CASE HISTORY

Discovery and development of sorafenib: a multikinase inhibitor for treating cancer

Scott Wilhelm, Christopher Carter, Mark Lynch, Timothy Lowinger, Jacques Dumas, Roger A. Smith, Brian Schwartz, Ronit Simantov and Susan Kelley

Abstract | Since the molecular revolution of the 1980s, knowledge of the aetiology of cancer has increased considerably, which has led to the discovery and development of targeted therapies tailored to inhibit cancer-specific pathways. The introduction and refinement of rapid, high-throughput screening technologies over the past decade has greatly facilitated this targeted discovery and development process. Here, we describe the discovery and continuing development of sorafenib (previously known as BAY 43-9006), the first oral multikinase inhibitor that targets Raf and affects tumour signalling and the tumour vasculature. The discovery cycle of sorafenib (Nexavar; Bayer Pharmaceuticals) — from initial screening for a lead compound to FDA approval for the treatment of advanced renal cell carcinoma in December 2005 — was completed in just 11 years, with approval being received ~5 years after the initiation of the first Phase I trial.

Metastasis
The spread of cancer cells through lymphatics/blood vessels to other sites or tissues in the body (for example, brain or liver).

Epigenetic events
Reversible heritable changes in gene function or other cell phenotype that occur without a change in DNA sequence.

Department of Cancer Research, Bayer Pharmaceuticals Corp., West Haven, Connecticut 06516, USA. Correspondence to S.W. e-mail: scott.wilhelm.b@bayer.com
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The mainstays of cancer treatment during the twentieth century were surgery, radiation and chemotherapy. However, surgery is not curative in cases of advanced metastatic disease, and radiation and chemotherapy are limited by severe side effects and a limited capacity to discriminate between healthy and cancerous cells. Furthermore, these treatment modalities offer limited therapeutic benefit to patients with solid tumours — the most common malignancies — which are inherently radio- and chemotherapy-resistant.

For the majority of the twentieth century, drug discovery programmes focused on the development of novel cytotoxic chemotherapeutics, owing to a lack of understanding of the molecular mechanisms that drive tumorigenesis. However, in the 1980s, several groups began to identify the key molecular changes responsible for malignant transformation. These changes included the discovery of both cancer-causing oncogenes and tumour-suppressor genes that normally hold cancer in check[1–7], and the identification of epigenetic events such as promoter methylation that predispose individuals to cancer by switching genes that regulate cell growth on or off[8]. Oncogenes are frequently activated by inherited or spontaneous gain-of-function mutations or fusions with other genes[8], or they can be aberrantly expressed due to amplification, increased promoter activity or protein stabilization[8], and so they have integral roles in the genesis of human tumours. The inherited or spontaneous loss of function of tumour-suppressor genes resulting from inactivating mutations or epigenetic events (for example, promoter hypermethylation) was also implicated in tumorigenesis at this time[3,8,9].

These landmark findings greatly contributed to our molecular understanding of cancer, and subsequent studies of oncogene function were instrumental in delineating many of the key signal transduction pathways. Several oncogenes were found to encode either growth factors (for example, *c-sis*)[10], constitutively active growth factor receptor tyrosine kinases (RTKs) (for example, epidermal growth factor receptor (EGFR, also known as ErbB); ErbB2 (also known as HER2/*neu*); and *fms*)[11–13], or non-receptor tyrosine kinases (for example, Src)[14] that relay signals through the ubiquitous MAPK (mitogen-activated protein kinase) intracellular signal-transduction pathway, which is composed of Raf, MEK (MAPK kinase) and ERK (extracellular signal-regulated kinase). Other oncogenes have since been confirmed as encoding constitutively activated components of the MAPK cascade (*Ki-ras*, *N-ras* and *B-raf V600E*)[15,16] or transcription factors that represent nuclear targets of this signal-transduction pathway (*myc*, *fos* and *jun*)[17–19]. The highly evolutionarily conserved MAPK

signalling pathway regulates normal cellular proliferation, survival, differentiation, adhesion and motility by relaying extracellular growth-factor signals to multiple downstream nuclear effectors (for example, transcription factors) via RTKs and Ras[20]. It therefore became clear that dysregulated activation of signalling through the MAPK pathway has a major role in human cancer, including several well-vascularized solid tumour types and haematological malignancies.

Our increased understanding of the aetiology of cancer at the molecular level since the 1980s has shifted the focus of drug discovery and development over the past decade away from non-specific chemotherapeutics and towards rationally designed drugs that target cancer-specific pathways. By inhibiting cancer-specific pathways, it was hoped that the new targeted agents would spare normal cells and thereby offer improved safety benefits over standard chemotherapeutics, while also providing a higher therapeutic index.

Target selection: Raf kinase

The Raf serine/threonine kinase isoforms (A-Raf, B-Raf and Raf1 (or C-Raf)) are the first kinases in the MAPK cascade and are pivotal regulators of cellular proliferation and survival[20]. In addition, it has recently been shown that wild-type Raf1 can prolong cell survival, independent of MAPK signalling, by direct interaction with anti-apoptotic and apoptotic regulatory proteins[20,21]. Dysregulated activation of these direct Raf pathways, which can be independent of Raf's kinase activity (that is, involve a mechanism other than protein phosphorylation), might also be implicated in tumorigenesis and the progression of several solid tumour types[20,21].

Dysregulated signalling through Raf kinase isoforms is detected in ~30% of human cancers[20]. Constitutive B-Raf activity can be caused by activating oncogenic mutations, such as the *b-raf* V600E mutation, which is prevalent in melanomas (63%)[22] and papillary thyroid carcinomas (up to 50%)[23,24]. Wild-type Raf1 is often hyperactivated in a wide range of human solid tumours as a consequence of constitutively active upstream oncogenic *ras* mutants, or the overexpression of upstream growth factors and/or their RTKs in the absence of oncogenic mutations. Furthermore, constitutively active *ras* oncogenes (particularly *k-ras*) are common in human solid tumours, including 90% of pancreatic, 45% of colorectal, and 30% of hepatocellular cancers (HCC), 35% of non-small-cell lung cancers (NSCLC), 15% of melanomas and 10% of kidney tumours[25]. Raf1 hyperactivation in the absence of oncogenic mutations is common in renal cell carcinoma (RCC) (50%)[26] and HCC (100% in 30/30 biopsies)[27], and is associated with poor prognosis in ovarian[28] and androgen-insensitive prostate cancer[29].

