Targeting non-malignant disorders with tyrosine kinase inhibitors

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Abstract | Receptor and non-receptor tyrosine kinases are involved in multiple proliferative signalling pathways. Imatinib, one of the first tyrosine kinase inhibitors (TKIs) to be approved, revolutionized the treatment of chronic myelogenous leukaemia, and other TKIs with different spectra of kinase inhibition are used to treat renal cell carcinoma, non-small-cell lung cancer and colon cancer. Studies also support the potential use of TKIs as anti-proliferative agents in non-malignant disorders such as cardiac hypertrophy, and in benign-proliferative disorders including pulmonary hypertension, lung fibrosis, rheumatoid disorders, atherosclerosis, in-stent restenosis and glomerulonephritis. In this Review, we provide an overview of the most recent developments — both experimental as well as clinical — regarding the therapeutic potential of TKIs in non-malignant disorders.

Fibrosis
The pathological formation of fibrous connective tissue as a repair response (for example, wound healing) or a separate active process that affects the normal architecture of the underlying organ or tissue (for example, lung fibrosis and heart fibrosis).

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doi:10.1038/nrd3297

Protein tyrosine kinases (PTKs) modulate a wide range of cellular events — including proliferation, metabolism, differentiation and apoptosis — under normal cell conditions and in diseased states[1,2]. The human protein kinase genome (also known as the kinome) contains 518 protein kinase genes, including genes that encode transmembrane receptor tyrosine kinases (RTKs) and soluble cytoplasmic tyrosine kinases (also known as non-receptor tyrosine kinases)[3]. The RTKs are a large family of PTKs that contain an intracellular catalytic protein tyrosine kinase domain and regulatory sequences, a transmembrane domain and an extracellular ligand-binding domain.

More than 58 mammalian RTKs have been identified, including the well-known insulin receptor, Fms-like tyrosine kinase 3 (FLT3), KIT, epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), colony-stimulating factor 1 receptor (CSF1R), and insulin-like growth factor 1 receptor (IGF1R)[2]. Phosphorylation of tyrosine residues in target RTKs is essential for maintaining cellular homeostasis and modulating gene expression in various intercellular and intracellular signalling pathways. RTKs are therefore important targets for basic research and drug development[2].

In this Review, we briefly consider RTK signalling pathways in cancerous and non-cancerous tissues, as well as the role of tyrosine kinase inhibitors (TKIs) in classical oncological indications, before focusing on their role in a range of non-malignant diseases. There are many non-malignant diseases for which TKIs have potential therapeutic utility. We provide an overview of recent developments in this field, highlighting areas with the strongest research evidence for their translational potential and thus a higher likelihood of success in the clinic. With this in mind, disorders that are not discussed here include cystic fibrosis[4], asthma[5], chronic obstructive pulmonary disease (COPD)[6], gastritis[7], psoriasis[8], papilloma[9], sepsis[10] and macular degeneration[11]. Readers are directed to the references cited above for further information on the potential use of TKIs in these disease areas.

RTK activation and signalling

Activation of RTKs is initiated by hormones or binding of growth factors to specific sites within the extracellular domain of the receptor. A well-described example of hormone-associated RTK activation is the phosphorylation of IGF1R and EGFR in certain breast cancers stimulated by oestrogen[12]. Various growth factors such as nerve growth factor (NGF), EGF, PDGF and VEGF mediate their cellular responses through binding and activation of specific RTKs[13]. Upon ligand binding, RTKs undergo a dimerization process (or a conformational change in the case of PTKs, such as the insulin receptor, that occur as pre-formed dimers) that results in autophosphorylation of the tyrosine kinase domains[1]. Such phosphorylation events activate the kinase, thereby increasing its intrinsic PTK activity, and produce new binding sites for proteins containing SRC homology 2 (SH2) domains and phosphotyrosine-binding (PTB) domains. Molecules recruited

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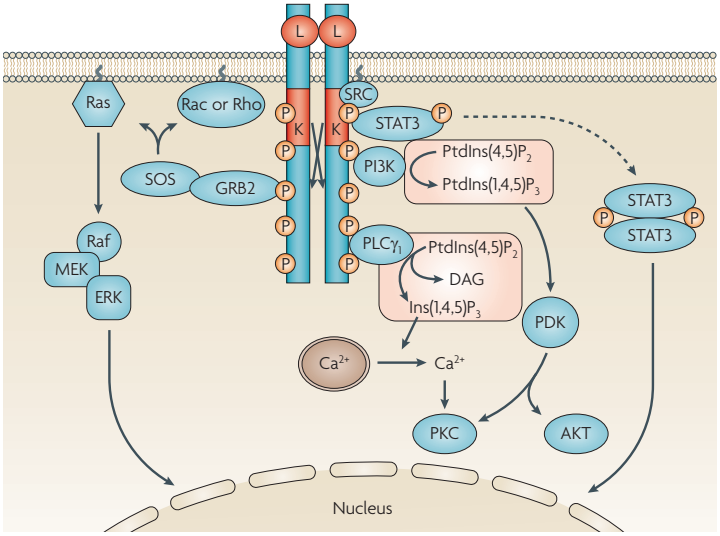

Figure 1 | **General signalling pathways of receptor tyrosine kinases in cancer.** Ligand (L) binding to the extracellular domain of the tyrosine kinase receptor causes dimerization that results in autophosphorylation and activation of the intracellular kinase domain (K). The ensuing phosphorylation of docking sites on the receptor leads to the recruitment of enzymes (for example, phosphoinositide 3-kinase (PI3K)), phospholipase Cγ1 (PLCγ1), SRC and signal transducer and activator of transcription 3 (STAT3)) and adaptor molecules (for example, growth factor receptor-bound protein 2 (GRB2)) and activation of downstream signalling pathways. These include the AKT (also known as PKB), mitogen-activated protein kinase, Rac and Rho, and Janus kinase (JAK)–STAT pathways. The signalling pathways regulate a diverse array of processes including transcription, translation, metabolism, cell proliferation, survival, differentiation and motility. Gene mutations or overexpression can trigger unregulated activation of receptor tyrosine kinases, leading to deregulation of downstream signalling pathways that can ultimately cause malignant proliferative disorders. DAG, diacyl glycerol; ERK, extracellular signal-regulated kinase; $Ins(1,4,5)P_3$, inositol-1,4,5-trisphosphate; MEK, mitogen-activated protein kinase–ERK kinase; PDK, phosphoinositide-dependent kinase; PKC, protein kinase C; $PtdIns(4,5)P_2$, phosphatidylinositol-4,5-bisphosphate; $PtdIns(4,5)P_3$, phosphatidylinositol-3,4,5-trisphosphate.

through these binding motifs are either enzymes that are tyrosine-phosphorylated and activated, such as SRC and phospholipase Cγ, or adaptor molecules (SH2 domain-containing transforming protein (SHC), growth factor receptor-bound protein 2 (GRB2) or E3 ubiquitin–protein ligase CBL) that link RTK activation to downstream signalling pathways[1].

The adaptor molecules then recruit additional effector molecules containing SH2, SRC homology 3 (SH3), PTB and pleckstrin homology domains to the activated receptor, resulting in the assembly of signalling complexes at the membrane and the activation of intracellular downstream signalling cascades[14,15]. Key signalling cascades activated by RTKs include the Ras–extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3K)–AKT pathway and the Janus kinase–signal transducer of activated transcription (JAK–STAT) pathway (FIG. 1).

Ultimately, the complex signalling network triggered by RTKs leads to either activation or repression of various subsets of genes, and thus RTKs regulate intercellular communication and control cell proliferation, mitogenesis, survival, differentiation, motility and metabolism[1,16]. The individual cellular consequences of RTK activation are complex, and depend on the cell type and the activated signal transduction pathway[1] (see FIG. 2, which shows RTKs in cardiomyocytes, smooth muscle cells and myofibroblasts, highlighting differing and shared signalling pathways).

RTK signalling in proliferative diseases

Unregulated activation of some of the major families of RTKs, through mechanisms such as point mutations, overexpression, or abnormalities in signalling patterns, are implicated in many malignant and benign-proliferative disorders and have therefore received considerable attention[17] (BOX 1). For example, deregulation of ERBB signalling is implicated in the aetiology of several epithelial cancers, and overexpression of ERBB2 (REF. 18) and other ERBB receptors occurs in several human cancers, including mammary carcinomas[19–21]. EGFR activation stimulates growth, desensitizes cells to apoptotic stimuli and regulates angiogenesis[19]. Gene amplification, overexpression and activating mutations of EGFR are seen in glioblastoma[22] and in prostate[23], breast[24], colorectal[25,26] and non-small-cell lung cancer[27] and other squamous carcinomas[28].

