Molecular mechanisms of cardiotoxicity of tyrosine kinase inhibition

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Abstract | Cancer therapy has progressed remarkably in recent years. In no area has this been more apparent than in the development of 'targeted therapies', particularly those using drugs that inhibit the activity of certain tyrosine kinases, activating mutations or amplifications of which are causal, or strongly contributory, to tumorigenesis. However, some of these therapies have been associated with toxicity to the heart. Here we summarize what is known about the cardiotoxicity of cancer drugs that target tyrosine kinases. We focus on basic mechanisms through which interruption of specific signalling pathways leads to cardiomyocyte dysfunction and/or death, and contrast this with therapeutic responses in cancer cells.

Cardiomyocytes
The contractile cells of the heart that generate force. These cells are terminally differentiated and so cardiomyocytes lost through injury can only be replaced by differentiation of progenitor cells.

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The small-molecule tyrosine kinase inhibitor (TKI) imatinib (Gleevec or Glivec) has revolutionized the treatment of patients with chronic myeloid leukaemia (CML)[1]. Indeed, on the basis of treatment success in CML and other haematological cancers (BOX 1), the concept has emerged that cancer is a manageable, if not curable, disease, similar to other chronic diseases. Prevention or aggressive treatment of co-morbid diseases that might be of greater risk to patients than the malignancy is therefore crucial. This includes complications of cancer treatments, particularly cardiovascular disease, which is a greater risk in young cancer survivors treated with anthracyclines or radiation than that of a second malignancy[2].

Although past studies of cardiotoxicity (herein defined as drug-mediated direct injury to cardiomyocytes resulting in compromised left ventricular (LV) function) have typically focused on anthracyclines or radiotherapy, more recently interest has turned to drugs that target tyrosine kinases. These agents fall into two general classes: humanized monoclonal antibodies directed against receptor tyrosine kinases or their ligands, and small-molecule TKIs[3]. During the past year it has become evident that some of these agents have associated cardiotoxicities and can, at least in some patients, cause symptomatic congestive heart failure (CHF) and, in others, asymptomatic LV dysfunction[4,5]. Therefore, tyrosine-kinase-targeted therapies can be further subdivided into drugs that have known or likely toxicity to the heart, and those that have a very low cardiotoxicity risk (TABLE 1).

It is important to stress that, with few exceptions, rates of cardiotoxicity associated with TKIs are not known because clinical trials have not included predefined cardiac endpoints (that is, they have not prospectively measured LV function before and during treatment). Therefore, the identification of cardiotoxicity and CHF has been based largely on medical history and physical examination, which are unreliable, particularly in patients with mild to moderate grades of CHF or with asymptomatic LV dysfunction[6,7]. This is complicated by the fact that it might be particularly difficult to diagnose CHF in patients with cancer, who have many reasons other than LV dysfunction to develop dyspnoea (shortness of breath), fatigue and oedema, which are the cardinal symptoms of CHF. As we discuss below, however, even when assessments of LV function have been included in clinical trials, rates of LV dysfunction are often reported in ways that make it difficult to place the findings in a clinically meaningful context. Furthermore, rates of heart failure that are determined in trials before the approval of a drug by the US Food and Drug Administration (FDA), from which patients with co-morbidities, particularly cardiovascular disease, are often excluded (for examples, see REFS 8–10), could underestimate rates that will be seen after approval when patients with cardiovascular disease or multiple risk factors are likely to be treated. Finally, clinical trials are typically of short duration (that is, months), whereas therapy with some of these agents might be lifelong.

At a glance

- Tyrosine kinase inhibitors (TKIs) have revolutionized the treatment of several malignancies, converting lethal diseases into manageable, if not curable, chronic diseases. This makes it essential to limit toxicities of these agents.
- The goal of tumour-cell killing by TKIs must be balanced against cardiotoxicity, because in some instances tumour cell death and preservation of cardiomyocyte health may be mutually exclusive.
- Cardiomyocytes are contractile and have an extremely high demand for ATP. As a result, they might be particularly susceptible to agents that perturb mitochondrial function, either as a primary or secondary effect. Therefore, alterations in mitochondrial function could have a role in the cardiotoxicities of some currently approved agents.
- Very few clinical trials have examined cardiotoxicities of TKIs in a prospective fashion with predefined cardiac endpoints, including left ventricular function. Therefore, there is a wide gap in our knowledge regarding the types of, and risk of, cardiotoxicity for most of these agents.
- Some current kinase targets in cancer are not expressed in cardiomyocytes and therefore have little or no direct role in cardiomyocyte survival. However, the current generation of TKIs is inherently non-selective, and the purposeful design of multi-targeted TKIs might allow a single agent to be more effective, and to be used in more types of cancer, but with this comes an increased risk of cardiotoxicity. In some cases this will probably be due to inhibition of 'bystander' targets that are not essential for the killing of tumour cells but that are involved in cardiomyocyte survival.
- Identification of the kinase that, when inhibited, is responsible for cardiotoxicity of an agent is important for future drug design, which should avoid these kinases where possible. Thus, greater selectivity of individual agents may require the use of more agents to treat a particular cancer, but cardiotoxicity as an 'off-target' effect should be minimized.

Cardiotoxicity of a targeted agent was first reported for trastuzumab (Herceptin), the monoclonal antibody that targets the ERBB2 receptor (also known as HER2)[11–13]. In addition, cases of heart failure have been reported after treatment of patients with imatinib[4,14], and adverse cardiac effects are mentioned in the prescribing information for dasatinib (Sprycel), sunitinib (Sutent), sorafenib (Nexavar) and bevacizumab (Avastin). It is clear, however, that cardiotoxicity is not a TKI 'class effect' because it seems to be uncommon with certain other TKIs, such as those that target the epidermal growth factor receptor (EGFR; also known as ERBB1) (TABLE 1). Therefore, toxicity needs to be determined for each agent on a case-by-case basis.

Although in most cases the overall cardiac risk of TKI therapy does not seem to be excessive, the precise clinical magnitude of the problem is not clear and the potential reversibility of the dysfunction is unknown. Recently, progress has been made in determining basic mechanisms underlying the cardiotoxicity of these drugs. There are two key features to clarify for each agent: first, the target responsible for cardiotoxicity and, second, the signalling pathway or pathways mediating the toxicity. None of the small-molecule inhibitors are truly selective, and some (for example, sunitinib) seem to be relatively non-selective[15]. Therefore, determining mechanisms of toxicity requires the identification of the specific target responsible. In some instances the results of such investigations have proved surprising, because kinases with no known role in the maintenance of cardiomyocyte 'health' have been identified as key targets (for example, ABL with imatinib)[4]. Once a tyrosine kinase is identified as having a key maintenance function in the heart, all subsequent agents that target that kinase will be suspect, and this hypothesis forms the basis of our classifying TKIs as 'likely' to have cardiotoxicity (TABLE 1). Therefore, we should be able to predict many agents that may be cardiotoxic.

The identification of targets mediating cardiotoxicity can also help to guide future drug development, because some of these kinases are likely to be 'bystander' targets that have no role in tumorigenesis — for example, ABL in gastrointestinal stromal tumours (GIST) — and there is therefore no need for a drug to inhibit them. The second requirement for delineating mechanisms of toxicity is that the signalling pathways that transduce the toxicity must be identified. In some instances, the pathway that leads to cardiomyocyte dysfunction or death will not be the same pathway that is crucial for tumour cell death. So strategies could be developed to block drug-induced activation of a pathway in the heart but to leave tumour-cell killing intact.

Here we review what is known about the basic mechanisms of cardiotoxicity of cancer therapies directed against tyrosine kinases. We discuss individual drugs, presenting data when they are available. We also discuss compounds that are in development or early phase clinical trials for which there are no conclusive human data but for which preclinical studies have either raised concerns of cardiotoxicity or alleviated them. For agents causing concern, we hope to motivate a consideration of prospective assessment of LV function in clinical trials.