In 1989, Kasid *et al.* demonstrated that disrupting the *raf1* gene using the specific antisense oligonucleotide (ASON) ISIS 5132 inhibits the growth of human lung, breast and ovarian tumour xenografts in athymic mice[30]. This provided the first proof-of-concept that the *raf1* gene is a valid anticancer target. ISIS 5132 is a 20-base phosphorothioate ASON designed to hybridize to the 3′-untranslated sequence of *raf1*, inducing its degradation, and decreasing Raf1 protein synthesis[30]. Interestingly, ISIS 5132 also decreased Raf1 activity and enhanced the sensitivity of several resistant human tumour lines to cytotoxic agents and radiation[30].

A collaboration between Bayer Pharmaceuticals and Onyx Pharmaceuticals provided further validation of Raf1 as a target for anticancer drug design. They demonstrated that mice with colon, pancreatic or fibrosarcoma human tumour xenografts harbouring oncogenic *k-ras*, and expressing an MEK construct that disrupted signalling from Raf to ERK, survived twofold longer than controls lacking this construct[31].

Medicinal and combinatorial chemistry

When the project team was formed in 1994, the reagents and assays were available to identify a Raf kinase inhibitor. A scintillation proximity assay for the high-throughput screening (HTS) and identification of selective Raf/MEK/ERK enzyme inhibitors had already been developed by McDonald *et al.* at Glaxo-Wellcome Inc., showing that this approach was feasible[32]. Tumour cell lines that contained oncogenic *k-ras* and/or *b-raf* mutations demonstrated upregulated signalling through the Raf–MEK–ERK pathway. Such tumour lines would be vital for performing the necessary *in vitro* and human tumour xenograft studies required to identify and select candidate Raf kinase inhibitors for further evaluation in Phase I clinical trials. So, the essential tools were available to proceed with the discovery and development of a novel targeted Raf1 kinase inhibitor for the treatment of cancer.

At the onset of the medicinal chemistry programme that led to sorafenib, there were no marketed kinase inhibitors in the field of oncology. However, before the approval of sorafenib, two first-generation targeted anticancer agents — imatinib (Gleevec; Novartis) and erlotinib (Tarceva; Genentech), which target Bcr–Abl and EGFR, respectively — had begun to show clinical benefit[33,34]. Several technologies designed to accelerate the identification of innovative and effective targeted drugs were crucial to these successes[35]. HTS, as described above for the identification of selective Raf/MEK/ERK inhibitors, is one of these key methodologies, which, along with combinatorial chemistry, has become an essential component of the contemporary cancer drug discovery process[35,36]. Another crucial component was the introduction of early ADME (absorption, distribution, metabolism and elimination) optimization in lead identification to facilitate preclinical proof-of-concept studies[37].

By 1994, Bayer and Onyx had engaged in a collaboration to discover novel therapies targeting the Ras–Raf–MEK–ERK pathway. HTS screening for Raf1 kinase inhibitory activity was initiated in 1995, and eventually led to the testing of about 200,000 compounds[38,39]. This effort generated 3-thienyl urea **1** as a promising lead compound, with a Raf1 IC_{50} (half maximal inhibitory concentration) of 17 μM (FIG. 1).

The Raf1 inhibitory potency of the lead 3-thienyl urea was improved tenfold (that is, to 1.7 μM; compound **2**)

Therapeutic Index
(Also known as therapeutic ratio or margin of safety). A comparison of the amount of a therapeutic agent that causes the therapeutic effect to the amount that causes toxic effects.

Scintillation proximity assay (SPA)
A process that uses fluoromicrospheres coated with streptavidin to detect phosphorylation of substrates by kinases, which has become an important technique in high-throughput screening (HTS) for new drugs.

Xenograft
Xenograft mouse models of cancer are created by injecting homogeneous human tumour cell lines into immunodeficient mice.

Combinatorial chemistry
Any of various technologies for the rapid synthesis of large collections of compounds to facilitate the identification of new active compounds for drug targets by high-throughput screening techniques.

IC_{50}
The half maximal inhibitory concentration, or the concentration of an inhibitor that is required for 50% inhibition of a biochemical or cellular target.

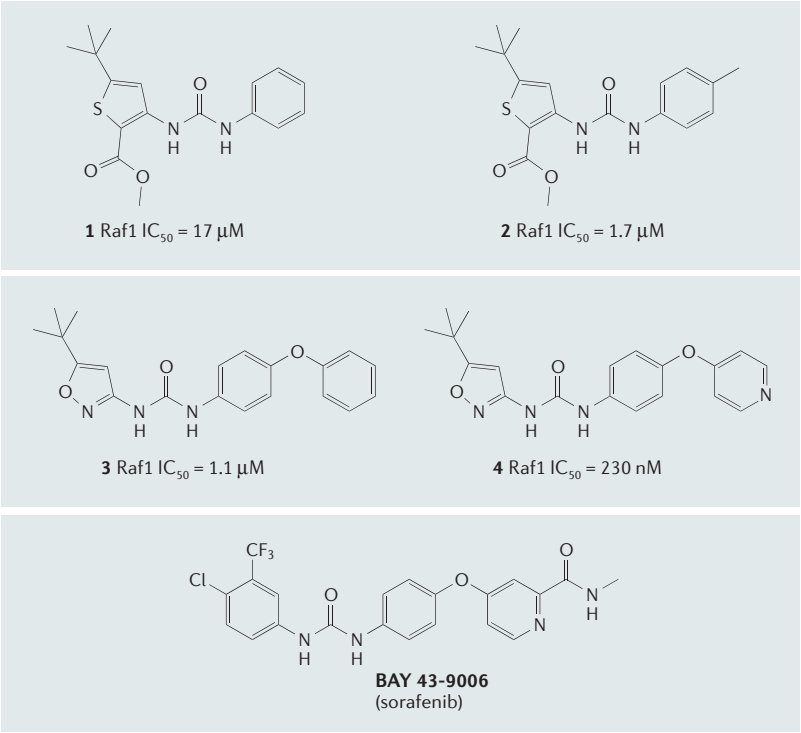


Figure 1 | **Summary of the chemical optimization of sorafenib.** The Raf1 inhibitor **1** was identified through high-throughput screening. Medicinal chemistry efforts described in the main text led to sorafenib.

