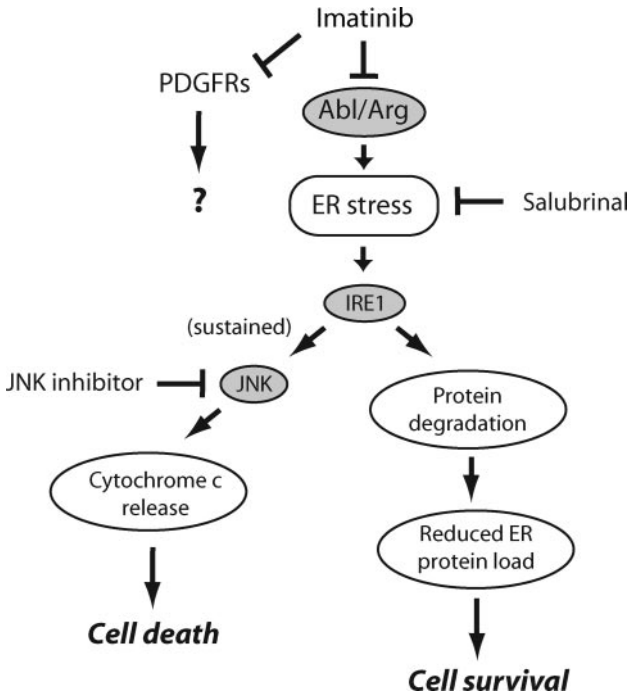

Figure 4. Pathways of imatinib-induced cardiomyocyte toxicity. Imatinib, via inhibition of Abl/Arg, leads via unclear mechanisms to induction of ER stress. This activates protein kinase IRE1, which upregulates factors involved in degradation of misfolded proteins in the ER, thereby attempting to restore homeostasis.[56] If ER stress is sustained, however, JNKs are activated, leading to activation of the intrinsic apoptosis program and cell death. Both salubrinal, an inhibitor of ER stress,[57] and JNK inhibition by a peptide antagonist protected against imatinib cardiomyocyte toxicity.[9] The role, if any, of inhibition of PDGFRs in imatinib-induced cardiotoxicity is unknown at this time.

the ER stress response that ultimately mediates cell death (Figure 4). Of note, imatinib-induced cell death was prevented by both salubrinal, a compound that protects cells from ER stress,[57] and JNK inhibition.[9]

TKIs often inhibit several tyrosine kinases, and it is important to identify the key target whose inhibition induces cell death. Imatinib targets Abl, ARG (Abl-related gene), PDGFRα/β, and c-Kit, but because c-Kit is not expressed in adult cardiomyocytes, the toxicity was presumably due to inhibition of either Abl/ARG, PDGFRs, or an unknown kinase. However, because lentivirus-mediated expression of an imatinib-resistant mutant of Abl blocked imatinib toxicity in cardiomyocytes, inhibition of Abl appeared to be the mechanism of imatinib cardiotoxicity.[9] This information led Fernandez and coworkers[44] to engineer a variant of imatinib, WBZ4, that no longer inhibited Abl but retained activity against c-Kit. Therefore, WBZ4 retained efficacy comparable to imatinib in treating mouse models of GIST (driven by c-Kit) but did not have associated cardiac dysfunction, presumably because Abl was not inhibited.[44] This type of approach could prove instrumental for future design of kinase inhibitors; however, the caveat is that redesign will be impossible in situations in which the kinase driving cancer progression is also critical for cardiomyocyte function or survival. In these situations, cardiotoxicity may be unavoidable, although treatable, it is hoped, if physicians are aware of the potential problem.

Sunitinib: A Multitargeted TKI

Sunitinib targets VEGF receptor (VEGFR)1–3, PDGFRα/β, c-Kit, FMS-like tyrosine kinase-3, colony-stimulating factor-1 receptor (CSF-1R), and the product of the human RET gene (RET, mutated in medullary thyroid carcinomas/multiple endocrine neoplasias).[19,59] Cardiac events (myocardial infarction, heart failure, or cardiovascular death) were observed in 11% of patients with imatinib-resistant GIST treated with sunitinib for a median of 30.5 weeks (range, 10.7 to 84.9), highlighting that cardiotoxicity with TKIs can take months to develop.[8] Heart failure developed in 8%, and 19% had a decline in LVEF of 15 EF% or more. Hypertension (>150/100 mm Hg) developed in 47%. Endomyocardial biopsy of the 2 index patients with heart failure showed cardiomyocyte hypertrophy and mitochondrial abnormalities but no cardiomyocyte necrosis and no fibrosis or inflammation. Importantly, the majority of patients were able to resume sunitinib after withholding therapy and institution of standard heart failure management. Studies in vitro and in mouse models showed significant mitochondrial dysfunction, and cardiomyocyte apoptosis was present only when sunitinib-treated animals were also hypertensive.[8]

Given the planned multitargeting of sunitinib and the lack of selectivity of type I TKIs in general, the cardiomyopathy may reflect on- and/or off-target effects. The mechanisms of sunitinib-associated LV dysfunction are not known at this time. However, it is important to identify these mechanisms given the efficacy of sunitinib in GIST and RCC.