Similarly, activating mutations and overexpression of PDGF and its receptors PDGFRα and PDGFRβ, as well as KIT, are linked to various human cancers, including gliomas[29], ovarian carcinomas[30], gastrointestinal stromal tumours (GISTs)[31,32] and myelomonocytic leukaemia[33]. Interestingly, PDGFRα expression levels are higher in more advanced forms of gliomas than in less malignant glial tumours[29]. Moreover, the PDGF system is proposed to have a pathogenic role in cardiopulmonary diseases such as atherosclerosis, lung fibrosis[34], bronchiolitis obliterans[35,36], in-stent restenosis[37] and pulmonary hypertension[38].

VEGFA, VEGFB, VEGFC, VEGFD, placental growth factor and their receptors (VEGFR1, VEGFR2 and VEGFR3) are implicated in angiogenesis that occurs in many solid tumours. These include renal cell carcinoma, lung cancer, breast cancer, colon cancer, hepatoma, bladder cancer, gastric cancer and prostate cancer[39–43]. There is also evidence for an involvement of FGF–FGFR and IGF–IGF1R signalling in tumorigenic processes[44,45].

TKIs in classical oncological indications

Alterations of RTK signal transduction in proliferative disorders led to the hypothesis that TKIs could have anticancer effects and, as a result, many such compounds have been developed[46]. Most of these are low-molecular-mass inhibitors designed to block the ATP-binding site and prevent the autophosphorylation of tyrosine kinases, thereby inhibiting activation of intracellular signal transduction pathways[17].

More than 20 tyrosine kinase targets that have been validated at the biochemical, cellular and preclinical level are currently being evaluated in clinical trials in several oncological indications. Furthermore, clinical

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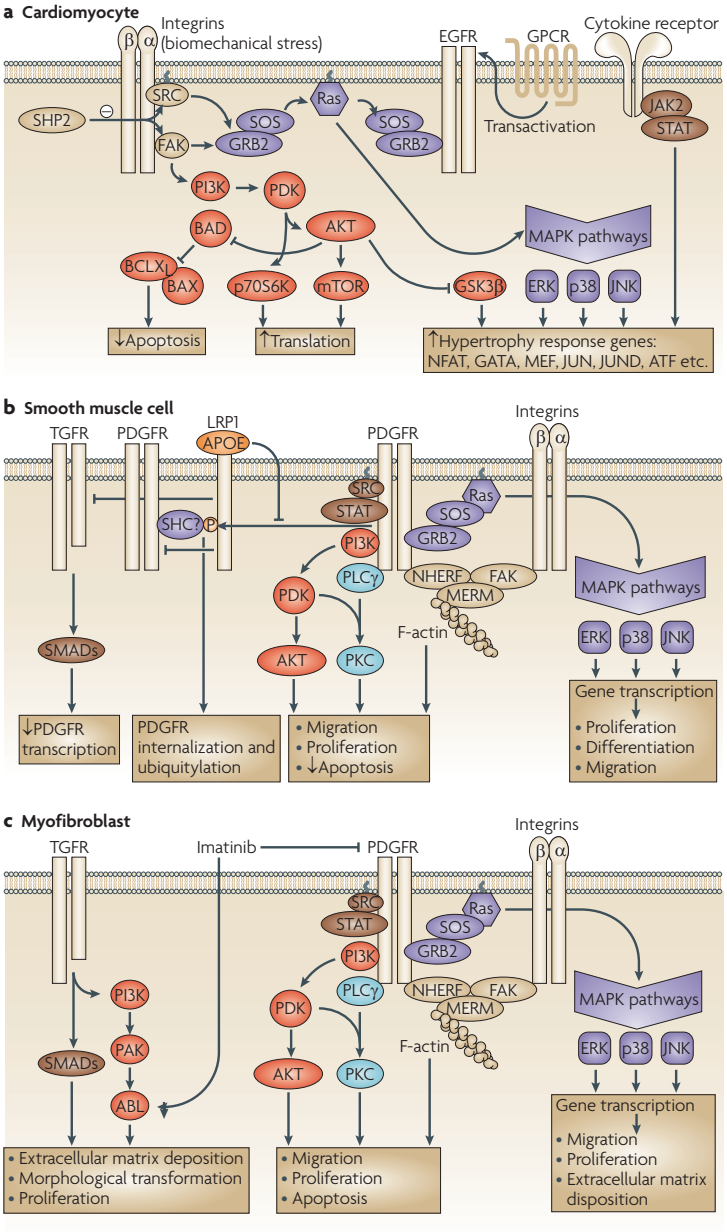

Figure 2 | **Comparison of receptor tyrosine kinase signalling in cardiomyocytes, smooth muscle cells and myofibroblasts. a** | In cardiomyocytes, receptor tyrosine kinase signalling is involved in the hypertrophic response to stretch. Integrin signalling in response to biomechanical stress leads to activation of focal adhesion kinase (FAK) and SRC, triggering the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) signalling cascades. Transactivation of epidermal growth factor receptor (EGFR) by G protein-coupled receptor (GPCR) signalling (for example, in response to prostaglandin E_2 in an inflammatory setting[206]) also activates the MAPK signalling pathway, and cytokines such as cardiotrophin, possibly released in response to biomechanical stress, activate the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway[207]. Ultimately, these signalling pathways suppress apoptosis, increase protein translation (thus increasing cellular volume) and increase the transcription of hypertrophy response genes. **b** | In the atherosclerotic response in smooth muscle cells, platelet-derived growth factor receptor (PDGFR) activates the PI3K and MAPK pathways and interacts with the cytoskeleton through the adaptor Na^+–H^+ exchange regulatory factor (NHERF). This modulates gene transcription, suppresses apoptosis and increases cell migration and proliferation. Low-density lipoprotein receptor-related protein 1 (LRP1) limits PDGFR activity by regulating its expression and degradation, both directly[192] and indirectly through transforming growth factor receptor (TGFR) inhibition[193]. **c** | The smooth muscle cell shares features with the myofibroblast. Whereas TGFβ plays a key part in fibrosis by activating myofibroblasts and inducing extracellular matrix deposition, PDGF is a potent mitogen for myofibroblasts. Imatinib has been suggested to block both TGFβ- and PDGF-mediated fibrotic signalling. APOE, apolipoprotein E; ATF, activating transcription factor; BAD, BCL2 antagonist of cell death; BAX, BCL2-associated X protein; $BCLX_L$, basal cell lymphoma extra large; ERK, extracellular signal-regulated kinase; GATA, GATA-binding transcription factor; GSK3β, glycogen synthase kinase 3β; JNK, Jun N-terminal kinase; MEF, myocyte-specific enhancer factor; MERM, merlin–ezrin–radixin–moezin family of cytoskeletal linkers; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; P, phosphotyrosine; PDK, phosphoinositide-dependent kinase; PKC, protein kinase C; PLC, phospholipase C; p70S6K, p70 ribosomal S6 kinase; SHP2, SRC homology 2-containing protein tyrosine phosphatase 2.

proof-of-concept has been achieved with several TKIs (TABLE 1). Many RTK inhibitors are also effective antagonists of serine/threonine kinases (for example, sorafenib (Nexavar; Bayer/Onyx) and sunitinib (Sutent; Pfizer)). So far no specific serine/threonine kinase inhibitor has entered clinical practice, although trials are underway[47]. Lack of target specificity is a major obstacle to the clinical development of many kinase inhibitors; several studies have shown that even inhibitors that were previously regarded as highly specific have a propensity to inhibit multiple targets[48,49], thus increasing the risk of undesirable side effects (BOX 2).