by a 4-methyl substitution on the phenyl ring. However, no variants of this 3-thienyl urea with IC_{50} values below 1 μM were identified while pursuing a traditional medicinal chemistry approach. A library of ~1,000 bis-aryl urea analogues of the lead compound was then constructed, using rapid parallel synthesis techniques. The objective was to further explore the structure–activity relationships (SAR) of the lead 3-thienyl urea compound, and to improve its Raf1 inhibitory potency. This combinatorial library was then screened against Raf1 to identify a new analogue, 3-amino-isoxazole (compound **3**), exhibiting a Raf1 kinase IC_{50} of 1.1 μM[39,40] (FIG. 1). From this point, the inhibitory potency of compound **3** was increased fivefold (Raf1 kinase IC_{50} 230 nM) by replacing its distal ring with a 4-pyridyl moiety (which produced compound **4**)[40] (FIG. 1). Compound **4** also showed decreased lipophilicity, improved aqueous solubility and significant activity in HCT116 proliferation assays. Furthermore, the inhibition of HCT116 cell proliferation by compound **4** was associated with decreased phosphorylation (that is, decreased activation) of MEK and ERK[31,41]. Compound **4** was found to be orally available in mice, and to inhibit the growth of HCT116 xenografts *in vivo*, thereby providing proof of principle for this new kinase inhibitor class.

Further SAR studies were then undertaken. This effort revealed that although the urea moiety was essential for Raf1 kinase inhibitory activity, aromatic replacements of the heterocyclic moiety of compound **4**, resulting in diphenyl ureas, were tolerated. Finally, modification of the distal pyridine ring, while maintaining the diphenylurea moiety, led to the identification of sorafenib[40,41] (FIG. 1).

In vitro biochemical assays confirmed that sorafenib is a potent *in vitro* inhibitor of Raf1 kinase (IC_{50} of 6 nM) (TABLE 1)[42]. Sorafenib was also shown to potently inhibit the wild-type B-Raf, and oncogenic *b-raf* V600E serine/threonine kinases, pro-angiogenic RTKs (vascular endothelial growth factor receptors (VEGFRs) 1/2/3, platelet-derived growth factor receptor-β (PDGFRβ) and fibroblast growth factor receptor 1 (FGFR1)) and other RTKs involved in tumorigenesis (c-Kit, Flt-3 and RET) *in vitro* (TABLE 1)[42,43]. With the exception of wild-type B-Raf, these molecular targets have been implicated in the aetiology of several forms of human cancer. However, sorafenib had no significant inhibitory effect on MEK1, ERK1, protein kinase B, protein kinase A, protein kinase Cα, protein kinase-Cγ, EGFR, HER2/*neu* or insulin-like growth factor receptor 1 (IGFR1) in biochemical assays (TABLE 1)[42].

X-ray crystallographic studies of the complexes formed between sorafenib and Raf1, wild-type B-Raf and *b-raf* V600E were published in 2004 (REF. 44). The distal 4-pyridyl ring of sorafenib occupies the ATP adenine binding pocket of the kinase domain, interacting with the conserved hinge region in a bidentate fashion[44]. The lipophilic trifluoromethyl phenyl ring at the opposite end of the molecule inserts into a hydrophobic pocket formed between the αC and αE helices and amino-terminal regions of the DFG motif and catalytic loop. The urea functionality forms two crucial hydrogen bonds, one with the backbone aspartate of the DFG loop, the other with the glutamate side chain of the αC helix. Although these X-ray data were not available at the time of the identification of sorafenib, it provided, after the fact, a clear rationale for most of the earlier SAR observations.

Interestingly, the interaction between sorafenib and Raf1–wild-type B-Raf turned out to show some similarity with the interaction between c-Abl and its inhibitor, imatinib[45]. The net effect of sorafenib's interactions with Raf kinase is to stabilize the DFG motif in an inactive conformation[44].

Pharmacological profile

As shown in FIG. 2, sorafenib directly blocks the autophosphorylation of several receptor tyrosine kinases (VEGFR1, 2 and 3, PDGFRβ, c-Kit and RET), as well as inhibiting downstream Raf kinase isoforms (wild-type Raf1, and B-Raf and mutant *b-raf* V600E) in cell lines. Wilhelm *et al.* demonstrated that sorafenib inhibited signalling through the MAPK pathway in a broad range of tumour cell lines harbouring oncogenic *k-ras* and/or *b-raf* mutations[42]. They also showed that sorafenib induced dose-dependent inhibition of the corresponding human tumours when grown as xenografts into athymic nude (*nu*/*nu*) mice[42]. Furthermore, they demonstrated that sorafenib potently inhibited VEGF- and PDGFβ-stimulated phosphorylation of VEGFR2 and PDGFRβ RTKs, respectively, in human cells (TABLE 1)[42]. Sorafenib induced complete tumour stasis in human

Table 1 | ***In vitro* inhibitory profile of sorafenib***

Kinase target	*In vitro* IC_{50} value (nM)
Biochemical (kinase) assay	
Raf-1	6
Wild-type B-Raf	25
Oncogenic b-raf V600E	38
VEGFR1	26
VEGFR2	90
Murine VEGFR3	20
Murine PDGFRβ	57
Flt-3	33
p38	38
c-Kit	68
FGFR1	580
MEK1, ERK1, EGFR, HER2/*neu*, c-met, IGFR1, PKA, PKB, CDK1/cyclin B, pim-1, PKCα, PKCγ	>10,000
Cellular kinase assay	
MEK phosphorylation in MDA MB 231 cells[*‡]	40
ERK1/2 phosphorylation in MDA MB 231 cells[‡]	90
MEK1/2, and p44/p42 MAPK phosphorylation in FRO cells[§]	500
RET phosphorylation in human NIH3T3 fibroblasts	47
Oncogenic V804L RET human thyroid carcinoma cells[‖]	110
Oncogenic V804M RET human thyroid carcinoma cells[‖]	147
Oncogenic b-raf V600E in human thyroid carcinoma cells	1,000
VEGFR2 phosphorylation in human NIH3T3 fibroblasts	30
VEGF–ERK1/2 phosphorylation in human HUVEC cells[¶]	60
PDGFRβ phosphorylation in HAoSMC[#]	80
VEGFR3 phosphorylation in mouse HEK-293 cells	100
Flt-3 phosphorylation in mouse HEK-293 cells with human ITDs[**]	20