ERBB2 inhibitors: trastuzumab and lapatinib

Trastuzumab is a humanized monoclonal antibody against the ERBB2 receptor tyrosine kinase, a member of the EGFR (ERBB) family, which is amplified and overexpressed in 25% of human breast cancers[13,16]. In the initial clinical trials it was evident that trastuzumab had significant cardiotoxicity, which manifests as a decrease in left ventricular ejection fraction (LVEF) or as symptomatic CHF. The incidence of cardiac dysfunction ranged from 4% to 7% with trastuzumab alone, to 27% when chemotherapy that included anthracyclines was administered concurrently[13,17]. Subsequently, five randomized trials have demonstrated a benefit for trastuzumab in ERBB2$^+$ patients with breast cancer receiving adjuvant chemotherapy after local regional treatment for a primary tumour[11,12,18]. In four of these trials, significant trastuzumab-associated cardiotoxicity was also observed, with an increase of 5–17% in the frequency of asymptomatic decreased LVEF and of 1–3% in the incidence of symptomatic CHF[19,20]. Risk factors for the development of trastuzumab-associated cardiac dysfunction included older age, concomitant or sequential treatment with anthracyclines, and marginal or low LVEF after chemotherapy[20,21]. As most trastuzumab-treated patients do not experience decreased cardiac function, additional factors such as genetic background, co-morbidities and, possibly, immune status must influence the risk of cardiac toxicity. Long-term studies suggest that trasuzumab-related cardiac dysfunction responds to medical therapy for CHF, and is at least partially reversible upon stopping treatment, often without

Congestive heart failure (CHF). A medical condition in which the contractile function of the heart muscle declines, leading to an enlarged heart with dilated chambers, impaired pumping (left ventricular dysfunction), fluid congestion in the lungs, fluid collection in the legs (oedema) and decreased perfusion of tissues. Patients may have low blood pressure, shortness of breath with exercise or when lying flat, and decreased kidney function.

Grades of CHF
The severity of CHF is graded on a clinical scale from I to IV. This scale was established by the New York Heart Association (NYHA). NYHA class I patients are asymptomatic, even with exertion, and class IV patients have significant symptoms even at rest.

Box 1 | **Haematological cancers: good targets for tyrosine kinase inhibitors**

Tyrosine-kinase-targeting therapies have had the greatest clinical success in the treatment of haematological malignancies, which include myeloproliferative diseases (MPDs), myelodysplastic syndromes, and acute lymphoid and myeloid leukaemias[3]. The MPDs are characterized by clonal haematopoiesis, overproduction of different myeloid lineage blood cells and abnormalities of haemostasis and thrombosis[92]. In MPD, blood cell differentiation is relatively normal, but there is a tendency to progress to acute leukaemia, which is characterized by a profound block in differentiation and overproduction of immature cells[93]. There are five principal MPDs: chronic myeloid leukaemia (CML, with overproduction of neutrophils); chronic eosinophilic leukaemia (CEL, in which eosinophils are overproduced), polycythaemia vera (PV, with increased erythrocyte production), essential thrombocythaemia (ET, with platelet overproduction) and chronic idiopathic myelofibrosis. Dysregulated tyrosine kinase signalling underlies the pathogenesis of most, if not all, of these diseases and, consequently, these patients usually respond dramatically to drugs that inhibit the relevant kinase (TABLE 1). CML is characterized by the Philadelphia (Ph) chromosome, the product of which is the BCR–ABL fusion tyrosine kinase. A subset of patients with CEL have fusion of platelet-derived growth factor receptor-α (PDGFRα) to FIP1L1 (REF. 94), and an acquired point mutation (V617F) in the non-receptor tyrosine kinase Janus kinase 2 (JAK2) is found in most patients with PV and about half of patients with ET and chronic idiopathic myelofibrosis[95].

There are also some related chronic myeloid disorders in which dysregulated tyrosine kinases have a causative role. Some cases of chronic myelomonocytic leukaemia (CMML) are associated with activation of PDGFRβ by fusion with TEL (also known as ETV6) or other partners[96]. Activating point mutations in KIT are found in some patients with systemic mast cell disease. Patients with 8p11 myeloproliferative syndrome have myeloproliferation frequently accompanied by non-Hodgkin lymphoma, in which both myeloid and lymphoma cells share fusions of the receptor tyrosine kinase FGFR1 (fibroblast growth factor receptor 1) to multiple partners[97]. Finally, tyrosine kinases have been implicated in the pathogenesis of some acute leukaemias, principally in Ph^+ acute lymphoblastic leukaemia (ALL), in which BCR–ABL is found, and in acute myeloid leukaemia (AML), in which about a third of patients have activating mutations in the FLT3 (FMS-related tyrosine kinase 3) receptor tyrosine kinase.

Gastrointestinal stromal tumour
(GIST). A rare cancer of the upper gastrointestinal tract (stomach and duodenum) arising from cells of neuroendocrine origin. Most GIST tumours contain activating mutations in *KIT* or *PDGFRα*, and so respond to imatinib and sunitinib therapy.

Left ventricular ejection fraction
(LVEF). A measure of the pumping ability of the heart, defined as the percentage change in the volume of the left ventricle (the main chamber of the heart that pumps blood coming from the lungs out to the body) when the ventricle contracts. A normal LVEF is 50–70%. EFs below 50% indicate LV dysfunction.

Antibody-dependent cell-mediated cytotoxicity
(ADCC). A mechanism of cell death mediated by antibody binding to a target cell, followed by recognition through Fc receptors on cytotoxic T-lymphocytes or natural killer cells, leading to target cell killing.

Dilated cardiomyopathy
A condition characterized by both dilation of the heart and reduced contractile function, often leading to CHF.

Pressure overload stress
A technique used in experimental animals that leads to a marked and sudden increase in blood pressure in the heart, which is typically induced by partially occluding the aorta with a suture.

additional medical intervention[22,23]. The mechanism of trastuzumab-induced cardiac dysfunction is not fully understood, but is distinct from that of anthracyclines, for which the cardiotoxicity correlates with total cumulative drug dose, is irreversible and is associated with changes in myocardial ultrastructure, including vacuolization and cardiomyocyte loss[24]. By contrast, trastuzumab-associated cardiotoxicity is at least partially reversible and is not associated with ultrastructural changes[22]. In theory, the cardiac effects of trastuzumab could occur through several mechanisms, including drug interactions with cytotoxic chemotherapeutic agents, antibody-dependent cell-mediated cytotoxicity (ADCC), ERBB2 receptor downregulation and inhibition of ERBB2 signalling in cardiomyocytes (FIG. 1).

ERBB2 in normal cardiac development and function. There is abundant laboratory evidence that ERBB2 has an important role in cardiomyocyte development and function. ERBB2 functions as a co-receptor for two other ERBB receptor tyrosine kinase family members, ERBB3 and ERBB4, and their peptide ligands, the neuregulins, all of which are expressed in cardiac tissue. Neuregulin 1 (NRG1) is produced by cardiac endothelial cells, binds to ERBB4 on cardiomyocytes and promotes heterodimerization with ERBB2, which leads to autophosphorylation of the ERBB2–ERBB4 heterodimer, increased tyrosine kinase activity and activation of various intracellular signalling pathways. Treatment of isolated neonatal cardiomyocytes with NRG1 activates the extracellular signal-regulated kinase (ERK)–MAPK (mitogen-activated protein kinase) and phosphatidylinositol 3-kinase (PI3K)–Akt pathways to promote cardiomyocyte proliferation, contractile function and survival[25], whereas activation of the Src–FAK (focal adhesion kinase) pathway increases cell–cell contact and mechanical coupling[26]. In mice, germline deletion of *Erbb2* (REF. 27), *Erbb4* (REF. 28) or *Nrg1* (REF. 29) is lethal in mid-gestation owing to failure of the ventricles to form properly, suggesting that ERBB2 signalling is required for embryonic cardiomyocyte proliferation. Mice with cardiac-specific deletion of *Erbb2* late in development are viable, but develop age-related dilated cardiomyopathy and have decreased survival after pressure overload stress, which mimics severe hypertension[30,31]. Cardiomyocytes from these mice have increased numbers of mitochondria and vacuoles, and increased sensitivity to anthracycline[30]. Although cardiomyocyte apoptosis in ERBB2-deficient hearts was low or absent, adenoviral-mediated expression of the anti-apoptotic protein $BCL\text{-}X_L$ in the hearts of newborn mice partially rescued heart chamber dilation and the impaired contractility[30].