Pulmonary vascular diseases

Pulmonary hypertension is characterized by excessive vasoconstriction and thickening of vessel walls due to proliferation of vascular smooth muscle cells. Current vasodilatory therapeutic approaches for the treatment of chronic pulmonary arterial hypertension (PAH; group I of the current WHO (World Health Organization) classification of pulmonary hypertension[50]) include prostanoids, endothelin receptor antagonists and phosphodiesterase 5 inhibitors[51–54]. These treatments provide symptomatic relief together with some improvement in prognosis. However, there is no strong evidence that

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Box 1 | **Overview of mechanistic similarities between cancer and non-malignant disease**

Most, if not all, cancers have acquired the same set of functional capabilities (sustained angiogenesis, insensitivity to anti-growth signals, self-sufficiency in growth signals, evasion of apoptosis, limitless replicative potential and metastasis)[195]. The same mechanisms of unregulated receptor tyrosine kinase (RTK) signalling that lead to malignant proliferative disorders can also cause benign proliferation of affected tissues. On the basis of this finding, a class of 'angiogenesis-dependent diseases', including not only cancers but also non-neoplastic diseases such as pulmonary arterial hypertension (PAH), has been proposed[196].

For example, upregulation or constitutive activation of platelet-derived growth factor receptor is implicated in certain types of cancer[29–33], and increased levels have also been shown in lung biopsy specimens from patients with severe PAH[55], in fibroblasts and blood vessels of patients with scleroderma[113] and in atherosclerotic lesions[177,178]. As another example of an RTK with a central role in both malignant and non-malignant diseases, the fibroblast growth factor receptor and its downstream signalling pathway have been implicated both in tumorigenic processes[44] and in smooth muscle cell hyperplasia in patients with PAH[79].

Endothelial cells in plexiform vascular lesions (a classical feature of PAH) have been characterized as hyperproliferative and apoptosis resistant, which is consistent with a neoplastic hypothesis[197]. For example, plexiform lesions express both vascular endothelial growth factor (VEGF) and its receptor, suggesting an autocrine action of VEGF, which is characteristic of neoplastic endothelial cell processes[99,198]. Microsatellite instability of growth-regulating genes in endothelial cells — encoding transforming growth factor-β receptor 2 and BCL-associated X protein (BAX) — has also been reported within plexiform lesions[199]. Finally, cancer cells rely primarily on glycolysis to generate ATP (known as the Warburg effect)[200], and endothelial cells from patients with PAH also display a high glycolytic rate[201].

these approaches have a direct anti-proliferative effect on pulmonary vascular smooth muscle cells, and so RTK pathways are attracting growing interest as alternative targets for PAH.

Several growth factors, including PDGF[38,55], FGF2 (previously known as basic FGF)[56], EGF[57] and VEGF[58,59], have been implicated in the abnormal proliferation and migration of pulmonary vascular cells. These growth factors — which act as potent mitogens and chemo-attractants for smooth muscle cells, fibroblasts and endothelial cells — usually form homodimers or heterodimers. These stimulate cell surface receptors, which activate the major signal transduction pathways, such as the Ras–MAPK pathway. The cellular response to stimulation by growth factors is proliferation, migration and resistance to apoptosis. Because of the extensive research into PAH, below we organize the scientific and clinical evidence for the role of tyrosine kinases in this field according to the type of growth factor.

Platelet-derived growth factor. Upregulation of PDGFRα and PDGFRβ protein levels has been shown in an ovine model of chronic intra-uterine pulmonary hypertension[60]; however, no difference has been observed between pulmonary hypertensive and control animals in the levels of pulmonary PDGFA or PDGFB mRNA. In lung biopsies from patients with severe PAH, PDGFA mRNA levels and PDGFRβ protein expression were increased[38,55].

The PDGFR inhibitor imatinib — which is approved for the treatment of chronic myelogenous leukaemia (CML) and GIST[61] and is also an inhibitor of the kinases BCR–ABL and KIT[62–64] — reversed advanced pulmonary vascular disease in two animal models of pulmonary hypertension[38]. In rats with monocrotaline-induced pulmonary hypertension, treatment with imatinib 4 weeks after induction of the disease resulted in 100% survival, compared with only 50% survival in sham-treated animals. Changes in right ventricular pressure and right heart hypertrophy were also reversed to near-normal levels in these rats. Imatinib prevented phosphorylation of PDGFR and suppressed activation of downstream signalling pathways. Similar results were obtained in chronically hypoxic mice treated with imatinib after full establishment of pulmonary hypertension.

In pulmonary artery tissue from patients with PAH, PDGFRα and PDGFRβ expression was significantly higher than in that from healthy donors[65]. Furthermore, clinical case reports have also indicated that imatinib has efficacy in patients with PAH[66–68], in whom pulmonary vascular resistance decreased mainly due to an increase in cardiac output. The increase in cardiac output is interesting given that potential cardiotoxicity (see the subsection on the cardiotoxicity of TKIs) has been attributed to some RTK inhibitors. However, the exact mechanisms of imatinib on right ventricular function under hypertrophic (and probably decompensated) conditions are not yet completely understood.

A Phase II trial investigating the safety and tolerability of imatinib in PAH has recently been finalized[69]. This proof-of-concept study indicated a good long-term safety and tolerability profile in patients with advanced PAH. Although imatinib did not produce a statistically significant improvement in the primary efficacy end point — the change in 6-minute walking distance — many secondary efficacy parameters (for example, the change in pulmonary vascular resistance and the change in cardiac output) indicated superiority of imatinib over placebo. Interestingly, a subgroup analysis suggested a greater efficacy of the drug in patients with more severe pulmonary hypertension — that is, those with a pulmonary vascular resistance at baseline of more than 1,000 dyn s cm^{-5}. This finding generated the hypothesis that patients with a higher degree of pulmonary vascular remodelling were more likely to respond than patients with mild to moderate disease. This criterion was therefore used when recruiting for the ongoing Phase III randomized, controlled trial.

Although the main effects of imatinib in PAH are attributed to inhibition of PDGFR, inhibitory effects on KIT and ABL kinases cannot be excluded. The KIT receptor is expressed on dendritic cells and mast cells that are

Right ventricular pressure
The pressure in the ventricular heart chamber on the right side, which also reflects the systolic blood pressure in the pulmonary artery and can be assessed non-invasively by echocardiography or invasively by right heart catheterization.

Cardiotoxicity
The occurrence of disturbances in heart electrophysiology and/or muscle damage resulting in cardiomyocyte dysfunction or death.

Decompensated
Describes the functional deterioration of a previously working structure or system. In the context of heart function, it refers to the progressive decline in the contractility of the myocardium, which can ultimately lead to death from cardiac failure.

Vascular resistance
The force that blood must overcome to maintain perfusion of the blood vessel, calculated as the ratio between pressure and flow.

Vascular remodelling
Structural changes of the vascular wall characterized by intimal, medial and adventitial thickening in response to hypoxia, inflammation, haemodynamic changes or vascular diseases.

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Table 1 | **Overview of selected tyrosine kinase inhibitors**

Compound	Main targets	Preclinical models	Development status	Refs
Imatinib (Gleevec/ Glivec; Novartis)	BCR–ABL, KIT and PDGFR	FDC-P1 cells with KIT mutants cloned from patients with AML	• Approved for CML and GIST • Pre-registration for ALL • Phase II for solid tumours, sarcoma and melanoma	208,209
Nilotinib (Tasigna; Novartis)	BCR–ABL, KIT and PDGFR	Increased survival of mice injected with *Bcr–Abl*-transformed haematopoietic cell lines or primary marrow cells	• Approved for CML, including for patients who are resistant or intolerant to imatinib • Phase III for melanoma and GIST	157,210
Sunitinib (Sutent; Pfizer)	FLT3, KIT, VEGFR and PDGFR	• MV4-11, RS4;11 and OC1-AML5 cells • NOD–SCID mice	• Approved for RCC and GIST • Pre-registration for pancreatic tumours • Reached Phase III for breast, lung and prostate cancers and HCC, but development discontinued	211–213
Sorafenib (Nexavar; Bayer/Onyx)	KIT, Raf and VEGFR	• MDA-MB-231, NCI-H460, Colo-205, HT-29, DLD-1 and A549 cells • NCr-nu/nu mice	• Approved for RCC and HCC • Phase III for thyroid tumours and NSCLC • Phase II for prostate cancer	214,215
Gefitinib (Iressa; AstraZeneca)	EGFR	HL-60, U-937 and Kasumi cells	• Approved for NSCLC • Phase II for other tumour types	27,216, 217
Semaxanib	FLT3, KIT, VEGFR and PDGFR	BaF3, MV4-11 and RS4;11 cells	Reached Phase III for colorectal cancer, but development discontinued	218–220
Midostaurin	FLT3, KIT, VEGFR and PDGFR	BaF3 cells with wild-type or mutant FLT3	Phase III for AML	221,222
Lestaurtinib	FLT3	BaF3 cells with wild-type or mutant FLT3	Reached Phase II for AML and solid tumours, but development discontinued	223,224
Tandutinib	FLT3, KIT and PDGFR	BaF3, Molm-13, Molm-14, HL-60, AML-193, KG-1 and THP-1 cells	• Phase II for glioblastoma • Discontinued for prostate cancer and AML	225,226