How to Assess New Agents in Development

One obvious question is whether LV dysfunction associated with TKIs is a class effect, analogous to that which is observed with anthracyclines. However, it is probably misleading to refer to the TKIs as a class because, unlike β-blockers or angiotensin receptor antagonists, the specific targets, and thus the biological effects, vary widely across the members of the TKI "class." Thus, each agent must be understood on a case-by-case basis, focusing on the specific kinases inhibited. With that understood, it is probably not surprising that LV dysfunction does not seem to be common to all (or probably even most) TKIs, as illustrated by the apparent lack of cardiotoxicity with EGFR inhibitors (Table 1). In addition, many TKI targets are not known to be expressed in the heart (eg, RET, FLT3, CSF-1R), and therefore cardiotoxicity is unlikely with agents targeting these kinases. However, there are a large number of TKIs on the horizon with targets that are expressed in the heart, for which we do not have (and may never have) good prospective data concerning cardiac effects. What strategies can identify agents that might induce cardiac dysfunction versus those not likely to? We believe that the best approach is to take a target-focused approach to examine what is known from the basic science literature that might raise concerns about cardiotoxicity of TKIs directed at popular kinase targets in cancer (Table 2). With the understanding that the phenotypes of mouse models deleted for a specific kinase may not necessarily correlate with the phenotype caused by the incomplete inhibition of kinase activity with a drug, we believe that findings in the mouse models should be instruc-

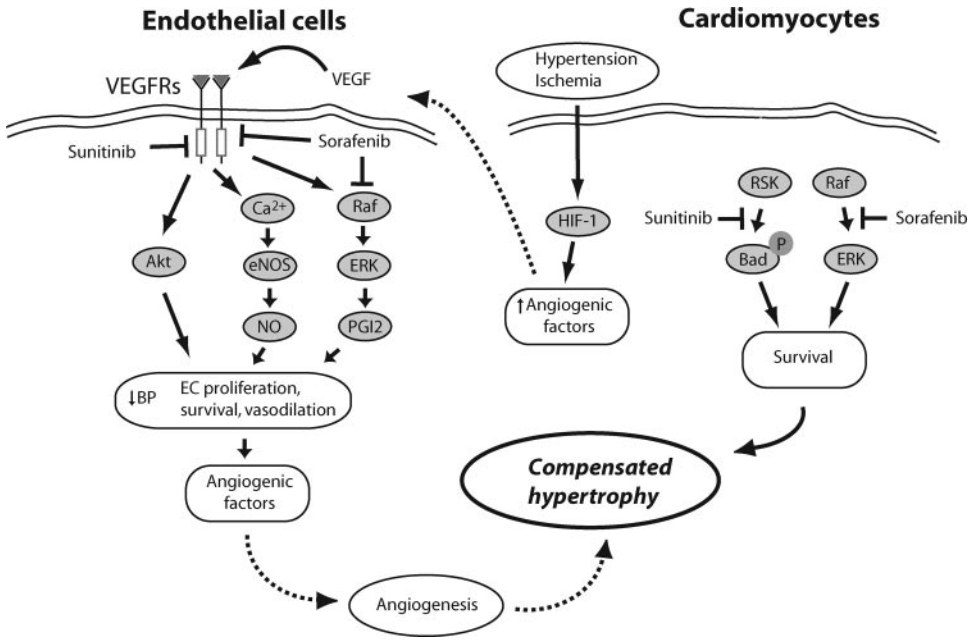

Figure 5. Mechanisms of VEGF/VEGFR-mediated protection of cardiomyocytes from pressure stress and ischemia and potential interactions of sunitinib and sorafenib. Hypertensive stress in the heart and the resulting relative ischemia/hypoxia of cardiomyocytes activate hypoxia-inducible factor 1 (HIF-1), leading to the release of angiogenic factors including VEGF.[32] VEGF activates VEGFRs on the surface of ECs, activating a number of pathways that induce vasodilation (NO and prostaglandin I_2), EC proliferation and survival (Akt and ERKs), and the further release of proangiogenic factors, all of which lead to angiogenesis in the heart, allowing compensated hypertrophy to occur.[31,33] Sunitinib and sorafenib inhibit VEGFRs in ECs, potentially blocking angiogenesis and leading to decompensated hypertrophy. Sunitinib, via an off-target effect, may also inhibit various cytoprotective pathways in the heart (eg, ribosomal S6 kinase [RSK], which would otherwise inhibit proapoptotic Bad). Sorafenib, by inhibiting the Raf/ERK pathway, could also induce apoptosis in ECs and cardiomyocytes. BP indicates blood pressure.