Molecular mechanisms of trastuzumab cardiotoxicity. Taken together, these results suggest that inhibition of cardiomyocyte ERBB2 signalling by trastuzumab might be a central mechanism of the cardiotoxicity induced by this agent, but other observations argue that the story could be more complex. If inhibition of ERBB2 signalling induces cardiomyocyte dysfunction, then cardiotoxicity would also be expected with small-molecule inhibitors of ERBB2 kinase activity. However, early clinical results with lapatinib (GW572016), a quinazoline compound that is an orally available dual kinase inhibitor of EGFR and ERBB2, suggest that it has minimal cardiotoxicity[32]. Because of the trastuzumab experience, prospective monitoring of cardiac function is an integral part of phase I–III trials of lapatinib. In a published phase I trial in 67 patients with metastatic breast cancer and other carcinomas, many of whom had previously been treated with anthracyclines, no drug-related reductions

Table 1 | **Cardiotoxicity of tyrosine-kinase-targeting drugs**

Agent	Class	Tyrosine kinase target(s)	Cancer target(s)	Other toxicity
Drugs with known or likely cardiotoxicity*				
Trastuzumab (Herceptin)	mAb	ERBB2	ERBB2$^+$ breast cancer	Infusion reactions, neutropaenia
Imatinib (Gleevec)	TKI	ABL1/2, PDGFRα/β, KIT	CML, Ph$^+$ B-ALL, CMML, CEL, GIST	Oedema, nausea, myelosuppression, immunosuppression (?)
Dasatinib (Sprycel)	TKI	ABL1/2, PDGFRα/β, KIT, Src family	CML	Myelosuppression, oedema, pleural/pericardial effusion, panniculitis, QT prolongation, bleeding
Nilotibib (Tasigna)	TKI	ABL1/2, PDGFRα/β, KIT	CML	Myelosuppression, hyperbilirubinaemia, rash, QT prolongation
Sunitinib (Sutent)	TKI	VEGFR1–3, KIT, PDGFRα/β, RET, CSF1R, FLT3	Renal cell carcinoma, GIST	Haemorrhage, hypertension, adrenal dysfunction, hypothyroidism
Sorafenib (Nexavar)	TKI	VEGFR2, PDGFRβ, KIT, FLT3, RAF1, BRAF	Renal cell carcinoma, melanoma	Skin rash, hypertension, haemorrhage, acute coronary syndromes
Bevacizumab (Avastin)	mAb	VEGFA	Colorectal cancer, NSCLC	Haemorrhage, gastrointestinal perforation, poor wound healing, hypertension, neutropaenia, arterial thromboembolism
Drugs with low cardiotoxicity				
Lapatinib (Tykerb)	TKI	EGFR, ERBB2	Breast cancer	Skin rash, diarrhoea
Gefitinib (Iressa)	TKI	EGFR	NSCLC	Skin rash, diarrhoea, nausea, interstitial lung disease
Erlotinib (Tarceva)	TKI	EGFR	NSCLC, pancreatic cancer	Skin rash, diarrhoea, nausea, interstitial lung disease
Cetuximab (Erbitux)	mAb	EGFR	Colorectal cancer, squamous cell carcinoma of head/neck	Skin rash, infusion reactions, interstitial lung disease, hypomagnesaemia
Panitumumab	mAb	EGFR	Colorectal cancer	Skin rash

*Rate of cardiotoxicity is known only for trastuzumab and lapatinib. B-ALL, B-cell acute lymphoblastic leukaemia; CEL, chronic eosinophilic leukaemia; CML, chronic myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; CSF1R, colony-stimulating factor 1 receptor; EGFR, epidermal growth factor receptor; FLT3, FMS-related tyrosine kinase 3; GIST, gastrointestinal stromal tumour; mAb, humanized monoclonal antibody; NSCLC, non-small-cell lung cancer; PDGFR, platelet-derived growth factor receptor; Ph$^+$, Philadelphia chromosome positive; QT prolongation, prolongation of the QT interval on electrocardiogram that may predispose to arrhythmia; RET, rearranged during transfection; TKI, tyrosine kinase inhibitor; VEGFR, vascular endothelial growth factor receptor.

in LVEF were observed[32], despite pharmacodynamic evidence of inhibition of ERBB2 signalling in breast cancer cell lines and patient tumour samples. Although a definitive safety profile for lapatinib must await the completion of several randomized phase III trials in breast cancer, it seems likely that the frequency of cardiac dysfunction will be low. Indeed, in a recent randomized trial in metastatic breast cancer, lapatinib was associated with only a 2.5% incidence of asymptomatic decreased LVEF[33]. How can this be reconciled with the more prominent cardiotoxicity of trastuzumab? One obvious possibility is that immune-mediated effects on cardiomyocytes are responsible.

Trastuzumab is an immunoglobulin G1 (IgG1) monoclonal antibody, which is the most effective IgG isotype for mediating complement-dependent cell lysis and ADCC, and has been shown to stimulate ADCC against ERBB2$^+$ tumour cells *in vitro*[34]. However, pertuzumab, another IgG1 monoclonal antibody that blocks ERBB2 dimerization, is associated with a low frequency of cardiac dysfunction in early clinical trials[35], indicating that ADCC might not explain trastuzumab cardiotoxicity. There could also be a pharmacokinetic difference in the duration or extent of inhibition of ERBB2 by trastuzumab and lapatinib, although existing studies suggest the kinetics of inhibition of ERBB2 signalling to be similar after single doses of either agent.

An alternative and intriguing explanation is that trastuzumab might trigger a unique intracellular signalling response in cardiomyocytes on binding to ERBB2. In breast cancer cells, trastuzumab inhibits autophosphorylation of ERBB2–ERBB3 heterodimers[36]. However, in rat cardiomyocytes, binding of a different anti-ERBB2 antibody not only reduced ERBB2 activation, but also triggered downregulation of BCL-X_L and increased expression of BCL-X_S, leading to loss of mitochondrial membrane potential, a reduction in the level of ATP, cytochrome *c* release and caspase activation[37].