A549, human lung carcinoma cell line; ALL, acute lymphoblastic leukaemia; AML, acute myelogenous leukaemia; AML-193, human acute monocytic leukaemia cell line; BaF3, murine pro-B cell line; CML, chronic myelogeous leukaemia; Colo-205, human colorectal cancer cell line; DLD-1, human colorectal adenocarcinoma cell line; EGFR, epidermal growth factor receptor; FLT3, Fms-like tyrosine kinase 3; GIST, gastrointestinal stromal tumour; HCC, hepatocellular carcinoma; HL-60, human promyelocytic cell line; HT-29, human colorectal adenocarcinoma cell line; Kasumi, human myelogenous leukaemia cell line; KG-1, human acute myelogenous leukaemia cell line; MDA-MB-231, human breast adenocarcinoma cell line; Molm, human acute monocytic leukaemia cell line; MV4-11, human cell line established from biphenotypic B-myelomonocytic leukaemia blast cells; NCI-H460, human large-cell lung cancer cell line; NOD–SCID, non-obese diabetic–severe combined immunodeficiency; NSCLC, non-small-cell lung cancer; OC1-AML5, human leukaemia cell line; PDGFR, platelet-derived growth factor receptor; RCC, renal cell carcinoma; RS4;11, cell line established from the bone marrow of a patient with acute lymphoblastic leukaemia; THP-1, human acute monocytic leukaemia cell line; U-937, human histiocytic lymphoma cell line; VEGFR, vascular endothelial growth factor receptor.

present in the vascular wall of pulmonary arteries from patients with PAH[70,71]. The ABL kinase can transactivate angiotensin 1 receptor[72] and modulate the transforming growth factor-β (TGFβ) pathway in a SMAD-independent manner[73]. Furthermore, ABL interferes with mitochondrial membrane potential and cytochrome c release in cardiomyocytes. This potential cardiotoxic effect could be diminished by expression of imatinib-resistant mutants of ABL[74]. So, an interesting approach to avoid the potential detrimental effect of imatinib is a chemical modification of the compound that eliminates its effect on ABL while retaining its efficacy against PDGFR and KIT[75]. Other clinically approved PDGFR inhibitors are sorafenib and sunitinib, which also inhibit other RTKs (TABLES 1,2).

Fibroblast growth factor. The family of FGF consists of 23 ligands (FGF1 to FGF23) and four receptors (FGFR1 to FGFR4). These receptors are known to have a central role in lung development. The most studied FGF in pulmonary hypertension is FGF2, which is a potential target for new treatments for the condition. FGF2 is ubiquitously expressed, and is upregulated in response to hypoxia and shear stress in pulmonary arterial smooth muscle cells[76,77]. It is overproduced in PAH and contributes to hyperplasia of smooth muscle cells. Upregulation of FGF2 has been reported in an ovine model of pulmonary hypertension with increased pulmonary blood flow[56]; however, no relevant changes in FGF2 levels occurred in chronically hypoxic mice[78]. Recently, the FGFR1 inhibitor SU5402 demonstrated reversal of established monocrotaline-induced pulmonary hypertension in rats[79]. However, due to the heterogeneity within the FGF receptor family, selective inhibitors are lacking. Further limitations to the potential clinical use of FGF inhibitors in pulmonary hypertension arise from the potential to induce

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Box 2 | **Selective versus non-selective tyrosine kinase inhibitors: which is superior?**

Some tyrosine kinase inhibitors (TKIs) target a wide range of kinases. As discussed below, it is unclear whether this lack of selectivity should be considered an advantage or a shortcoming of these drugs[202]:

- Classical pharmacology suggests that, for a targeted treatment with minimized undesirable side effects, the molecular pathway involved in the disease process should be addressed as specifically as possible.
- Conversely, biological pathways commonly involve regulatory loops and redundant pathway activation. Recently, multi-component therapeutics for networked systems, such as tyrosine kinase signalling, have been suggested.
- Combination drugs that affect multiple targets simultaneously control complex disease systems more effectively, are less prone to the development of resistance and are the standard of care in many important therapeutic areas[203].
- A prominent example in this context is imatinib, originally used for its ability to inhibit BCR–ABL in patients with chronic myelogenous leukaemia harbouring mutated BCR–ABL. Recently, it was shown that the main mechanism of action is dependent on concomitant inhibition of the KIT pathway, another key target of imatinib[204]. The second disease for which imatinib is indicated, gastrointestinal stromal tumours, is mainly caused by a gain-of-function mutation in the *KIT* proto-oncogene, and therefore benefits from the BCR–ABL-independent mode of action of imatinib[205].
- In non-malignant but chronic proliferative diseases, such as pulmonary hypertension or lung fibrosis, many receptor tyrosine kinase pathways (for example, platelet-derived growth factor, fibroblast growth factor, epidermal growth factor and vascular endothelial growth factor) are simultaneously activated, suggesting that multi-target TKIs could be superior to selective inhibitors of a single receptor.

epithelial-to-mesenchymal transition and high levels of reactive oxygen species, which are associated with proliferative and inflammatory conditions[80].

Epidermal growth factor. The EGF receptor (also known as ERBB1 and HER1) belongs to the ERBB family of RTKs, which includes ERBB2 (also known as HER2), ERBB3 (also known as HER3) and ERBB4 (also known as HER4). In pulmonary hypertension, EGF-dependent proliferation and migration of smooth muscle cells requires the extracellular matrix component tenascin C[81]. EGF colocalizes with tenascin C in the obstructive lesions of patients with PAH[82], indicating the role of EGF in the progression of this disease. Although EGFR expression does not change under conditions of pulmonary hypertension, several EGFR inhibitors, such PKI166 (REF. 57) and the approved drugs gefitinib and erlotinib[83] (TABLE 1), improved monocrotaline-induced pulmonary hypertension in rats. However, no benefit was shown with the dual EGFR–HER2 inhibitor lapatinib[83], and none of these inhibitors improved hypoxia-induced pulmonary hypertension in mice. Previous investigations that had a strong predictive value regarding the future clinical success of investigational drugs for PAH mostly showed efficacy in both of these rodent models of pulmonary vascular disease, acknowledging the multiple signalling pathways that are operative in human disease. So, also considering the absence of EGFR upregulation in human tissue of patients with PAH[83], it could be suggested that the EGF system does not represent a promising target for the treatment of PAH.

Vascular endothelial growth factor. The best-characterized member of the VEGF family, VEGFA, exists as a homodimeric glycoprotein comprised of two identical 23-kDa subunits[85], which correspond to the $VEGF_{165}$ (165 residues beyond the signal sequence) isoform[86]. VEGFA and its isoforms — including the isoforms with 121, 189 and 206 amino acids[87–89] — activate tyrosine kinase receptors VEGFR1 (also known as FLT1)[90] and VEGFR2 (also known as KDR and FLK1)[91], as well as neuropilin 1 (NRP1) [92] and NRP2 (REF. 93). By contrast, VEGFB and placental growth factor (PlGF) bind to VEGFR1 (REFS 94,95), and VEGFC[96] and VEGFD[97] bind to VEGFR2 and VEGFR3. As for all tyrosine kinases, signalling mechanisms involve protein kinase C, PI3K, ras–raf–ERK and AKT, thereby affecting cellular proliferation, permeability and migration[43].

VEGF is expressed in the plexiform lesions of lungs from patients with PAH[98,99]. Experimental data for pulmonary hypertension show conflicting results, with both VEGF upregulation (in hypoxia-induced pulmonary hypertension) and downregulation (in monocrotaline-induced pulmonary hypertension). Interestingly, inhibiting VEGFR with a combination of hypoxia and SU5416 (semaxanib) aggravated pulmonary hypertension, mainly due to the occlusion of small pulmonary arteries by proliferating endothelial cells[100,101]. Inhibition of VEGF may also alter lung structure, as shown by emphysema formation after SU5416 application in rodents[102,103]. Therefore, the role of chronic VEGF inhibition in pulmonary hypertension should be investigated carefully. For a review on the role of VEGF in the pulmonary system, see REF. 104. Approved agents that target the VEGF signalling pathway comprise small-molecule inhibitors (sorafenib, sunitinib (TABLE 1) and pazopanib (Votrient; GlaxoSmithKline)), monoclonal antibodies against VEGF (bevacizumab (Avastin; Genentech)), and VEGF-specific Fab fragments (ranibizumab (Lucentis; Genentech)), which could be tested in preclinical models of pulmonary hypertension to evaluate their therapeutic or detrimental potential.