*See REFS. 42,43,91,92 for further details. [‡]Human breast carcinoma cells containing G463V *b-raf* and *k-ras* oncogenes. [§]ARO and FRO human thyroid carcinoma cells. [‖]Human thyroid carcinoma cell lines with RET mutations (V804L or V804M) conferring resistance to anilinoquinazolines and pyrazolopyrimidines. [¶]Human umbilical vein endothelial cells (HUVEC). [#]Human aortic smooth muscle cells (HAoSMC). [**]Internal tandem duplication Flt-3 mutants found in human acute myeloid leukaemia. CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; FGFR, fibroblast growth factor receptor; HER, human epidermal growth factor receptor; IGFR, insulin growth factor receptor; ITD, internal tandem duplications Flt-3 mutants; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; PDGFR, platelet-derived growth factor receptor; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; VEGFR, vascular endothelial growth factor receptor.

colon tumour xenograft models (HT-29 and Colo-205 (both of which are *b-raf V600E*-positive) and DLD-1 (*k-ras* positive)) at doses of 30 and 60 mg per kg[42]. It also induced complete tumour stasis in a breast carcinoma xenograft model (MDA-MB-231 containing G463V *b-raf* and *k-ras* oncogenes). In addition, sorafenib inhibited the growth of a number of human xenografts, including ovarian (SK-OV-3, overexpresses EGFR and HER2/*neu*), pancreatic (*k-ras*-positive Mia PaCa 2), melanoma (LOX, UACC 903 and 1205 Lu containing *b-raf V600E*) and thyroid (containing oncogenic RET)[42,43,46]. Although sorafenib inhibited the growth of two NSCLC xenograft models (A549 and NCI-H460), inhibition of the MAPK pathway was not evident in these studies[42]. Sorafenib's inhibitory activity against human colon Colo-205 xenografts was also not associated with a detectable reduction in phosphorylated ERK[42]. Therefore, sorafenib can inhibit the growth of some tumour types by mechanisms other than a direct tumour antiproliferative effect mediated by MAPK pathway inhibition — most probably by exerting potent antivascular effects[42,46].

Sorafenib's tumour growth-inhibitory effects could in fact be attributed to inhibition of tumour angiogenesis, as demonstrated by a significant reduction in microvessel area and microvessel density in the Colo-205 xenograft model[42]. Sorafenib also inhibited the growth of renal tumours in the Renca murine model by disrupting the tumour vasculature[47]. Presumably, inhibition of endothelial cell VEGFR2 and pericyte PDGFRβ activity by sorafenib contributed to its anti-angiogenic effects in these models.

Angiogenesis
The growth of new blood vessels from pre-existing vessels. Angiogenesis is a normal process in growth and development but is also a fundamental process required for the growth of tumours.

Pericytes
Elongated contractile cells found in association with arterioles outside the basement membrane.

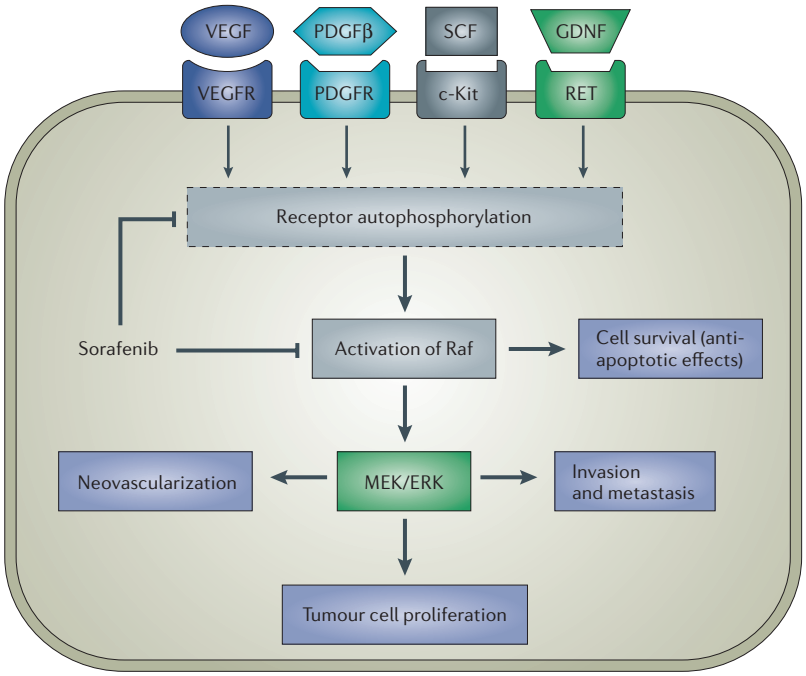


Figure 2 | **Cellular targets of sorafenib.** Sorafenib blocks receptor tyrosine kinase signalling (VEGFR, PDGFR, c-Kit and RET) and inhibits downstream Raf serine/threonine kinase activity to prevent tumour growth by anti-angiogenic, antiproliferative and/or pro-apoptotic effects. ERK, extracellular signal-regulated kinase; GDNF, glial-derived neurotrophic factor; MEK, mitogen-activated protein kinase kinase; PDGFR, platelet-derived growth factor receptor; SCF, stem cell factor; VEGFR, vascular endothelial growth factor receptor.