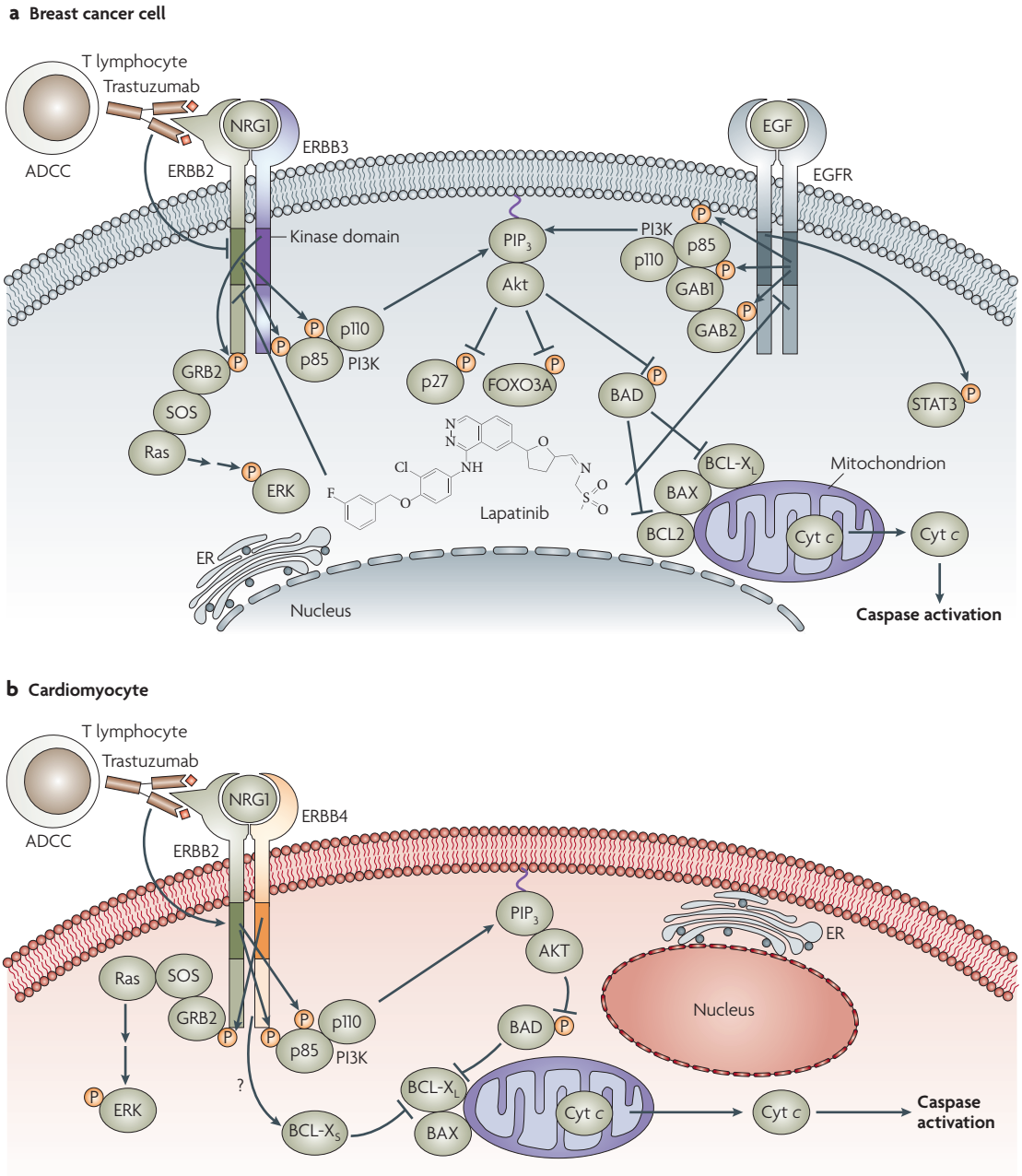

Figure 1 | **ERBB2 signalling and inhibition in breast cancer and cardiomyocytes. a** | Oncogenic signalling in a breast cancer cell can be mediated by overexpressed ERBB2, either alone or as a heterodimer with ERBB3, and also by epidermal growth factor receptor (EGFR, also known as ERBB1) homodimers. These events lead to the activation of the Ras–ERK (extracellular signal-regulated kinase), phosphatidylinositol 3-kinase (PI3K)–Akt and signal transducer and activator of transcription 3 (STAT3) pathways. Akt has a central oncogenic role, mediating inhibitory phosphorylation of p27, forkhead box O3A (FOXO3A) and BCL2-antagonist of cell death (BAD). Inhibition of ERBB2 signalling by trastuzumab impairs all downstream events, in particular reversing BAD inhibition, leading to BCL2 associated X protein (BAX) oligomerization at the mitochondrial membrane, release of cytochrome *c* (Cyt *c*) and caspase activation. In addition to inhibiting ERBB2 signalling, trastuzumab might also mediate anti-tumour effects through T lymphocytes and antibody-dependent cell-mediated cytotoxicity (ADCC). Lapatinib inhibits signalling through both the ERBB2–ERBB3 and the EGFR complexes. **b** | Signalling in cardiomyocytes through the ERBB2–ERBB4 heterodimers is essential to cell proliferation during development and to contractile function in the adult. Although several of the same signalling pathways (Ras–ERK, PI3K–Akt) are activated in cardiomyocytes as in breast cancer cells, inhibition of these pathways might not cause cytotoxicity. Rather, an increase in the ratio of BCL-X_S to BCL-X_L induced by anti-ERBB2 antibodies might trigger BAX oligomerization, mitochondrial membrane depolarization, ATP depletion and contractile dysfunction. In addition, ADCC might contribute to trastuzumab cardiotoxicity. ER, endoplasmic reticulum; GAB1, GRB2-associated binding protein 1; GRB2, growth factor receptor-bound protein 2; NRG1, neuregulin 1; PIP_3, phosphatidylinositol trisphosphate; SOS, son of sevenless.

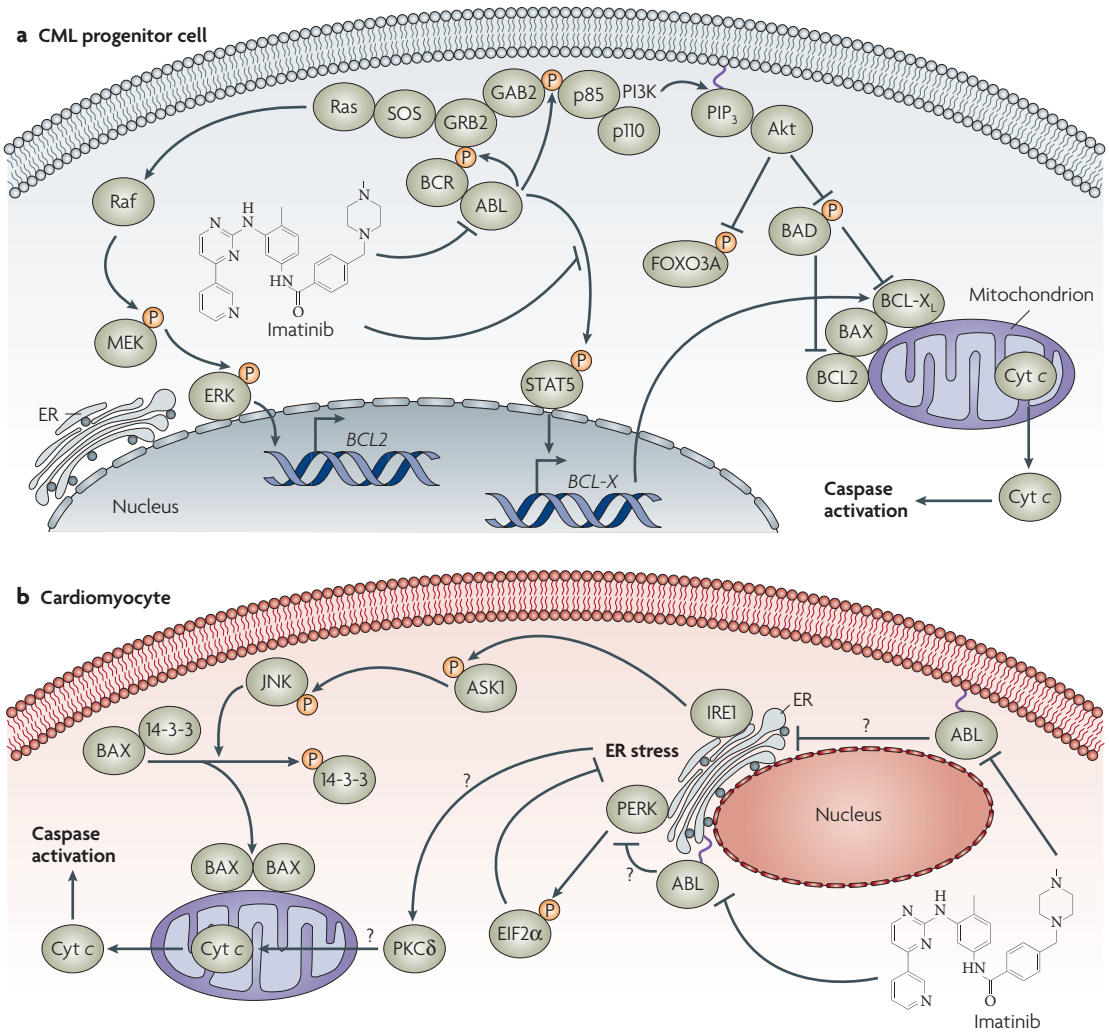

Figure 2 | **ABL signalling and inhibition in chronic myeloid leukaemia cells and cardiomyocytes. a** | Constitutive signalling in chronic myeloid leukaemia (CML) progenitor cell, through the cytoplasmic BCR–ABL tyrosine kinase, leads to activation of Ras–ERK (extracellular signal-regulated kinase), phosphatidylinositol 3-kinase (PI3K)–Akt and signal transducer and activator of transcription 5 (STAT5) pathways. Several of these pathways converge on anti-apoptotic mechanisms: the Ras–ERK pathway stimulates the expression of *BCL2*, STAT5 activates *BCL-X*, and Akt inhibits BCL2-antagonist of cell death (BAD) and forkhead box O3A (FOXO3A). Imatinib blocks all BCR–ABL-dependent phosphorylation and signalling events, leading to reversal of pro-survival effects and activation of apoptosis. **b** | In cardiomyocytes ABL (localized to plasma membrane or endoplasmic reticulum, ER) seems to maintain ER homeostasis by mechanisms that are not yet clear. The ABL kinase inhibitor, imatinib, induces ER stress, leading to activation of the PKR-like ER kinase (PERK) and IRE1 pathways, and to overexpression of protein kinase Cδ (PKCδ). PERK phosphorylates the eukaryotic translation initiation factor 2α (EIF2α) as part of a protective response, and on sustained ER stress IRE1 activates Jun N-terminal kinases (JNKs), leading to phosphorylation of 14-3-3 and release of BAX followed by mitochondrial depolarization, ATP depletion, cytochrome *c* (Cyt *c*) release, and features of necrotic and apoptotic cell death. 14-3-3, 14-3-3 protein; ASK1, apoptosis signal-regulating kinase 1; BAX, BCL2 associated X protein; GAB2, GRB2-associated binding protein 1; GRB2, growth factor receptor-bound protein 2; MEK, mitogen-activated ERK kinase; PIP_3, phosphatidylinositol trisphosphate; SOS, son of sevenless.