tive. Herein we will briefly review several important tyrosine kinase targets in cancer that are current focuses of drug development and discuss the phenotypes resulting from manipulation of these kinases in mouse models. We will also discuss any available clinical data on the cardiac effects of TKIs inhibiting these same targets.

Receptor Tyrosine Kinases

VEGF/VEGFR Inhibition

VEGF is a central regulator of angiogenesis, acting on its cognate tyrosine kinase receptor, VEGFR2. It has now been estimated that of the ≈200 different types of human cancers, ≈60% overexpress VEGF, resulting in tumor progression and metastasis.[60] Proof of principle of the effectiveness of anti-VEGF therapy in cancer came when Hurwitz et al[3] reported improved overall survival and progression-free survival in metastatic colon cancer patients treated with chemotherapy plus bevacizumab (the mAb that sequesters VEGF) versus chemotherapy alone. Many solid tumors also express additional angiogenic factors including PlGF (a homolog of VEGF that is the ligand for VEGFR1), fibroblast growth factor, PDGF, transforming growth factor-α, and hepatocyte growth factor, all of which act in part by upregulating expression of VEGF.[61,62] Multitargeted TKIs such as sunitinib and sorafenib inhibit PDGFRs in addition to VEGFRs.[14]

All of the VEGF/VEGFR-targeted therapeutics lead to blockade of endothelial cell (EC) proliferation (thereby blocking angiogenesis) and to EC apoptosis (causing regression of vessels).[62] Hypertension has emerged as an important side effect of all VEGF/VEGFR inhibitors, with the incidence of hypertension ranging from 16% to 47%.[8,63] Because VEGFR signaling is an important regulator of nitric oxide production by ECs, it is likely that impaired nitric oxide production leading to endothelial dysfunction is 1 factor driving the hypertension seen in patients.[64] Regardless of mechanism, the trials clearly demonstrate that a certain basal level of VEGF/VEGFR signaling is necessary for maintaining normal function of ECs and the vasculature.[62] Furthermore, large-vessel thrombosis, particularly in the elderly, has been noted with bevacizumab, suggesting that platelet/EC interactions are also altered when VEGF/VEGFR signaling is inhibited.[65]

In patients with poorly controlled hypertension, inhibition of VEGF/VEGFR signaling may be even more serious. Studies in mice demonstrate that angiogenesis is a key component of a normal adaptive response to a pressure load (Figure 5).[31–33] Two studies employing a "VEGF-trap" strategy similar in concept to bevacizumab demonstrated that pressure overload resulted in reduction of myocardial capillary density, global contractile dysfunction, cardiac fibrosis, and eventually decompensated heart failure.[31,33] Therefore, special attention to blood pressure management seems prudent in patients treated with VEGF/VEGFR inhibitors.

PDGFR Inhibition

PDGFRs are expressed ubiquitously, including on cardiomyocytes and ECs. Mutations and overexpression of PDGFRs play key roles in a number of cancers including chronic myelomonocytic leukemia, GIST, glioblastoma, and osteosarco-

ma.[12] Thus far, it is unclear whether cardiac effects occur with inhibition of PDGF signaling. Data in mice suggest that exogenous delivery of PDGF to the heart may be protective in the ischemic heart (Table 2).[39–41] However, it is unknown whether inhibition of endogenous PDGFR signaling by TKIs in patients will be detrimental. Ongoing clinical trials involving agents that inhibit PDGFRs should provide clues to the role of PDGFRs in cardiomyocytes and suggest whether there is toxicity with its inhibition and the form the toxicity might take.