In other human tumour xenograft models (MDA-MB-231 breast; HT-29 colon; PLC/PRF/5 HCC)[48], sorafenib's activity involved both inhibition of signalling through the MAPK pathway (that is, an antiproliferative effect) and inhibition of angiogenesis. Sorafenib was also recently found to induce apoptosis in several human cancer lines, including ACHN renal carcinoma, HT-29 colon carcinoma, MDA-MB-231 breast carcinoma, A549 NSCLC, PLC/PRF/5 and HepG2 HCC[48], and KMCH cholangiocarcinoma cells, and Jurkat (acute T-cell), K562 (chronic myelogenous) and MEC-2 (chronic lymphocytic) leukaemia cells. In these cancer lines, sorafenib acted by downregulating the levels of the anti-apoptotic protein MCL1 (myeloid cell leukaemia sequence 1)[49,50]. Furthermore, sorafenib induced apoptosis of human melanoma cell lines by an MEK/ERK- and caspase-independent mechanism, which involved the nuclear translocation of apoptosis-inducing factor[51]. The induction of tumour cell apoptosis might represent another mechanism of action for sorafenib in a broad range of tumour types, and this effect seems to involve inhibition of ERK-independent activities of Raf1.

Clinical development in RCC

As the molecular targets of sorafenib are involved in the aetiology of many common malignancies, it was first evaluated in a mixed population of patients with several forms of advanced solid tumours.

Phase I. The first Phase I clinical trial evaluating oral sorafenib tablets (as a tosylate salt) in patients with advanced solid tumours was initiated in July 2000 (REF. 31). A total of four single-agent Phase I trials, evaluating sorafenib oral doses ranging up to 800 mg twice daily (bid), have now been completed and published[52–55]. From these Phase I trials, the optimum regimen and maximum tolerated dose were determined as continuous oral administration of sorafenib at doses of 400 mg bid[56]. Dose-limiting toxicities in these studies included grade 3 diarrhoea and fatigue at 800 mg bid, and grade 3 skin toxicity at 600 mg bid[56]. Overall, sorafenib was well tolerated and the majority of adverse events were mild to moderate in severity and easily manageable[52–56]. In these Phase I trials, 11 patients with metastatic RCC were evaluated for tumour response[52–55] (RECIST). Early signals of antitumour activity were detected in one patient with metastatic RCC, who had a confirmed partial response (PR), which was sustained for 104 days with sorafenib 600 mg bid[54], and two additional RCC patients who experienced sustained (≥2 years) stable disease[52,53].

Sorafenib also demonstrated favourable tolerability and promising preliminary antitumour activity in Phase I combination trials with standard chemotherapeutic agents, including oxaliplatin[57], 5-fluorouracil and leucovorin[58], paclitaxel/carboplatin[59], gemcitabine[60], doxorubicin[61], taxotere[62], CPT-11[63] and dacarbazine (DTIC)[64]. In these trials, the addition of sorafenib did not significantly increase the toxicity of the chemotherapeutic agent. Sorafenib also did not increase the toxicity of interferon when co-administered with this cytokine[65,66].

Rationale for RCC as target indication. RCC is a notoriously chemoresistant, hypervascularized tumour type, often associated with upregulated Raf1, EGFR, VEGF and VEGFR activity[26]. Furthermore, overexpression of VEGF and EGFR are associated with poor prognosis in RCC[67,68]. Loss of function of the von Hippel–Lindau tumour-suppressor gene (*VHL*) in RCC results in the upregulation of hypoxia-inducible factors (HIF1 and HIF2), and consequent aberrant overexpression of transforming growth factor-α (TGFα), and its receptor EGFR, and VEGF[69,70]. In preclinical models, the TGFα–EGFR autocrine loop has been shown to promote the growth of $VHL^{-/-}$ RCC tumours, and VEGF expression promotes neoangiogenesis[69,70]. Therefore, there was a strong rationale to support the use of an oral multikinase inhibitor, such as sorafenib, for the treatment of advanced RCC.

Phase II/III RCC. On the basis of the promising preliminary activity in RCC patients across the Phase I trials, Bayer and Onyx decided to evaluate sorafenib monotherapy as a treatment for RCC by enriching the recruitment of RCC in an accruing Phase II trial, with a randomized discontinuation trial (RDT) design. The very high rate of RCC patients who were progression-free after 12 weeks of dosing in this Phase II trial led to the initiation of the Phase III study to assess the safety and activity of sorafenib. These two randomized controlled

Apoptosis
Programmed cell death.

RECIST criteria
Response Evaluation Criteria in Solid Tumors are standardized, radiographic criteria for determining tumour response or progression in clinical trials of cancer drugs.

Randomized discontinuation trial (RDT) design
A two-phase trial: in Phase I all patients are openly treated with the medication being evaluated; in Phase II, those with stable disease are randomly assigned to continue the same treatment or switch to placebo.