Unlike most tissues, neurons and cardiomyocytes are relatively resistant to apoptosis induced by cytochrome *c* release and caspase activation, possibly owing to increased expression of X-linked inhibitor of apoptosis (XIAP) and decreased expression of apoptotic protease activating factor 1 (APAF1)[38]. Consequently, antibody-mediated inhibition of ERBB2 might regulate mitochondrial integrity through the BCL-X proteins, leading to ATP depletion and contractile dysfunction without profound changes in cardiomyocyte ultrastructure. An ERBB2 kinase inhibitor such as lapatinib might alter or abolish the effect of trastuzumab on BCL-X family proteins in cardiomyocytes, raising the interesting possibility that lapatinib might ameliorate trastuzumab cardiotoxicity. This would provide additional motivation for a trial that combines these two agents[39].

Box 2 | **Non-cardiac toxicity with tyrosine-kinase-targeting drugs**

Rational use of tyrosine-kinase-targeting therapy requires an understanding of mechanism-dependent (on-target) and mechanism-independent (off-target) toxicity. Unlike in conventional chemotherapy, off-target toxicity is uncommon with tyrosine kinase inhibitors (TKIs). Toxic side effects include nausea, vomiting, muscle cramps, hypophosphataemia and hepatotoxicity with imatinib; interstitial lung disease with gefitinib and erlotinib; antibody infusion reactions, reversible posterior leukoencephalopathy with bevacizumab; and pulmonary events with FMS-related tyrosine kinase 3 (FLT3) inhibitors (TABLE 1). The myelosuppression observed with ABL inhibitors in patients with chronic myeloid leukaemia (CML) probably reflects impaired malignant haematopoiesis and not inhibition of bone marrow KIT, because this effect is rarely seen in patients with gastrointestinal stromal tumours (GIST) treated with these drugs.

It is also important to identify on-target adverse effects of TKIs outside the cardiovascular system. Hypothyroidism is observed in more than a third of patients treated with sunitinib for GIST and metastatic renal cell carcinoma[68], which may be due to the inhibition of rearranged during transfection (RET), leading to thyroid follicular cell apoptosis. In patients treated with bevacizumab, gastrointestinal perforation, poor wound healing and haemorrhage are probably directly related to the inhibition of vasculogenesis. Epidermal growth factor receptor (EGFR) antibodies and TKIs both cause papulopustular rash, dry skin, and hair and periungual abnormalities that require dose reduction or interruption in 8–17% of patients. These agents cause growth arrest, apoptosis and decreased cell migration in EGFR-expressing basal keratinocytes through inhibition of the mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K)–Akt and Janus kinase (JAK)–STAT (signal transducer and activator of transcription) pathways[98]. Finally, imatinib has significant but pleiotropic effects on the immune system, including inhibition of T-cell proliferation and activation[99–103], impaired monocyte[104] and natural killer cell[105] function, and increased antigen presentation by dendritic cells[106]. The target or targets underlying these effects are unclear, but may include ABL1/2 and/or the T-cell-receptor-associated tyrosine kinases ZAP70 and SYK. The clinical consequences of imatinib-induced immunomodulation are unclear, but could be manifested by impaired anti-viral immunity[106] and by altered graft-versus-leukaemia responses in patients treated with allogeneic stem-cell transplantation[67].

ABL inhibitors: imatinib, dasatinib and nilotinib

Imatinib, dasatinib and nilotinib are ATP-competitive small-molecule inhibitors of the ABL kinase that have been developed principally for the treatment of CML (TABLE 1), in which the leukaemic cells express the BCR–ABL fusion protein encoded by the Philadelphia chromosome (Ph) (BOX 1). Imatinib and nilotinib bind to the inactive (unphosphorylated) conformation of the ABL kinase domain, but the potency of nilotinib for BCR–ABL inhibition is about 20-fold greater than that of imatinib. In addition to ABL, both drugs also inhibit ARG (ABL-related gene or ABL2), platelet-derived growth factor receptor-α and β (PDGFRα/β) and KIT.

Imatinib was approved by the FDA in 2001 for the treatment of CML on the basis of data from the IRIS (International Randomized Study of Interferon versus STI571) trial, a randomized phase III comparison of imatinib with interferon-α and low-dose cytarabine in newly diagnosed patients with chronic phase CML[40]. Imatinib is also indicated for treatment of Ph$^+$ acute lymphoblastic leukaemia (ALL), chronic myelomonocytic leukaemia with PDGFRβ fusion proteins, chronic eosinophilic leukaemia with PDGFRα fusion proteins (BOX 1) and GIST with mutations in KIT or PDGFRα. Nilotinib is currently in phase II–III trials for imatinib-refractory CML. Dasatinib is at least 100-fold more active against BCR–ABL in cell-based assays, in part because it inhibits ABL in both the inactive and active (phosphorylated) states. In addition to inhibiting ABL, ARG, PDGFRα, PDGFRβ and KIT, dasatinib is a potent inhibitor of all Src family kinases[41]. Both dasatinib and nilotinib are active against some imatinib-resistant BCR–ABL mutants, and dasatinib was recently approved by the FDA for treatment of imatinib-refractory CML and Ph$^+$ ALL[42]. In CML cells, BCR–ABL activates various signalling pathways, including Ras–Raf–MAPK, PI3K–Akt and signal transducer and activator of transcription 5 (STAT5). Treatment of CML cells with ABL kinase inhibitors does not simply revert them to normal, but induces apoptotic cell death (FIG. 2a), in part through impaired expression of anti-apoptotic factors, such as BCL2 and BCL-X_L, and decreased inhibitory phosphorylation of pro-apoptotic factors, such as forkhead box O3A, (FOXO3A) and BCL2-antagonist of cell death (BAD).

Cardiotoxicity of imatinib. Ten patients developed CHF while taking imatinib, raising the possibility that imatinib might have associated cardiotoxicity[4]. Electron microscopic analysis of heart biopsy specimens from two patients showed nonspecific abnormalities in mitochondria and, unusually, cytosolic vacuoles containing membranous debris, some of which were quite large. Such vacuoles are not typical of heart failure owing to coronary artery disease or dilated cardiomyopathies of unknown cause. However, some of the patients had significant co-morbidities, raising the possibility that the heart failure could have been the result of these rather than the drug. The overall incidence of CHF in the IRIS trial was about 1%, and there was no difference between the imatinib and interferon arms. However, the lack of prospective monitoring of cardiac function, and the fact that most patients randomized to the interferon arm 'crossed over' to imatinib therapy, would have made it more difficult to detect a potential cardiotoxic effect of imatinib in this trial. That said, the data are reassuring, because they suggest that CHF of sufficient severity to require hospitalization is uncommon. Recently, extremely low rates of cardiotoxicity for imatinib were reported in letters written in response to work by Kerkela *et al.*[4] Our concerns about determining (and reporting) the incidence of heart failure on the basis of retrospective reviews of case report forms with minimal or no LV function data, which are delineated in our response to these letters[43], are in general agreement with concerns raised earlier by others[44,45], and are addressed further below.

Studies in cultured cardiomyocytes showed that imatinib leads to significant mitochondrial dysfunction with loss of membrane potential, release of cytochrome *c* and markedly impaired energy generation with significant declines in ATP concentration. This was associated with cell death that had features of apoptosis (positive TUNEL (terminal deoxynucleotidyl transferase biotin-dUTP nick-end labelling) staining) but that morphologically resembled necrotic cell death, with cytosolic vacuolization, loss of cell membrane integrity and minimal activation of caspase 3. Mitochondria isolated from hearts of mice treated with imatinib were also abnormal and showed increased calcium-induced opening of the mitochondrial permeability transition pore. Cardiomyocytes require enormous amounts of ATP because they are contractile.

Mitochondrial permeability transition pore
A mitochondrial pore that, when opened by pathological stresses, leads to collapse of the mitochondrial membrane potential, disruption of energy production, and mitochondrial swelling and rupture with release of cytochrome *c*.