EGFR Inhibition

EGFR (or ERBB1) is mutated and/or overexpressed in many different types of tumors including gliomas and carcinoma of the breast, ovary, and lung.[17] More than 80% of non–small-cell lung cancers (the leading cause of cancer death worldwide) harbor gene amplifications or mutations of the EGFR.[66] To date, we are aware of no reported cases clearly implicating mAbs or TKIs targeting EGFR (Table 1) in inducing heart failure or LV dysfunction in patients. There is also relatively little in the basic science literature that raises concerns. That said, Rockman and coworkers[67] showed that treatment of mice with the EGFR TKI erlotinib, an agent approved for the treatment of lung and pancreatic cancer (Table 1), enhanced myocardial injury induced by isoproterenol infusion. They concluded that EGFR signaling may be protective in settings of catecholamine excess. Furthermore, vigilance for potential cardiotoxicity may be prudent with the TKI canertinib, which is now in clinical trials. This combination ERBB inhibitor causes irreversible inhibition of EGFR and, more importantly, ERBB2 and ERBB4 by a unique mechanism, forming covalent bonds with cysteine residues in the active site of the kinases.

c-Kit Inhibition

c-Kit is the receptor tyrosine kinase for stem cell factor. Point mutations and overexpression or deletions of portions of this receptor are seen in a wide variety of cancers, which include acute myeloid leukemia, GIST, small-cell lung cancer, and sarcomas (Table 1).[12] Not surprisingly, a large number of trials are ongoing with Kit inhibitors.

Kit is normally expressed on hemangioblasts, which are the precursors for both hematopoietic stem cells and endothelial progenitor cells. Studies in the c-Kit$^{W/W-v}$ mouse (in which 1 c-Kit allele is deleted and the other encodes a protein with reduced kinase activity) demonstrated that c-Kit is necessary for proper homing of bone marrow–derived proangiogenic stem/progenitor cells to regions of infarction (Table 2). This homing, in turn, is necessary to prevent adverse remodeling after myocardial infarction.[34,35] These data raise potential concerns about the use of c-Kit inhibitors in cancer patients with a history of coronary artery disease. Possibly balancing that concern is the finding that imatinib reduced intimal hyperplasia after vascular injury in the femoral artery of mice.[36] The finding was thought to be due to reduced homing of bone marrow cells, which are vascular smooth muscle cell progenitors, to the site of injury. The cardiovascular effects of c-Kit inhibition in patients remain to be elucidated.

Nonreceptor Kinases

Abl Inhibition

In addition to imatinib, dasatinib (Sprycel, Bristol-Myers Squibb) and nilotinib (Tasigna, Novartis) also target Abl/Arg, PDGFRs, and c-Kit. Both are effective in treating Philadelphia chromosome–positive imatinib-resistant CML. Nilotinib prolongs the QT interval, and sudden deaths have been reported rarely (www.fda.gov/cder/Offices/OODP/whatsnew/nilotinib.htm). Dasatinib, which also targets the Src family of NRTKs, is associated with fluid retention including pericardial and pleural effusions. However, the mechanism has yet to be clarified. Dasatinib has also been implicated in the development of heart failure with up to 4% of patients in some series developing congestive heart failure (dasatinib prescribing information; https://www.sprycell.com/pdf/pi.pdf). Although this could certainly be due to inhibition of Abl,[9,44] dasatinib also inhibits Src and was recently reported to inhibit a number of other kinases,[68] and any of these could play a role as well.

Raf Family Inhibition

Raf (rapidly accelerated fibrosarcoma) is a family of cytoplasmic serine/threonine (as opposed to tyrosine) kinases consisting of Raf-1, A-Raf, and B-Raf.[69] Activating mutations of B-Raf occur in 66% of melanomas and to a lesser degree in a variety of solid tumors including the thyroid, colon, and ovary. Raf-1 is rarely mutated in cancers but is overexpressed in some, including squamous cell cancer of the head and neck. The Raf kinases, in particular B-Raf, are upstream of the prosurvival ERKs.[70] Many RTK oncogenic mutants signal via Raf-1/ERKs to induce cell cycle entry and proliferation and to block apoptosis. Thus, the Raf family is a very popular target of small-molecule inhibitors.

Raf-1 plays a protective role in the heart, particularly in settings of pressure overload stress (Table 2). In mice, deletion of Raf-1 in the heart led to a dilated, hypocontractile heart with enhanced cardiomyocyte apoptosis and fibrosis.[38] Furthermore, when mice with a mutation that blocked Raf signaling (via cardiac-specific expression of a dominant inhibitory Raf-1) were subjected to pressure overload, there was cardiomyocyte apoptosis and significant mortality.[37] The roles of B-Raf and A-Raf in the heart are not clear.