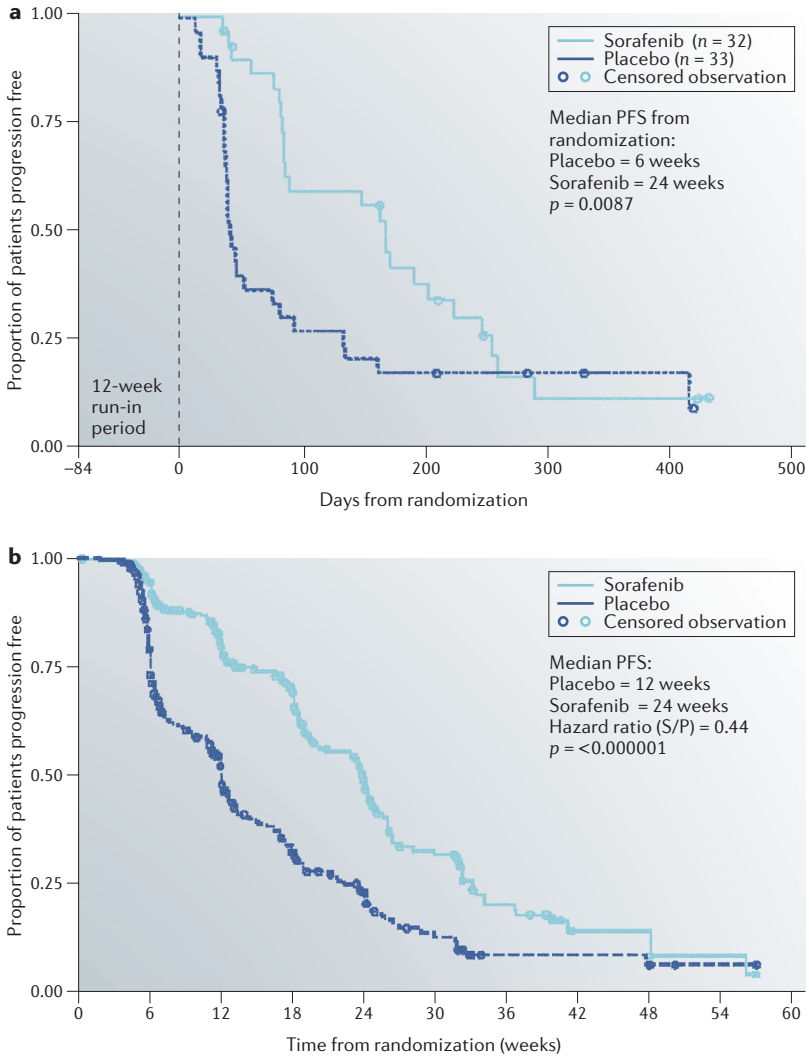


Figure 3 | **Clinical activity of sorafenib in cancer efficacy trials.** Sorafenib significantly prolonged progression-free survival (PFS) in comparison with placebo treatment in patients with advanced renal cell carcinoma participating in a Phase II randomized discontinuation trial (**a**), and a Phase III trial (**b**). Part **a** reproduced, with permission, from REF. 71 © (2006) American Society of Clinical Oncology.

trials confirmed sorafenib's activity against RCC by showing that it significantly prolonged progression-free survival (PFS) compared with placebo in patients with advanced disease[71,72] (FIG. 3a,b). These trials also confirmed that sorafenib was well tolerated by patients with advanced RCC.

In the Phase II RDT, which involved 502 patients with multiple tumour types, a total of 202 RCC patients were evaluated. In this trial, significantly more patients treated with sorafenib (16/32; 50%) were progression-free at 12 weeks post-randomization, compared with those on placebo (6/33; 18%; p <0.0077)[71]. Furthermore, sorafenib treatment significantly prolonged the median PFS fourfold over placebo treatment (24 weeks versus 6 weeks; p = 0.0087)[71] (FIG. 3a). The Phase III Treatment Approaches in Renal cancer Global Evaluation Trial (TARGETs) is the largest randomized controlled trial in RCC performed to date, and involved 903 patients (intention-to-treat cohort) with advanced RCC[72]. In this trial, sorafenib was associated with a twofold increased median PFS compared with placebo treatment (24 versus 12 weeks; p <0.000001)[72] (FIG. 3b). This PFS benefit was independent of gender, age, prior therapy, MSKCC (Memorial Sloan-Kettering Memorial Cancer Center) risk group[73], baseline Eastern Cooperative Oncology Group performance status and time since diagnosis[72]. Furthermore, patients receiving sorafenib experienced a 39% improvement in overall survival relative to those on placebo treatment (p <0.018; hazard ratio 0.72), according to a planned survival analysis[72], although the pre-specified threshold for statistical significance was not reached (O'Brien–Fleming stopping boundary: p ≤0.0005). Nevertheless, the 28% reduction in the risk of death indicates a trend towards improved survival, consistent with the PFS results. In both of the randomized and controlled RCC trials, sorafenib was associated with a low PR rate, but the vast majority of treated patents did experience some degree of tumour shrinkage[71,72].

The TARGETs safety findings were generally consistent with those of the RDT studies. In TARGETs, the most common adverse events were dermatological (rash 40% and hand–foot skin reaction 30%), gastrointestinal (diarrhoea 43%) and constitutional (fatigue 37%)[72]. These events were mostly grades 1–2 in severity. Importantly, the comparative incidence rates of fatigue, nausea, anorexia, vomiting and constipation were similar in the sorafenib and placebo groups. Although hypertension was more prevalent among patients receiving sorafenib (17%) than placebo (2%), almost all cases of sorafenib-related hypertension were grade 1–2 in severity[72].

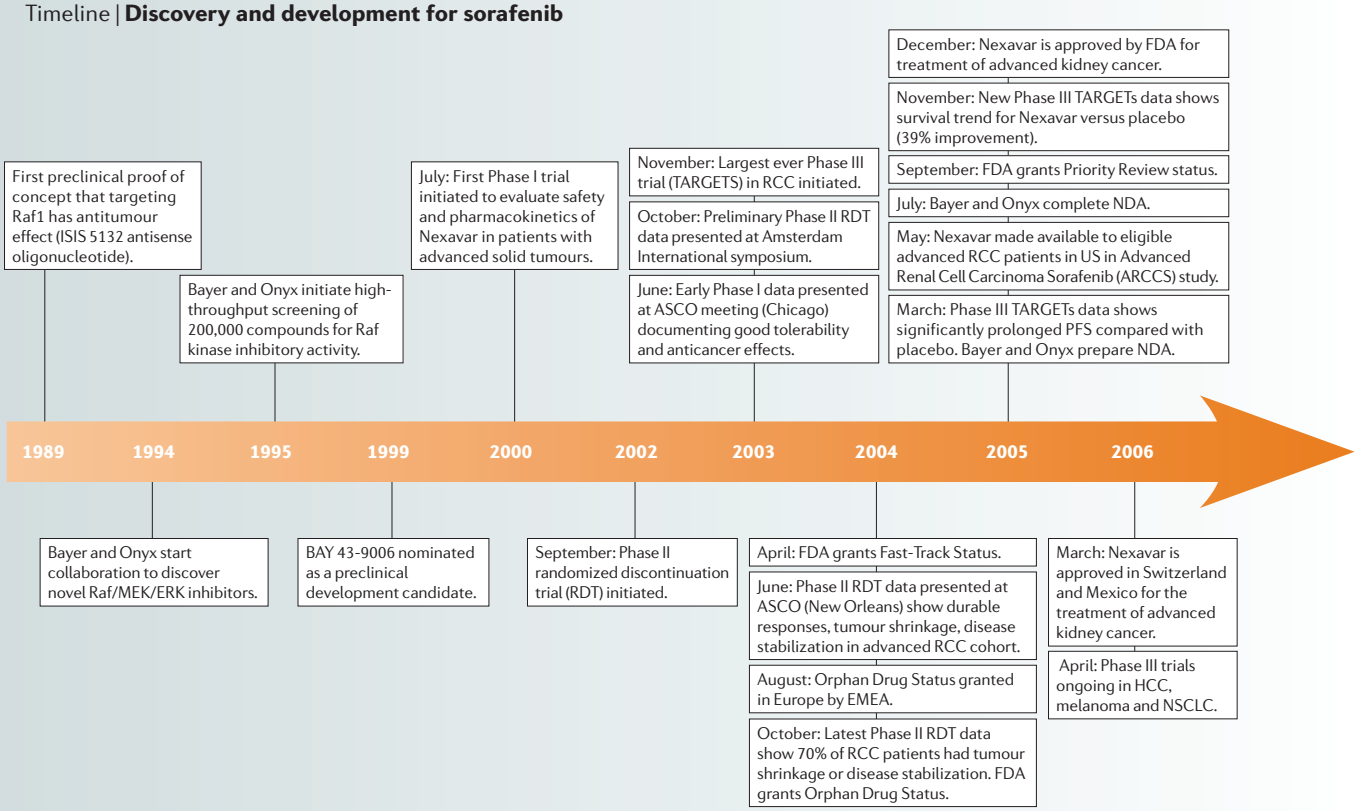
These Phase II/III results established oral sorafenib (400 mg bid) as a safe and effective new treatment for metastatic RCC and formed the basis for its FDA marketing approval in December 2005 for the treatment of advanced RCC. The sorafenib discovery and development cycle — from initial lead compound to approval — took ~11 years (TIMELINE).