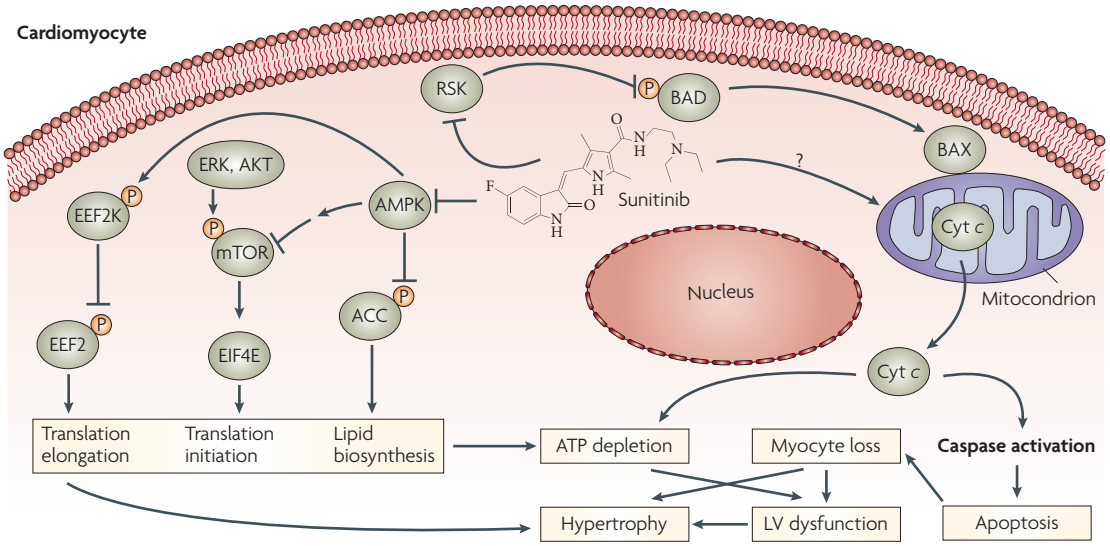

Figure 3 | **Potential role of off-target effects in mediating cardiotoxicity of multi-targeted tyrosine kinase inhibithors.** Sunitinib, through inhibition of ribosomal S6 kinase (RSK), could lead to the release of the pro-apoptotic factor BCL2-antagonist of cell death (BAD) from RSK-mediated inactivation. This would lead to BCL2 associated X protein (BAX) activation and cytochrome c (Cyt c) release, culminating in the activation of the intrinsic apoptotic pathway and, possibly, to ATP depletion. Myocyte loss and ATP depletion would lead to left ventricular (LV) dysfunction. Normally, in the setting of energy compromise, AMP-activated protein kinase (AMPK) would inhibit the energy consuming processes of protein translation and lipid biosynthesis through inhibition of eukaryotic elongation factor-2 (EEF2), mammalian target of rapamycin (mTOR) and/or acetyl-Coenzyme A carboxylase (ACC). However, sunitinib-mediated inactivation of AMPK could release EEF2, mTOR and ACC from inhibition, exacerbating any ATP depletion. In addition, because AMPK seems to be crucial in the response of cells to hypoxia, cardiomyocytes survival may be adversely affected by sunitinib in settings in which hypoxia occurs, most notably during ischaemia. Increased activity of EEF2 and mTOR in the presence of sunitinib could also promote hypertrophy that would likely already be triggered by the myocyte loss and LV dysfunction. Possible additional effects of sunitinib on mitochondrial function are indicated by a question mark. EIF4E, eukaryotic translation initiation factor 4E; EEF2K, eukaryotic elongation factor-2 kinase; ERK, extracellular signal-regulated kinase.

Indeed, it has been estimated that if energy generation ceased *in vivo*, all ATP stores would be exhausted within seconds[46]. This makes cardiomyocytes highly sensitive to perturbations in energy generation. By contrast, imatinib did not induce cell death in cardiac fibroblasts, which are non-contractile[4]. The mixed features of apoptotic and necrotic death are consistent with the fact that apoptosis is an energy-requiring process and, if energy stores are inadequate, cells often default to necrotic death[47,48].

To address whether the cardiomyocyte toxicity of imatinib was a nonspecific effect or a consequence of inhibition of one of its tyrosine kinase targets, we expressed the imatinib-resistant mutant, ABL T315I (REF. 49), in cardiomyocytes, and found that it partially rescued the cells from imatinib-induced toxicity[4]. Because ABL is known to induce apoptosis, the fact that it seemed to provide a survival signal in cardiomyocytes was surprising. However, it seems that ABL can signal cell survival and cell death, and that cell type and possibly strength of stimulus and level of activation are involved in determining this. For example, ABL mediates oxidant stress-induced death in fibroblasts, but is protective in osteoblasts[50]. In addition, deletion of either ABL or ARG has been shown to be protective in various cell types subjected to oxidant stress, whereas deletion of both increased cell death[51–53]. Owing to the extremely high activity of the electron transport chain in cardiomyocytes, it is believed that these cells are constantly threatened by oxidant stress, and it is tempting to speculate that ABL might be involved in defence against this. It will be crucial to identify the target or targets of ABL that transduce this survival signal in order to better understand the mechanisms of imatinib toxicity.

The identification of ABL as the relevant target predicts that newer ABL inhibitors, such as dasatinib, might also have associated cardiotoxicity. In support of this, 4% of patients taking dasatinib for a median duration of 6 months were reported to have developed CHF or LV dysfunction, and half of these cases were grade III or IV (moderate to severe, requiring treatment)[54]. The same might be expected of nilotinib, although so far there are limited clinical data about its adverse event profile in patients[10]. The relatively low reported frequency of cardiac dysfunction in patients taking ABL kinase inhibitors seems to contrast with the effects of imatinib treatment in mice, in which cardiomyocyte drop out, diminished contractile function and changes in cardiomyocyte ultrastructure were observed uniformly[4]. There could be several explanations for this discordance, including species-specific differences in cardiomyocyte ABL signalling, and effects of genetic modifiers on the cardiac response to imatinib in a genetically uniform mouse strain compared with genetically heterogeneous patients.

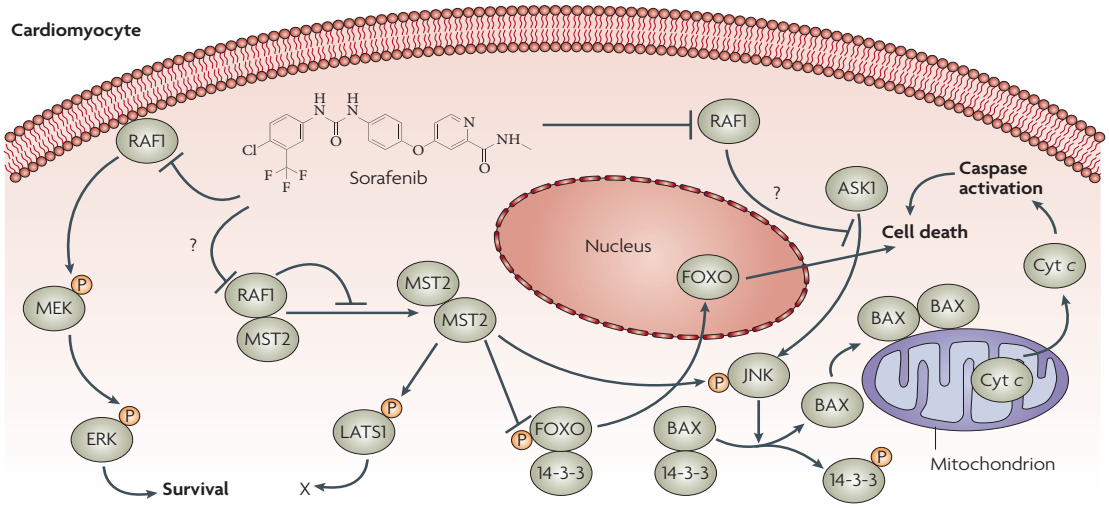

Figure 4 | **Putative signalling pathways of sorafenib cardiotoxicity.** Sorafenib-mediated inhibition of RAF1 (and BRAF, not shown) kinase activity will disrupt signalling through the extracellular signal-regulated kinase (ERK) kinase cascade, which is believed to have a role in cell survival in the heart, especially under conditions of stress. RAF1-mediated inhibition of two pro-apoptotic pathways in the heart, regulated by apoptosis signal-regulating kinase 1 (ASK1) and mammalian sterile 20 kinase 2 (MST2), is not dependent on RAF1 kinase activity because kinase-dead RAF1 is as effective as wild-type RAF1. It is not clear whether sorafenib will disrupt the RAF1–MST2 protein–protein interaction (indicated by the question mark). If so, this would trigger dimerization and activation of MST2 and subsequent activation of three pro-apoptotic mediators, Jun N-terminal kinase (JNK), forkhead box O (FOXO) and large tumour suppressor, homologue 1 (LATS1). If inhibition of the RAF1–ASK1 interaction also occurs (also indicated by the question mark), this would trigger activation of JNK, JNK-mediated phosphorylation of 14-3-3 proteins and subsequent release of BCL2 associated X protein (BAX), which would lead to cytochrome *c* (Cyt *c*) release from mitochondria and cell death. Downstream targets of LATS1 (indicated by an X), possibly mediating cell death in terminally differentiated mammalian cells, including cardiomyocytes, are not clear at present. MEK, mitogen-activated ERK kinase.