Other targets for pulmonary vascular disease. Other tyrosine kinases that may be interesting targets for the treatment of pulmonary hypertension include SRC, which is activated by reactive oxygen species and interacts with the carboxy-terminus of bone morphogenetic protein receptor type 2 (REF. 105), which is known to play an important part in PAH. ABL kinase, another potential target, is downstream of SRC kinase and is involved in angiotensin 1 signalling in smooth muscle cells[72].

Epithelial-to-mesenchymal transition
The trans-differentiation of epithelial cells to mesenchymal cells, characterized by loss of cell adhesion, repression of E cadherin expression and increased cell mobility. It can occur during normal development, as a repair mechanism, or as a mechanism of disease.

Plexiform lesion
A histological hallmark of idiopathic pulmonary arterial hypertension and related conditions. It is a distal pulmonary vascular structure that resembles kidney glomerulae, and its function is not well understood.

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Table 2 | **Non-malignant diseases with involvement of deregulated RTK signalling**

Indication	Deregulated RTK pathway targets	Compounds that modulate these targets	Development status
Pulmonary hypertension	PDGFR[38,55,65]	Imatinib (Gleevec/Glivec; Novartis)[38,66–68]	Phase III
	PDGFR and VEGFR[66–68,98,99]	Sorafenib (Nexavar; Bayer/Onyx)[153,227,228]	Phase Ib
	PDGFR, VEGFR and FGFR[66–68,79,98,99]	SU5614[79]	Phase IIb
Idiopathic pulmonary fibrosis	PDGFR[229]	BIBF-1120[110]	Phase II
	VEGFR[230]	Imatinib[109]	Reached Phase II, but development discontinued
Obliterative bronchiolitis	PDGFR[35]	Imatinib[36] and CGP 53716 (REF. 35)	Preclinical
	VEGFR[231]	PTK/ZK[36]	Preclinical
Cardiac hypertrophy and remodelling	EGFR[154,155]	Lapatinib (Tykerb; GlaxoSmithKline)[83]	Preclinical
		Gefitinib (Iressa; AstraZeneca)[83]	Preclinical
		Erlotinib (Tarceva; Genentech/Roche)[83]	Preclinical
In-stent restenosis	PDGF[37]	Imatinib[166]	Phase II
Macular degeneration	VEGF[232]	Ranibizumab (Lucentis; Genentech)[233]	Approved
		Bevacizumab (Avastin; Genentech)[234]	Reached Phase II, but development discontinued tyrosine kinase.

EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor; RTK, receptor

Last value carried forward analysis
A method to analyse outcome measures at a given time point even if patients have dropped out of the observation prematurely, whereby the parameter that was last observed is carried forward to the end-point analysis.

pa_{O2}
The partial pressure of oxygen in arterial blood. In medicine, it reflects the gas exchange efficiency of the cardiopulmonary system.

Event rate
The number of predefined events occurring in the treatment group (usually in comparison to the corresponding rate in a control group) in clinical trials. The difference in event rate between both groups determines the therapeutic effect and/or the risk to patients.

Cyclosporin A
A calcineurin inhibitor used as a standard immunosuppressive agent for the prevention of graft rejection in transplant medicine.

Tyrosine kinases in lung fibrosis

The proliferation of myofibroblasts in pulmonary blood vessels, bronchi and alveolar walls in pulmonary fibrosis is associated with increased levels of growth factors in lung tissue and broncho-alveolar lavage. Imatinib inhibits the profibrogenic activity of TGFβ, which prevents bleomycin-mediated[73,106] and irradiation-induced[107] lung fibrosis in animal models, and decreases parenchymal fibrosis in asbestos-induced pulmonary fibrosis[108].

The results of a double-blind, randomized, placebo-controlled trial of imatinib in 119 patients with idiopathic pulmonary fibrosis (IPF) have recently been published[109]. Disappointingly, patients treated with imatinib did not have a significantly different survival rate or change in lung function to those given placebo over the course of the 96-week follow-up period. However, the trial was not entirely negative as imatinib-treated patients showed significantly improved oxygenation at 48 weeks compared with patients receiving placebo. In the last value carried forward analysis, the positive effects on pa_{O2} were even sustained at 96 weeks. The event rate for the placebo group was 31.7%, suggesting that the study was underpowered to detect a difference (in time to death or time to disease progression) between groups. Moreover, the study had several clear limitations: the required background treatment was not well defined, patients suffered from only mild to moderate disease, the proportion of histologically proven IPF was below 50% and the drop-out rate was above 50%. Thus, although disappointing on first sight, this negative trial clearly does not preclude further consideration of TKI treatment studies in IPF. This notion is further supported by the benign side-effect profile of imatinib in IPF patients, who experienced that same rate of serious adverse events as the placebo group.

BIBF-1000 inhibits the receptors for PDGF, FGF2 and VEGF, blocking a similar pathway to that inhibited by imatininb and preventing bleomycin-induced pulmonary fibrosis in a rat model of the disease[110]. BIBF-1120, a variant of this inhibitor that targets the receptor kinases of PDGF, FGF2 and VEGF[111], is currently under investigation in ovarian, fallopian tube and primary peritoneal cavity cancer (ClinicalTrials.gov identifier: NCT00710762), but has been shown to cause diarrhoea, nausea, anorexia and bleeding. A 12-month, Phase II study investigating the effects of BIBF-1120 on pulmonary forced vital capacity in patients with IPF is also currently underway (ClinicalTrials.gov identifier: NCT00514683).

Bronchiolitis obliterans

The major limitation to long-term survival of lung allograft recipients is obliterative bronchiolitis, a manifestation of chronic rejection. The exact molecular and cellular mechanisms contributing to obliterative lesion formation — which includes epithelial damage, peribronchial inflammation and increasing bronchiole obliteration — remain unknown. However, RTK inhibitors may provide a novel therapeutic strategy for the prevention of chronic rejection.

In the heterotopic rat tracheal allograft model[35], intragraft PDGFRA and PDGFRB mRNA expression increased during the progressive loss of respiratory epithelium and airway occlusion in non-treated allografts compared with syngeneic grafts. Treatment with the PDGFR-selective PTK inhibitor CGP 53716 reduced myofibroproliferation and inhibited the development of obliterative bronchiolitis by more than 50%, both alone and in combination with suboptimal doses of cyclosporin A. CGP 53716 did not affect airway wall inflammatory cell proliferation, or the number of graft-infiltrating $CD4^+$ and $CD8^+$ T cells and $ED3^+$ (a surface marker) macrophages, suggesting that its effects are mediated by inhibition of myofibroproliferation rather than immunosuppression.

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EX2077.0007

Experimental obliterative airway disease was reduced with the VEGFR inhibitor PTK/ZK (also known as PTK787 and ZK222584) alone, or imatinib alone, and the development of obliterative airway disease was completely prevented with combined use of these two drugs[36]. However, late treatment failed to show efficacy. Blocking VEGF RTK action (using PTK/ZK) diminished allograft inflammation by reducing the number of lymph vessels in the allograft airway wall and reducing the activation of allograft blood vessels. PDGFR blockade with imatinib inhibited the growth of smooth muscle cells in the proliferating lesion[36]. In mouse tracheal allografts — which were characterized by a loss of epithelium and by airway occlusion — simultaneous treatment with antibodies against VEGFR1 and VEGFR2 also lowered the degree of luminal occlusion.

Systemic sclerosis

Systemic sclerosis is a rare disease of the connective tissue characterized by multi-organ fibrosis and dysfunction. The presence of stimulatory auto-antibodies targeting PDGFR in the serum of patients with scleroderma[112] may indicate a pathogenic role of this signalling pathway. This idea is supported by increased PDGFR levels in the fibroblasts and blood vessels of patients with scleroderma[113]. In vitro, imatinib inhibits the proliferation of normal dermal and sclerodermal fibroblasts[114]. The safety, tolerability and efficacy of imatinib in combination with cyclophosphamide was recently investigated in a small clinical study of patients with scleroderma-related pulmonary disease[115]; the combination was well tolerated and improved pulmonary function in mild but not severe restrictive lung disease.