The Raf family is a target of sorafenib and a number of other drugs in development.[71] Approved for both metastatic RCC and hepatocellular carcinoma,[63] sorafenib also inhibits VEGFR2/3, FLT3, c-Kit, and PDGFRs (Table 1). In clinical trials, sorafenib was associated with acute coronary syndromes, including myocardial infarction, in 2.9% of patients versus 0.4% in placebo-treated patients (sorafenib prescribing information; http://www.univgraph.com/bayer/inserts/nexavar.pdf). As with other anti-VEGF/VEGFR agents, hypertension is a significant problem. Overall, the aforementioned basic science findings suggest vigilance for cardiovascular side effects with sorafenib, particularly in patients with poorly controlled hypertension. Interestingly, gain-of-function mutations in Raf-1 and other components of the Raf pathway are causal in some cases of hypertrophic cardiomyopathy (Noonan and LEOPARD syndromes),[72]

raising the prospect that inhibitors targeting Raf-1 might be useful in these rare mendelian syndromes.

JAK/STAT Inhibition

The Janus kinase (JAK) family of NRTKs takes part in the regulation of cellular responses to numerous growth factors and cytokines including members of the interferon family and interleukins. JAKs are also activated by angiotensin II in cardiomyocytes. There are 4 JAKs, and they reprogram gene expression by phosphorylating and activating STATs, of which there are 7.[73]

Mutated RTKs and NRTKs (eg, Abl) often utilize JAK/STAT signaling to drive tumor growth and in some cases tumor angiogenesis. Furthermore, an activating point mutation (V617F) in JAK2 is present in nearly every patient with the myeloproliferative disorder polycythemia vera and in approximately half of patients with essential thrombocythemia and chronic idiopathic myelofibrosis.[12] The mutant JAK2 appears to act, at least in part, by activating STAT3 and STAT5 in leukemic cells.

In the heart, it is generally believed that JAK/STAT signaling is protective (Table 2).[42,43,74] Mice lacking cardiac STAT3 had enhanced susceptibility to myocardial ischemia, doxorubicin-induced cardiotoxicity, and age-related heart failure. STAT3 appears to be critical for maintaining cardiac capillary density via multiple mechanisms.[42] These findings suggest potential for cardiotoxicity of JAK inhibitors, particularly if they have activity against multiple JAK family kinases.

The most advanced JAK inhibitor in clinical development is lestaurtinib (CEP-701), which targets JAK2 and FLT3 (mutated in ≈30% of cases of acute monocytic leukemia) but also inhibits others including neurotrophin receptors. It is primarily being tested in patients with acute monocytic leukemia.[75] A NDA is expected to be filed in 2008.[6] Phase I/II trials of this agent have thus far not raised major concerns over cardiotoxicity; however, to our knowledge, these studies do not include monitoring of cardiac function. Given the apparent lack of selectivity of lestaurtinib, off-target and on-target effects are a concern.

Conclusions and Future Directions

Much still needs to be learned about the cardiac effects of agents that inhibit tyrosine kinase activity. With the number of drugs in development and on the market, a collaborative interdisciplinary approach between oncologists and cardiologists would greatly further our understanding of these agents. As with any developing field, careful clinical observations and investigation of symptom constellations suggestive of congestive heart failure will provide early recognition of possible cardiotoxicity. These vital clinical observations help to guide and inform basic scientists in the search for mechanisms of cardiotoxicity.

One important area for future research is the refinement of approaches to improve the selectivity of drugs in development. Methods now exist to screen TKIs for inhibitory activity against very large numbers of kinases (>200).[68] The majority of TKIs studied have been found to inhibit many more kinases than intended. Because many of these play no role in tumorigenesis, the risk of toxicity is increased with no apparent gain in therapeutic efficacy. However, as knowledge of cancer and cardiomyocyte biology improves, supported by the striking advances in kinase crystal structure determination, we should be better able to tailor treatment to very specific cancer targets, reducing the risk of off-target effects. When it is impossible to "design around" a critical target of tumorigenesis, knowledge of the role of that kinase in the heart and the effects of its inhibition will serve to alert clinicians to potential problems.

One final unresolved issue is the question of reversibility of the cardiotoxicity associated with tyrosine kinase inhibition, as opposed to anthracycline cardiotoxicity, and the basic mechanisms underlying these differences. Some degree of reversibility has been seen with both trastuzumab and sunitinib,[8,76] and, as noted above, a number of patients have been able to return to treatment after temporary cessation of therapy and/or institution of standard heart failure management. However, a dissenting report has surfaced recently regarding trastuzumab.[77] At this time, we simply do not have adequate long-term follow-up of patients treated with sunitinib to be able to assess the degree and duration of reversibility. This is obviously a critical issue to address because, if true, patients may be able to be maintained on these potentially life-saving therapies, albeit with very careful follow-up. Ultimately, this will require a close partnership between cardiologists and oncologists.

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Disclosures

None.

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