Signalling mechanisms of imatinib cardiotoxicity. As in the case of trastuzumab, mitochondrial dysfunction seems to have a central role in the cardiotoxic response to imatinib, but the mechanism seems to arise as a result of the induction of endoplasmic reticulum (ER) stress by the drug, which was evident both in cells in culture and in the hearts of mice treated with imatinib. ER stress, which is ordinarily initiated by unfolded proteins in the ER, activates two distinct signalling pathways mediated by PRK-like ER kinase (PERK) and by IRE1 (also known as ERN1), a dual protein kinase and endoribonuclease[55]. Imatinib-treated cardiomyocytes had increased phosphorylation of the PERK substrate eukaryotic translation initiation factor 2α (EIF2α), a protective response that shuts down general protein translation but upregulates expression of chaperones. Salubrinal, a drug that prevents dephosphorylation of EIF2α, attenuated imatinib-induced mitochondrial dysfunction, suggesting that the unfolded protein response contributes to the cardiotoxicity of imatinib (FIG. 2b). In addition, prolonged ER stress leads to recruitment of Jun N-terminal kinases (JNKs) through activation of the IRE1 signalling pathway[56,57], and a selective peptide inhibitor of JNKs largely protected cardiomyocytes from imatinib-induced cell death. Imatinib treatment also led to a marked increase in the expression of protein kinase Cδ (PKCδ), a kinase with pro-apoptotic effects in the heart[58]. Although neither a peptide inhibitor of PKCδ, nor a non-selective PKC small-molecule inhibitor, was able to rescue cardiomyocytes from imatinib-induced cell death[4], the reagents available are not optimal. Given its markedly increased expression, it is possible that PKCδ has some role in the cardiotoxicity of imatinib.

The pathway linking ABL inhibition with the induction of ER stress is not clear. Several studies in fibroblasts suggest that a portion of the myristoylated form of ABL (ABL Ib) is localized to the ER, and participates in the activation of PKCδ and mitochondrial depolarization in the response to oxidants and ER stress[59,60]. However, ABL kinase activation was implicated in these processes, whereas inhibition of ABL activity triggers cardiomyocyte ER stress. Because ER stress responses are also seen in imatinib-treated CML cell lines, in which BCR–ABL is cytoplasmic[61], this might not require localization of ABL to the ER. Further studies will be necessary to clarify the underlying mechanisms.

Other studies in animal models suggest that imatinib and related agents could have important applications in cardiovascular and pulmonary diseases unrelated to ABL and/or ARG inhibition. Specifically, imatinib markedly reduced radiation-induced pulmonary fibrosis and reversed pulmonary hypertension induced by the drug monocrotaline by means of effects attributed to the inhibition of PDGFR signalling[62,63]. In addition, imatinib reduced neointima formation after vascular injury, and this was attributed to the inhibition of KIT in progenitor cells, preventing their homing to the site of injury[64].

Neointima
The intima is the layer of the arterial wall immediately adjacent to endothelial cells. After catheter intervention, a hypercellular intima can re-form, leading to re-obstruction of the vessel.

Box 3 | **Potential cardiotoxicity of drugs that mediate or modulate apoptosis**

Although these agents do not generally inhibit tyrosine kinases, they could have cardiotoxicities. No agents in this category have been approved for clinical use, but this is an area of active development by the pharmaceutical industry. So far, the possibility that modulation of apoptotic factors could be accomplished *in vivo* and could produce significant effects has mostly been studied in animal models of acute ischaemic injury (stroke and myocardial infarction). Peptide inhibitors of caspases can reduce ischaemic injury[107,108], and one small-molecule, non-peptide inhibitor, IDN6556 (Pfizer), had entered phase II clinical trials. However, for cancer, the goal is to increase, rather than inhibit, apoptosis. Various strategies are being pursued, including small molecules that lead to the activation of caspase 3, and small molecules that are inhibitors of pro-survival pathways such as Akt[107].

Another area of interest is the inhibition of the IAP (inhibitor of apoptosis) family. IAPs are overexpressed in some cancers, and the cells of such cancers are relatively resistant to apoptosis. However, virus-mediated gene transfer of IAPs to the brain has been shown to reduce ischaemic injury in stroke models[107]. Thus, strategies for cancer therapeutics targeting apoptotic pathways are often diametrically opposed to strategies for cardiovascular therapeutics[109]. Agents targeting apoptotic pathways in cancer will clearly be of concern for potential cardiovascular toxicity.

Multi-kinase inhibitors: sunitinib and sorafenib

Many tumours depend on vascularization for growth and metastasis, but also require the action of tyrosine kinases in the tumour cell for proliferation and survival. The receptor tyrosine kinases vascular endothelial growth factor receptor (VEGFR) and PDGFR have been implicated in tumour angiogenesis. Theoretically, an agent that inhibited angiogenesis and one or more tyrosine kinases involved in tumour proliferation could have broad anticancer activity from this dual pharmacological effect, particularly in cancers that are well vascularized. This was the principle behind the development of sunitinib and sorafenib.

Sunitinib. Sunitinib (Sutent) is an orally active TKI that inhibits VEGFR1–3, PDGFRα/β, KIT, FMS-related tyrosine kinase 3 (FLT3), colony-stimulating factor 1 receptor (CSF1R) and rearranged during transfection (RET) receptor tyrosine kinases, and is an example of the recent trend towards drugs that target multiple receptor kinases[65]. Although this makes sense from a cancer treatment perspective, it is possible that these agents will carry a greater risk of cardiotoxicity than more selectively targeted agents, such as imatinib. In addition, making inhibitors less selective by design is also likely to result in the inhibition of several off-target kinases. Indeed, based on an approach that tested the binding affinity of several small-molecule inhibitors to 119 tyrosine and serine/threonine kinases, this clearly seems to be the case with sunitinib, which is expected to inhibit several off-target kinases at concentrations achieved in patients[15] (see below).

During the past few months, the results of two sunitinib trials containing LV function data have been published[5,66]. One trial, in patients with advanced GIST, reported no change in mean LVEF after a median treatment duration of 8 weeks. The prescribing information for sunitinib[67] notes that 11% of patients had declines in LVEF to below the lower limit of normal (50%)[66]. The other trial, in patients with metastatic renal cell carcinoma, reported that 10% of patients had declines in LVEF after a median treatment duration of 6 months, but there were no clinical sequelae[5]. However, LVEF values were not reported. Any LV dysfunction could be exacerbated or even caused by the recently reported hypothyroidism that can occur as a result of sunitinib treatment[67,68] (BOX 2; TABLE 1). The potential role of sunitinib-induced hypertension, which can be significant, as a contributory factor to CHF also needs to be evaluated.

Of the targets reported for sunitinib in the prescribing information, only the PDGFRs are known to be expressed in cardiomyocytes, and although overexpression of PDGF can signal cardiomyocyte survival[69], endogenous PDGF and its receptors have not been reported to do so. This raises the possibility that an off-target effect of the drug could account for the apparent cardiotoxicity. Scanning the kinase interaction map identifies several candidates, including the ribosomal S6 kinase (RSK) family, which signals survival through inhibitory phosphorylation of the pro-apoptotic factor BAD, and AMP-activated protein kinase (AMPK), which has been reported to transduce pro-survival signals in the heart[46,70]. Indeed, the inhibitor-binding assay predicts that inhibition of RSKs and AMPK by sunitinib could be seen at clinically relevant concentrations[15]. FIGURE 3 illustrates hypothetical mechanisms by which sunitinib might lead to cardiotoxicity. If any of these are correct, this would represent one example of potential problems with non-selective inhibitors and their inherent off-target effects. This agent needs to be carefully examined for risk and severity of cardiotoxicity.