Glomerulonephritis

The proliferation of mesangial cells is a prominent feature of many glomerular diseases, including IgA nephropathy and membranoproliferative glomerulonephritis[116]. PDGF, one of the best-characterized growth factors in renal disease, exerts a potent mitogenic activity on mesangial cells[117], suggesting PDGF inhibition as a therapeutic opportunity in the treatment of proliferative glomerular disease. Imatinib diminished cryoglobulin production and reversed the renal and systemic lesions in a murine model of cryoglobulinaemia and associated membranoproliferative glomerulonephritis[118]. It also ameliorated renal disease and improved survival in murine lupus nephritis[119]. Blockade of PDGFR activity reduced mesangioproliferative changes in experimental mesangial proliferative glomerulonephritis[120,121].

Rheumatoid arthritis

Therapeutic options for rheumatoid arthritis have increased tremendously in the past decade, and several different targets have been identified. Unfortunately, about 40% of patients with rheumatoid arthritis receiving biological therapies fail to achieve an American College of Rheumatology 70 response, and resistance to these drugs is a substantial problem for some patients[122]. Most biological therapies currently approved for the treatment of rheumatoid arthritis are also cumbersome to administer, requiring injection or infusion.

Orally bioavailable small-molecule TKIs may have therapeutic potential in rheumatoid arthritis, as PTKs are involved in various aspects of signal transduction in the disease. In murine collagen-induced and rat adjuvant-induced models of rheumatoid arthritis[123], inhibition of JAK3 preserves cartilage. Furthermore, recent clinical studies investigating inhibitors of two kinases, JAK3 and spleen tyrosine kinase (SYK)[124–126], have shown SYK inhibition produces significant clinical benefits in patients with active rheumatoid arthritis receiving methotrexate therapy[126]. In addition, the KIT inhibitor masitinib demonstrated efficacy in patients who had active rheumatoid arthritis that was refractory to treatment with disease-modifying anti-rheumatic drugs[127]. Recently, the importance of Bruton's tyrosine kinase has been highlighted in a mouse model of rheumatoid arthritis, in which META060 — a multi-kinase inhibitor of Bruton's tyrosine kinase, SYK, PI3K and glycogen synthase kinase — inhibited bone and cartilage destruction[128].

Transplant rejection

Recent improvements in graft survival are largely attributable to developments in immunosuppressive therapies and the availability of numerous immunosuppressive drugs. However, these therapies are frequently associated with severe side effects, which are due in part to the ubiquitous expression of the molecular targets of the immunosuppressive drugs[129].

JAK3 represents an excellent alternative target for clinical immunosuppression because its expression is limited to cells of the immune system[126,130]. JAK3 inhibitors effectively inhibit systemic immune responsiveness and acute allograft rejection. They also prolong cardiac allograft survival in a rodent model[131], as well as prolonging kidney allograft survival in a primate model of kidney transplantation[132,133]. JAK3 inhibition is also associated with a narrow side-effect profile[132]. Combining JAK3 inhibitors with calcineurin inhibitors (currently used in transplant medicine to prevent rejection) facilitates a synergistic interaction[131,132,134], without the side-effect profile that is typical of calcineurin antagonists[135]. In addition, aortic allograft vasculopathy has been reduced in rat models using tyrphostin AG-1295, a specific PDGFR TKI[136].

Data from solid-organ transplant rejection models[137] show that inhibition of the SRC family of tyrosine kinases is another attractive target for the prevention of acute rejection, which is achieved by prolonging the survival of allografts that are not matched for major histocompatibility complex[137]. A matter of concern could be that patients receiving long-term TKI treatment and at the same time being actively listed for organ transplantation (as may occur for patients with pulmonary hypertension or fibrosis) could face unforeseen side effects such as impaired wound healing and/or deregulated host defence mechanisms. However, the wound-healing effects of small-molecule TKIs are reversible upon withholding of the drug, and are dependent on drug half-life. Nevertheless, careful consideration should be given to the design of trial protocols on how treatments should be adjusted once the patient is offered a new organ.

American College of Rheumatology 70 response
An American College of Rheumatology 70 (ACR 70) response requires a patient to have a 70% reduction in the number of swollen and tender joints, and a reduction of 70% in three of the following five parameters: physician global assessment of disease, patient global assessment of disease, patient assessment of pain, C-reactive protein or erythrocyte sedimentation rate, and degree of disability in health assessment questionnaire score.

Active rheumatoid arthritis
A chronic, systemic inflammatory disorder that primarily affects synovial joints but may induce diffuse inflammation in the lungs, pericardium, pleura and skin.

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EX2077.0008

The cardiovascular system

Cardiotoxicity of TKIs: proof or suspicion? In recent years, cancer treatment has experienced a breakthrough with the clinical use of small-molecule TKIs. However, some of these agents are now associated with heart toxicity, including congestive heart failure (CHF) and asymptomatic left ventricular dysfunction[138]. A report published in 2006 described ten cases of heart failure after treatment with imatinib in patients suffering from CML without a prior history of heart disease[74]. However, in this report it was not possible to ascertain the total number of patients that were treated with imatinib during the observation period who had not developed any heart failure. Furthermore, when used in cultured cardiomyocytes and in mice at doses that reproduced plasma concentrations observed in humans treated with typical clinical doses, imatinib caused mitochondrial dysfunction with loss of membrane potential, cytochrome c release into the cytosol, impaired energy generation with reduced ATP content, and finally cell death[74,138]. While the preclinical findings based on molecular, cellular and animal studies were conclusive, the quality of the patient data was less robust, making conclusions regarding the propensity of imatinib to cause heart failure in patients questionable. In particular, confounding factors (for example, pre-existing cardiac diseases, previous pharmacotherapy with potentially cardiotoxic agents and concomitant cardiovascular risk factors) were not described in detail for the patients for whom cardiotoxic effects of imatinib were suggested. Furthermore, myocardial biopsies from only two out of ten patients showed cardiomyocyte damage similar to that found in experimental mouse tissue.

Supporting this notion, in a 5-year follow-up study[139] of 553 patients receiving imatinib for CML, only one patient developed CHF that was considered to be drug related. Furthermore, continuous use of imatinib for up to 6.5 years did not lead to increased rates of serious adverse events compared with shorter-term use[140]. A large clinical study in patients with GIST reported no cardiac events that could be attributed to imatinib treatment[141], and other studies were also unable to confirm cardiotoxicity in patients treated with imatinib[142,143].

Cardiotoxicity is nonetheless an important safety consideration. Other TKIs, such as sunitinib, dasatinib (Sprycel; Bristol–Myers Squibb), bevacizumab and sorafenib, also seem to have adverse cardiac effects, but through different mechanisms[144]. For example, the multikinase inhibitor sorafenib is known to induce acute coronary syndromes, such as myocardial infarction. These agents inhibit kinases that are not the intended drug target, leading to so-called off-target toxicity. However, inhibitors targeting EGFR seem to have little, if any, cardiotoxic effects[138], suggesting that cardiotoxicity is not a class effect of TKIs. For a detailed review of the cardiotoxicity of TKIs, see REF. 138.

Further studies are needed to clarify the underlying mechanisms leading to TKI cardiotoxicity, and to guide strategies — both at the level of drug discovery and in the clinic — to prevent the development of heart failure. The likely benefit of TKI therapy must be balanced against the risk of cardiotoxicity for each indication; cardiotoxicity is of greater concern in non-malignant than malignant diseases because the risk–benefit balance can shift in the absence of the poor prognosis associated with many malignant diseases. Careful cardiovascular monitoring and treatment at the first sign of myocardial damage may help to manage this problem.

Potential impact of tyrosine kinases on myocardial hypertrophy and remodelling. Besides the known undesirable cardiac side effects of TKIs, there is also evidence that members of this class of drugs may have beneficial effects on the heart, particularly in the hypertrophied myocardium. Cardiac hypertrophy results from a response to stress, such as hypertension and myocardial injury. Chronic hypertension leads to ventricular hypertrophy, which enables the heart to maintain a normal stroke volume despite the increase in afterload. Over time, however, the heart undergoes pathological changes resulting in ventricular failure.