Current development

RCC. Clinical trials are ongoing to further evaluate sorafenib in RCC and address a number of issues. Firstly, a Phase II trial is underway to compare sorafenib with interferon, the current standard of care for RCC. The use of combinations of targeted agents that inhibit different cancer pathways is an attractive approach that could further improve survival in notoriously resistant tumours such as RCC. To address this possibility, trials are underway to evaluate sorafenib in combination with other targeted agents, including a Phase II trial in combination with the anti-VEGF monoclonal antibody bevacizumab (Avastin; Genentech/Roche). The combination of sorafenib with the EGFR RTK inhibitor erlotinib is also being evaluated in a Phase II trial.

Two randomized, double-blind, placebo-controlled Phase III adjuvant trials are being planned in patients

Timeline | **Discovery and development for sorafenib**



ASCO, American Society of Clinical Oncology; ERK, extracellular signal-regulated kinase; HCC, hepatocellular carcinoma; MEK, mitogen-activated protein kinase kinase; NDA, New Drug Application; NSCLC, non-small-cell lung cancer; PFS, progression-free survival; RCC, renal cell carcinoma

with resected primary RCC at high or intermediate risk of relapse to resolve whether sorafenib monotherapy improves survival. The ASSURE trial will randomize patients to sorafenib, sunitinib (Sutent; Pfizer) or placebo for 1 year. In the SORCE trial, patients will be randomized to one of three arms: placebo for 3 years; oral sorafenib (400 mg bid) for 1 year followed by placebo for 2 years; or oral sorafenib for 3 years. This trial will address whether length of exposure to sorafenib correlates with survival. In addition, this trial will aim to identify biological parameters that predict benefit from sorafenib. The primary endpoint for this 8-year trial will be metastasis-free survival.

Other tumours. Because sorafenib targets multiple kinases involved in tumour growth and angiogenesis, and has broad preclinical activity in several models of human cancer[42], it would be anticipated to be of clinical benefit in several solid tumour types. In a Phase II trial, sorafenib monotherapy demonstrated a median time to progression (TTP) of 5.7 months and a median overall survival of 9.2 months in 137 HCC patients with good hepatic function[74]. A Phase III trial of sorafenib monotherapy is also ongoing for the treatment of advanced HCC. In a Phase II trial, in which 52 patients with metastatic, refractory NSCLC received sorafenib monotherapy[75], 30 patients experienced stable disease and there was evidence of tumour shrinkage in 15 patients[75]. Sorafenib monotherapy has also shown promise in early Phase II trials in patients with iodine-refractory metastatic papillary and follicular thyroid carcinomas[76], hormone-refractory prostate cancer[77,78] and metastatic breast cancer[79]. Single-agent sorafenib is also being evaluated through the National Cancer Institute's Cancer Therapy Evaluation Program (NCI-CTEP) for the treatment of other tumours, including head and neck cancer, sarcoma, small-cell lung cancer (SCLC) and mesothelioma[80].

The favourable tolerability of sorafenib in single-agent trials has led to its evaluation in combination with other anticancer therapies, including cytotoxic chemotherapy, immunological agents and anti-angiogenic agents. A Phase I trial explored the combination of sorafenib with carboplatin and paclitaxel in patients with solid tumours[59]. On the basis of preliminary evidence of efficacy, a further 105 patients with malignant melanoma were enrolled in this trial. Sorafenib in combination with carboplatin and paclitaxel was well tolerated and demonstrated promising preliminary antitumour activity in this cohort of patients with malignant melanoma[59]. On the basis of these results, two Phase III placebo-controlled trials have been initiated to evaluate sorafenib in combination with carboplatin and paclitaxel in patients with metastatic

malignant melanoma. Sorafenib is also currently being evaluated in combination with carboplatin/paclitaxel in a Phase III placebo-controlled trial in patients with NSCLC. Sorafenib has shown promise in combination with dacarbazine in patients with metastatic melanoma, according to the recently reported preliminary results of a Phase II trial[81].

In addition, sorafenib is being evaluated in combination with the humanized antivascular endothelial growth factor receptor monoclonal antibody bevacizumab in Phase I trials[82,83]. Preliminary results have suggested that this combination has clinical activity in patients with advanced solid tumours; however, the dose-limiting toxicities are under investigation[82,83]. The NCI-CTEP is further evaluating sorafenib in combination with anticancer agents including interferon-α, erlotinib, temsirolimus and cetuximab (Erbitux; ImClone/Bristol-Myers Squibb) in a wide range of tumour types.