Sorafenib. A second member of the multi-kinase inhibitor family is the agent sorafenib (Nexavar). This drug is known to induce acute coronary syndromes, including myocardial infarction, in 2.9% of treated patients (compared with 0.4% of placebo-treated patients)[71]. There have also been cases that seem to have resulted from sorafenib-induced cardiotoxicity unrelated to an acute coronary syndrome (D. Lenihan, personal communication) but again, the overall risk is not known. In addition to inhibiting several growth factor receptors (VEGFR2, VEGFR3, FLT3, KIT and PDGFRs), sorafenib inhibits RAF1 and BRAF. Raf family kinases are MAPK kinase kinases (MAP3Ks) functioning in the pro-survival ERK cascade[72], but also have ERK-independent pro-survival effects (FIG. 4). RAF1 inhibits two pro-apoptotic kinases, apoptosis signal-regulating kinase 1 (ASK1) and mammalian sterile 20 kinase 2 (MST2), both of which have central roles in oxidant stress-induced injury[73–76]. Intriguingly, however, neither of these effects requires RAF1 kinase activity, and both are mediated by inhibitory protein–protein interactions. For example, RAF1 binding to MST2 prevents dimerization and activation of the kinase and recruits a phosphatase that inactivates MST2 (REF. 77). So a key question from the perspective of cardiotoxicity is whether sorafenib simply inhibits RAF1 kinase activity, or whether it also disrupts the RAF1–ASK1 and/or RAF1–MST2 interactions. If the latter proves true, cardiotoxicity might be an even greater concern than if only the ERK cascade is inhibited.

The roles of BRAF and ARAF in the heart are not known, but the importance of RAF1 has been demonstrated in mouse models through conditional cardiac-specific deletion of *Raf1*, or through cardiac-specific expression of a kinase-inactive mutant of RAF1. Deletion of *Raf1* in the heart led to a dilated, hypocontractile heart with enhanced cardiomyocyte apoptosis and fibrosis[78]. These phenotypes could be largely prevented by ablation of ASK1. This might suggest that kinase activity is not critical to RAF1 pro-survival effects (and so sorafenib cardiotoxicity might be minimal). Indeed, mice with cardiac-specific expression of the kinase-dead, dominant-negative RAF1 mutant had entirely normal hearts, suggesting that normal inhibition of ASK1 and MST2 was intact and that RAF1 kinase activity was not necessary[79]. However, when these animals were stressed by pressure overload, cardiomyocyte apoptosis was markedly increased and mortality was high. So, although RAF1 kinase activity might not be important in the absence of stress, in the stressed heart it does seem to provide protection.

There are additional concerns related specifically to inhibition of VEGF–VEGFR signalling in the heart that may be particularly relevant to patients with poorly controlled hypertension receiving sorafenib or sunitinib. Two studies used adenoviral delivery of a decoy VEGF receptor to demonstrate that if VEGF–VEGFR signalling was disrupted during the imposition of a pressure load on the heart, capillary density was reduced and this was associated with marked contractile dysfunction, fibrosis and heart failure[80,81]. These studies show that a normal angiogenic response is necessary to maintain a normal response of cardiomyocytes to pressure load, and when this does not occur, hearts transition rapidly from compensated hypertrophy to heart failure. Interestingly, inhibition of the normal angiogenic response to pressure overload was recently shown to be mediated by the upregulation of p53 (REF. 82), raising concerns about strategies that might increase the activity of p53 in the heart (REF. 83), particularly in hypertensive patients.

Other targets with possible cardiotoxicity

There is significant preclinical evidence to indicate that inhibition of several other tyrosine kinases might result in cardiotoxicity. In addition, therapies targeting apoptotic pathways, which are regulated by many tyrosine kinases, could also cause cardiac dysfunction (BOX 3).

Janus kinase 2. An activating point mutation (V617F) in the non-receptor tyrosine kinase Janus kinase 2 (JAK2) is present in almost all patients with the myeloproliferative disorder polycythaemia vera and in about half of patients with essential thrombocythaemia and chronic idiopathic myelofibrosis[3] (BOX 1). The mutant JAK2 seems to function, at least in part, by activating members of the STAT family of transcription factors, including STAT3 and STAT5, in the leukaemic cells. In the heart, it is generally believed that JAK–STAT signalling is protective. For example, AG-490, a relatively non-selective JAK2 inhibitor, reduced apoptosis in a rat model of myocardial infarction[84]. In mice, homozygous null mutations in *Jak1*, *Jak2* or *Stat3* cause lethality. However, mice engineered to lack STAT3 specifically in the heart showed increased susceptibility to cardiac injury caused by myocardial ischaemia, and were more prone to develop age-related heart failure[85,86]. STAT3 seems to be crucial to maintaining cardiac capillary density through several mechanisms, including direct regulation of VEGF expression, induction of pro-angiogenic cytokines and suppression of anti-angiogenic gene programmes in the heart. Mice lacking STAT3 in the heart are also more susceptible to doxorubicin-induced cardiotoxicity, and overexpression of STAT3 protects against this[85–87]. Although JAK1 or tyrosine kinase 2 (TYK2) might be able to compensate for the inhibition of JAK2 in the heart (JAK3 is not expressed), these findings raise concerns about possible cardiotoxicity of JAK2 inhibitors, particularly if they have activity against several JAK family kinases.

The most advanced JAK inhibitor in clinical development is lestaurtinib (CEP-701), which is a relatively selective inhibitor of FLT3 and JAK2. There are no reports of significant cardiotoxicity in phase I–II trials of lestaurtinib in patients with acute myeloid leukaemia and activating FLT3 mutations[88], but prospective monitoring of cardiac function has not been carried out.

KIT. KIT, the receptor for stem cell factor, is inhibited by imatinib, dasatinib, nilotinib, sunitinib and sorafenib (TABLE 1). It is expressed on haemangioblasts, which are precursors for haematopoietic stem cells and endothelial progenitor cells (EPCs). Proper functioning of the KIT receptor seems to be necessary for the mobilization of EPCs to sites of injury, such as that following myocardial infarction. Concerns about inhibiting KIT were raised recently by studies of the $Kit^{W/W-v}$ mouse, a compound heterozygote in which one allele is deleted and the other has reduced kinase activity. This mouse, when subjected to myocardial infarction, had markedly impaired post-myocardial infarction repair and survival, which was attributed to failure to recruit bone-marrow-derived stem/progenitor cells that are pro-angiogenic to the infarct zone[89,90]. This raises concerns that inhibition of KIT might aggravate pathological remodelling of the heart post-myocardial infarction and prevent repair. Therefore, inhibiting KIT seems to prevent beneficial homing to areas of injury in the heart just as it seemed to inhibit detrimental homing to sites of vascular injury[64]. This could be relevant in patients treated with KIT inhibitors (TABLE 1).

Conclusions and future directions

We have attempted to outline real and theoretical concerns about cardiotoxicity of therapies that primarily target tyrosine kinases. Real concerns, based on studies with TKIs in cells and mice, are tempered by the fact that there are few cases in which we have adequate data on the clinical risk of cardiotoxicity, and we do not believe there is any reason why this knowledge deficit should not be corrected. Theoretical concerns are tempered by the fact that they are mostly based on studies performed in animals in which the gene of interest has been deleted, and the direct translation of these findings into predictions in the clinic is not possible. For example, mice with only one *Erbb2* allele deleted were indistinguishable from

wild-type mice, suggesting that reduced levels of ERBB2-dependent signalling are sufficient for the cardiomyocyte maintenance function, and that the moderate inhibition that accompanies drug treatment, as opposed to complete loss of function, might be better tolerated. Furthermore, these mouse models are genetically homogeneous, and toxicities in patients will be much more sporadic and variable in severity owing to genetic variability.

We have also tried to underline what is probably the most important concern — the move towards drugs targeting multiple kinases. This creates many opportunities for toxicity, not only as a result of the inhibition of desired targets but, probably much more importantly, also because of inhibition of off-target kinases. Ultimately, the cardiomyocyte is highly sensitive to perturbations in energy generation, largely because of its huge energy demand. Not surprisingly, mitochondrial dysfunction and energy depletion seem to be a common theme in the cardiotoxicities described above. However, unlike in anthracycline cardiotoxicity, there is at least the potential of recovery or reversibility with some TKIs if the cells can withstand the period of energy depletion without undergoing apoptosis or necrosis. Although at present there are no published data on reversibility of small-molecule TKI-induced LV dysfunction, sporadic cases clearly indicate that this might be quite striking, at least in some patients. Mechanisms of this phenomenon, if real, need to be determined, because they could be the key to being able to keep patients on these vital therapies. Clearly, early detection of LV dysfunction and institution of treatment could be key in preventing reversible cardiomyocite injury from proceeding to irreversible cell loss. Although we have not provided many answers, we hope that we have highlighted key uncertainties, and we would answer the question asked by van Heeckeren and colleagues[91]: 'Is it time for oncologists to get to know their cardiologists?' with a definite 'Yes'. Only with a close collaboration of these two disciplines can the issues be effectively addressed.

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Competing interests statement
The authors declare competing financial interests: see web version for details.

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