Studies to unravel the underlying mechanism of cardiac hypertrophy have increasingly implicated focal adhesion kinase (FAK), a non-receptor tyrosine kinase, in the pathogenesis of cardiac hypertrophy and remodelling. FAK mediates the first phase of the hypertrophic response to stretch in cardiac myocytes[145] by regulating the activation of myocyte enhancer factor 2 and Jun N-terminal kinase–Jun pathways, which have a key role in the early activation of the hypertrophic genetic programme[146]. Tyrosine protein phosphatase non-receptor type 11 (PTPN11; also known as SHP2), an SH2-containing tyrosine phosphatase, negatively regulates the growth of cardiomyocytes by downregulating the FAK–SRC pathway and the mammalian target of rapamycin (mTOR) pathway[147]. Consistent with this, SRC and many MAPKs, including ERK1–ERK2 and p38, are activated in cardiac hypertrophy[148,149], and studies in a rat model of genetic cardiac hypertrophy have suggested a role for Ras–MAPK signalling[150].

The involvement of the PI3K–phosphatase and tensin homologue (PTEN) signalling pathway — a common downstream pathway of many tyrosine kinases — in cardiac pathobiology was reviewed recently[151]. Attenuation of pressure overload-induced cardiac hypertrophy upon inhibition of JAK2 suggests that this non-receptor tyrosine kinase is also involved in the pathogenesis of the condition[152]. Although data regarding the signalling pathways involved in right ventricular hypertrophy are scarce, a study in rats has implicated Raf–ERK activation in the monocrotaline-induced model of this disease[153], suggesting some commonalities in the mechanisms underlying right and left ventricular hypertrophy. Clearly, intracellular signalling pathways involving tyrosine or serine/threonine kinases are activated during pathological cardiac hypertrophy — as shown for EGFR[154,155], for example — and so these kinases could be potential therapeutic targets. Several small-molecule selective TKIs or multi-kinase inhibitors are available that could be tested for therapeutic efficacy in cardiac hypertrophy. However, cardiac toxicity, including some early reports suggesting cardiotoxic effects of imatinib[74,156], remains a concern.

Right ventricular hypertrophy
The thickening of the right ventricular myocardium in response to increased afterload, mostly due to pulmonary hypertension.

Left ventricular hypertrophy
The thickening of the left ventricular myocardium that occurs either physiologically in response to exercise or as a pathological reaction to increased afterload (for example, in aortic stenosis, aortic insufficiency and systemic hypertension) or hypertrophic cardiomyopathies.

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EX2077.0009

Recently, the field of cancer therapeutics has witnessed the development of next-generation potent TKIs such as dasatinib and nilotinib (Tesigna; Novartis), which have higher target specificity at lower dosage. However, they also have toxicities, such as neutropenia and thrombocytopenia[157,158]. The undesirable side effects of other TKIs, such as sunitinib and sorafenib, have also been extensively investigated. Cardiovascular events and congestive heart failure were observed in patients with GIST receiving sunitinib[159], and subsequent studies with sunitinib reported an adverse effect on cardiac function and the development of heart failure[160,161]. In patients treated with sunitinib or sorafenib for renal cell carcinoma, cardiac events and electrocardiography changes were observed[162,163], but no statistically significant difference was shown in survival between patients who experienced cardiac events and those who did not. The authors of the studies suggest that cardiac damage due to treatment with sunitinib or sorafenib is a largely underestimated phenomenon, but is manageable if patients receive careful cardiovascular monitoring and treatment.

In summary, receptor or non-receptor kinases (tyrosine or serine/threonine kinases) are implicated in the pathobiology of cardiac remodelling. There may be a potential therapeutic application for small-molecule inhibitors, but toxicity should be carefully considered. Whereas TKIs seem to cause cardiotoxicity due to off-target effects, they also have considerably different, therapeutic effects on the maladaptive hypertrophy in left and right ventricular hypertrophy.

In-stent restenosis

Stenosis is an abnormal narrowing in blood vessels, which can be treated by balloon dilatation. Stents are expandable meshes (either bare metal or coated) that prevent reoccurrence of stenosis after angioplasty. Restenosis occurs when vascular smooth muscle cells migrate from the tunica media, through the injured endothelium, and proliferate to generate the neointima, which clogs the blood vessel lumen. Lymphocyte infiltration seems to underlie the inflammatory component of this event, with the involvement of growth factors and cytokines, including PDGF, FGF and to a lesser extent IGF1, transforming growth factor-h (TGF-h) and EGF.

All of these factors, which are derived from platelets, mononuclear cells, endothelial cells and smooth muscle cells, may have a role in early cellular events leading to restenosis. However, it is generally accepted that PDGF in smooth muscle cells of the tunica media is the main driver[37,164], with FGF also playing an important part. PDGF, together with other factors released from aggregating platelets and activated monocytes at the site of injury and from smooth muscle cells themselves, acts as a chemoattractant and a proliferating agent responsible for the migration of the smooth muscle cells. As a result, PDGFR kinase inhibitors have been developed to prevent smooth muscle cell proliferation, restenosis and accelerated atherosclerosis in the transplanted heart. A study in pigs investigated the effect of coating stents with tyrphostin AGL-2043, an inhibitor of the PDGFβ RTK, KIT and FLT3 (REF. 165). This showed that in-stent neointimal hyperplasia in porcine coronary arteries was reduced by 50% after 28 days. Lumen area was also preserved in animals with AGL-2043-coated stents but not in animals with stents lacking AGL-2043.

The efficacy of systemic imatinib in the prevention of recurrent restenosis was investigated in a double-blind, randomized, placebo-controlled study involving 180 patients with in-stent restenosis[166]. Patients received imatinib (600 mg per day) for 10 days, starting 2 days before repeat intervention (that is, before a second catheter investigation). The primary end point of the study was a reduction in angiographic restenosis at follow-up angiography, and repeat angiography was performed in 160 of 180 patients (88.9%). The combined rate of death or myocardial infarction at 1 year was 1.0% for both groups. Imatinib did not affect the angiographic restenosis rate compared with placebo or the need for target lesion revascularization[166]. These and other early clinical data support the finding that systemic imatinib therapy does not affect the risk of recurrence in patients with in-stent restenosis[167,168]. However, chronic local administration of imatinib or other tyrosine kinase inhibitors using drug-eluting stents could represent a future approach worth further consideration[169].

Atherosclerosis

According to the response-to-injury hypothesis of atherosclerosis pathogenesis, PDGF released from platelets aggregating at sites of endothelial injury plays a key part in the migration of vascular smooth muscle cells from the tunica media into the intima, as well as the subsequent proliferation of vascular smooth muscle cells at this site[170]. However, the currently accepted view of atherogenesis is local chronic inflammation with lipoprotein retention and the infiltration and activation of various immune cells[171].

PDGF is expressed by nearly every cell type of the atherosclerotic arterial wall, as well as by infiltrating inflammatory cells. Increased levels of PDGFs (A to D), particularly PDGFA[172,173] and PDGFB[174,175], are found in atherosclerotic lesions[176,177]. Expression of PDGFRα[177] and PDGFRβ[178] is also increased in vascular smooth muscle cells in the atherosclerotic vessel wall. However, mechanisms leading to increased PDGF and PDGFR expression during atherosclerosis are unknown.

PDGF is one of many factors secreted into the blood vessel wall by activated immune cells. Gain- and loss-of-function experiments that activate or inhibit signalling through PDGFRβ suggest a role for PDGF in animal models of atherosclerosis. For example, in a model of acute arterial injury induced by balloon catheterization, neointimal accumulation of smooth muscle cells was reduced by neutralizing PDGF antibodies[37]; PDGFB aptamers[179], PDGFR kinase inhibitors[180,181], and PDGFR-neutralizing antibodies[182,183] also showed similar effects in preclinical models. PDGFR kinase inhibitors suppressed restenosis following angioplasty in hypercholesterolaemic minipigs[184] and reduced atherosclerosis induced by chronic cardiac transplant rejection in rats[185]. In apolipoprotein E (APOE)-deficient mice, the formation of atherosclerotic lesions was inhibited

Tunica media
The middle layer of an artery or vein that is made up of smooth muscle cells and elastic tissue. It is localized between the tunica intima and tunica adventitia.