Future development

Future issues for the development of sorafenib include the identification and validation of appropriate biomarkers for improved patient selection, prognostication and/or as response endpoints. Preliminary biomarker evaluations from a Phase II trial suggest that high baseline pERK levels are indicative of sorafenib response in HCC[74]. In addition, soluble VEGFR2 (sVEGFR2) levels were significantly decreased 3 weeks after initiating sorafenib treatment in patients with advanced RCC in TARGETs[84]. This effect was sustained after 8 weeks of sorafenib treatment. There was also a weak correlation between the observed reduction in sVEGFR2 and target lesion reduction[84]. These and other putative markers identified by genomic profiling are being further evaluated. Finally, ongoing and future preclinical and clinical studies should soon cast light on sorafenib's mechanism of action in different tumour types, and determine whether inhibition of its other target kinases confers activity against other human malignancies. Possible target malignancies include acute myeloid leukaemia and chronic myeloid leukaemia (Flt-3 and c-Kit are targets)[85,86], imatinib-resistant gastrointestinal tumours (c-Kit is the target)[87], and papillary thyroid carcinoma (RET is the target)[88].

It is anticipated that its favourable tolerability profile will make sorafenib suitable for longer-term administration, either as a monotherapy or in combination with other therapies.

Conclusion

The drug discovery and development cycle of sorafenib, a rationally designed, targeted anticancer agent, took ~11 years. Sorafenib was approved by the FDA for the treatment of advanced RCC in December 2005, just 5 years after the initiation of the first Phase I clinical trials. The identification of a protein kinase involved in tumorigenesis and progression, and the adoption of state-of-the-art HTS and combinatorial chemistry approaches to identify novel compounds (*bis*-aryl-ureas) that inhibit this target kinase (that is, Raf1), greatly facilitated this process. The use of appropriate *in vitro* cancer cell lines and models of human cancer (that is, with upregulated MAPK signalling) were instrumental in identifying the anticancer activity of sorafenib and ensuring that it translated its early promise into the clinical setting.

Among the molecular targets of sorafenib are several RTKs involved in angiogenesis (VEGFR1, 2 and 3, and PDGFRβ) and tumorigenesis (Flt-3, c-Kit and RET). These additional molecular targets of sorafenib might be responsible for its broad-spectrum activity in several models of human cancer. It is clear from the clinical trials reported above that the repertoire of tumour types for which sorafenib could be applicable is rapidly expanding. The multiple molecular targets of sorafenib might account for its antitumour effects, as a single agent or in combination treatment, in clinical trials in RCC[71,72], HCC[74], melanoma[59,64,89] and NSCLC[90]. Similarly the multikinase inhibitory profile of sorafenib leads to effects in cancer cells as well as endothelial cells and pericytes of the tumour vasculature. Sorafenib inhibits tumour growth and angiogenesis by inhibiting cellular proliferation, and inducing apoptosis. Anticancer agents with multiple targets, or combinations of anticancer agents that act on different pathways, might be more effective against cancers with multiple molecular drivers, such as RCC, and it could be less likely that resistance to these drugs will develop in tumours than to agents targeting a single pathway.

The early realization that targeted agents like sorafenib can show clinical efficacy without the evidence of significant tumour shrinkage was also crucial to its successful clinical development. This observation about the drug's activity enabled inclusion of clinical endpoints such as PFS and TTP in clinical trials of sorafenib, to better demonstrate its disease-stabilizing effects. If clinical trials of sorafenib had only incorporated standard RECIST or endpoints defined by the World Health Organization, which rely on the extent of tumour shrinkage as a measure of response, sorafenib might not have reached Phase III trials. Although only a small number of RCC patients treated with sorafenib had PRs in the RDT and TARGETs clinical trials, the majority of patients had some degree of tumour shrinkage, and sorafenib significantly prolonged PFS compared with placebo in these trials[71,72].

In conclusion, in 2005, sorafenib, a molecularly targeted agent that inhibits Raf isoforms and VEGFR1, 2 and 3 RTKs, became the first new treatment to be approved by regulatory authorities for advanced RCC in more than a decade. Sorafenib also inhibits several other RTKs, such as PDGFRβ, c-Kit, Flt-3 and RET. It is conceivable that sorafenib's clinical activity is due to its capacity to inhibit multiple kinases involved in ubiquitous signalling pathways, which are dysregulated in cancer. With its novel mechanisms of action, favourable safety profile and combinability demonstrated so far with several standard chemotherapeutic agents, sorafenib has potential clinical utility in a broad range of tumour types.

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

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CORRIGENDA

Discovery and development of sorafenib: a multikinase inhibitor for treating cancer

Scott Wilhelm, Christopher Carter, Mark Lynch, Timothy Lowinger, Jacques Dumas, Roger A. Smith, Brian Schwartz, Ronit Simantov and Susan Kelley

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On page 836, the following sentence implies that an assay reported in 1999 by McDonald and colleagues was used to discover Raf kinase inhibitors that led to sorafenib: "When the project team was formed in 1994, the reagents and assays were available to identify a Raf kinase inhibitor. A scintillation proximity assay for the high-throughput screening (HTS) and identification of selective Raf/MEK/ERK enzyme inhibitors had already been developed by McDonald *et al*. at Glaxo-Wellcome Inc., showing that this approach was feasible[32]."

The authors wish to correct the text as follows: "When the project team was formed in 1994, the reagents and scintillation proximity assay technology were availble to format a high-throughput assay for the identification of Raf kinase inhibitors. A similar approach was also used by McDonald *et al*. at Glaxo-Welcome Inc., who identified Raf kinase inhibitors using a Raf/MEK/ERK SPA HTS assay[32]."

The authors also wish to clarify that the primary objective of the randomized discontinuation trial (RDT) discussed on page 839 and 840 of the article was to test for disease-stabilizing activity of sorafenib in metastatic colorectal cancer (CRC), with secondary endpoints for activity in other solid tumour types using a design by Ratain. Analysis of the RDT data indicated no disease-stabilizing activity in metastatic CRC patients, but confirmed the early clinical signals observed in metastatic renal cell carcinoma (RCC) patients, and led to the study being refocused towards patients with metastatic RCC.