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EX2077.0010

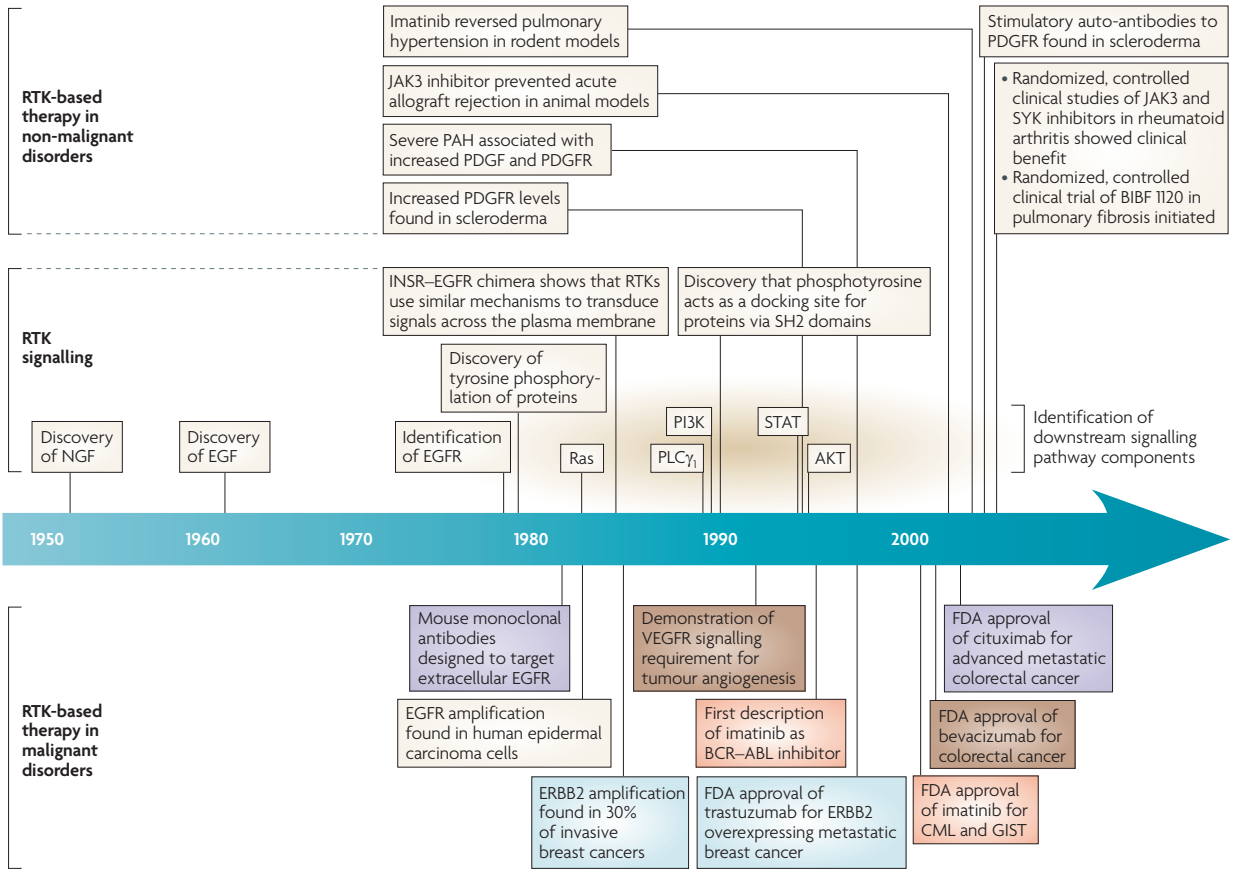

Figure 3 | **Timeline breakthrough discoveries in RTK signal transduction in health and disease (cancerous and non-malignant).** CML, chronic myelogenous leukaemia; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; GIST, gastrointestinal stromal tumour; INSR, insulin receptor; JAK, Janus kinase; NGF, nerve growth factor; PAH, pulmonary arterial hypertension; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; RTK, receptor tyrosine kinase; SH2, SRC homology 2; STAT, signal transducer and activator of transcription; SYK, spleen tyrosine kinase; VEGFR, vascular endothelial growth factor receptor.

by PDGFRβ-specific neutralizing antibodies[186] and delayed by a PDGFR kinase inhibitor[187]. Conversely, infusion of PDGFB or local transfection of a PDGFB expression vector increased smooth muscle cell proliferation and intimal thickening in rat and pig models of arterial injury[188,189]. So far, the evidence supports a role for PDGFRβ in neointimal proliferative responses to arterial injury, whereas a role for PDGFRα has not been demonstrated.

Low-density lipoprotein (LDL) receptor-related protein 1 (LRP1) is a multifunctional transmembrane receptor expressed by numerous cell types that can bind a biologically diverse range of ligands. Studies of LRP1 have provided the most support for a pivotal role of PDGFB, PDGFRβ, and smooth muscle cell migration and proliferation in atherogenesis. Co-immunoprecipitation studies suggest a molecular interaction between LRP1 and PDGFRβ[190], and LRP1 becomes tyrosine phosphorylated upon binding of PDGFB to PDGFRβ[190,191]. Results from cell culture experiments also suggest that when APOE binds to LRP1, PDGFB-induced phosphorylation of LRP1 and PDGFB-dependent smooth muscle cell migration and proliferation are inhibited. In mice lacking LDL receptors (an excellent model for the study of atherosclerosis), specific knockout of LRP1 in smooth muscle cells causes a dramatic increase in PDGFRβ expression and autophosphorylation in these cells, resulting in a markedly enhanced atherosclerotic response, which can be reduced by imatinib treatment[192]. These results suggest that LRP1 normally limits PDGFRβ expression and function in vascular smooth muscle cells. This function may involve two independent, synergistic mechanisms: first, reduction of PDGFRβ expression through inhibition by LRP1 of thrombospondin-mediated TGFβ activation and signalling through SMAD2–SMAD3 (REF. 193); and second, LRP1-mediated regulation of endocytosis and CBL-mediated ubiquitylation of PDGFRβ[194].

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EX2077.0011

Taken together, these studies demonstrate an atheroprotective role of LRP1, which seems to be fully or partially mediated by the suppression of PDGFRβ expression and signalling in vascular smooth muscle cells. Thus, although TKIs might seem promising for the treatment or prophylaxis of atherosclerosis, such an approach should be considered with caution to avoid promoting or exacerbating atherogenesis. As this is a disease that can take decades to establish, TKIs would need to be used over long time spans, necessitating a good safety and tolerability profile. This could be achieved by newly designed TKIs (as the safety profile of currently available agents does not fulfil this criterion). Alternatively, current agents could be used prophylactically at dosages that are substantially below those needed in acute and short-term disorders such as cancer.

Conclusions and outlook

TKIs have proven efficacy for the treatment of chronic myeloid leukaemia, renal cell carcinoma, non-small-cell lung cancer, colon cancer and various other forms of oncological disorders. There is additional evidence that RTK signalling also has an important pathophysiological role in the regulation of cellular differentiation, proliferation and apoptosis in non-malignant proliferative diseases (FIG. 3).

Several preclinical findings suggest a therapeutic potential in so-called benign-proliferative disorders, including pulmonary hypertension, lung fibrosis, rheumatoid disorders, atherosclerosis, in-stent restenosis and glomerulonephritis. However, the current development status of TKIs in non-malignant disorders remains somewhat preliminary and/or mixed. Clinical evaluation is most advanced in the field of pulmonary hypertension (a Phase II randomized, controlled trial is finalized and enrolment in a Phase III trial is to be completed in 2011), interstitial lung diseases (imatinib did not affect survival rates or lung function in patients with IPF), rheumatoid disorders (case reports of individual patients) and coronary artery disease (Phase II trials completed). Further promising preclinical evidence is available for bronchiolitis obliterans, in-stent restenosis, atherosclerosis and transplant rejection. However, the rather concerning risk–benefit profile of the currently approved TKIs (which had been acceptable so far in view of the limited prognosis of most malignant disorders) is not always justifiable when the drug is used on a long-term basis in comparably benign disorders such as PAH or interstitial lung diseases.

It is thus mandatory either to define strict safety margins for future clinical trial protocols in benign-proliferative disorders or, even better, to seek for disease-specific TKI profiling, thereby avoiding undesirable effects such as myelosuppression, cardiotoxicity, fluid retention, hepatic function abnormalities, gastrointestinal side effects and rash. A suggested translational research approach for the development of TKIs in benign-proliferative disorders (as previously used for pulmonary hypertension) could be to first identify the role of specific RTKs in cell culture and in disease-relevant animal models, followed by confirmation of results in human tissue from diseased organs and selection of disease-specific TKIs from the compound pipelines of pharmaceutical companies for subsequent clinical development.

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

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