PERSPECTIVES

TIMELINE

The discovery of receptor tyrosine kinases: targets for cancer therapy

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Receptor tyrosine kinases are a subclass of cell-surface growth-factor receptors with an intrinsic, ligand-controlled tyrosine-kinase activity. They regulate diverse functions in normal cells and have a crucial role in oncogenesis. Twenty years ago, the first primary structure of a receptor tyrosine kinase, the epidermal growth factor receptor, was elucidated. The characterization of both the molecular architecture of receptor tyrosine kinases and the main functions of these proteins and their ligands in tumorigenesis opened the door to a new era in molecular oncology and paved the way to the development of the first target-specific cancer therapeutics.

In the 20 years since the isolation of the cDNA encoding the epidermal growth factor receptor (EGFR) and the deduction of its amino-acid sequence, intensive research efforts have led to important insights into the molecular mechanisms of receptor tyrosine kinase (RTK) function. Moreover, substantial advances have been made in understanding the key roles of RTKs in the signalling pathways that govern fundamental cellular processes, such as proliferation, migration, metabolism, differentiation and survival, as well as those that regulate intercellular communication during development. RTK activity in resting, normal cells is tightly controlled. When they are mutated or structurally altered, however, RTKs become potent oncoproteins: abnormal activation of RTKs in transformed cells has been shown to be causally involved in the development and progression of many human cancers. Consequently, RTKs and their growth-factor ligands have become rational targets for therapeutic intervention using humanized antibodies and small-molecule drugs. In recent years, RTK-based cancer therapies — for example, for the treatment of metastatic breast cancer, gastrointestinal stromal tumours and non-small-cell lung cancer — have reached widespread clinical use and have thereby demonstrated the power of gene-based therapy development.

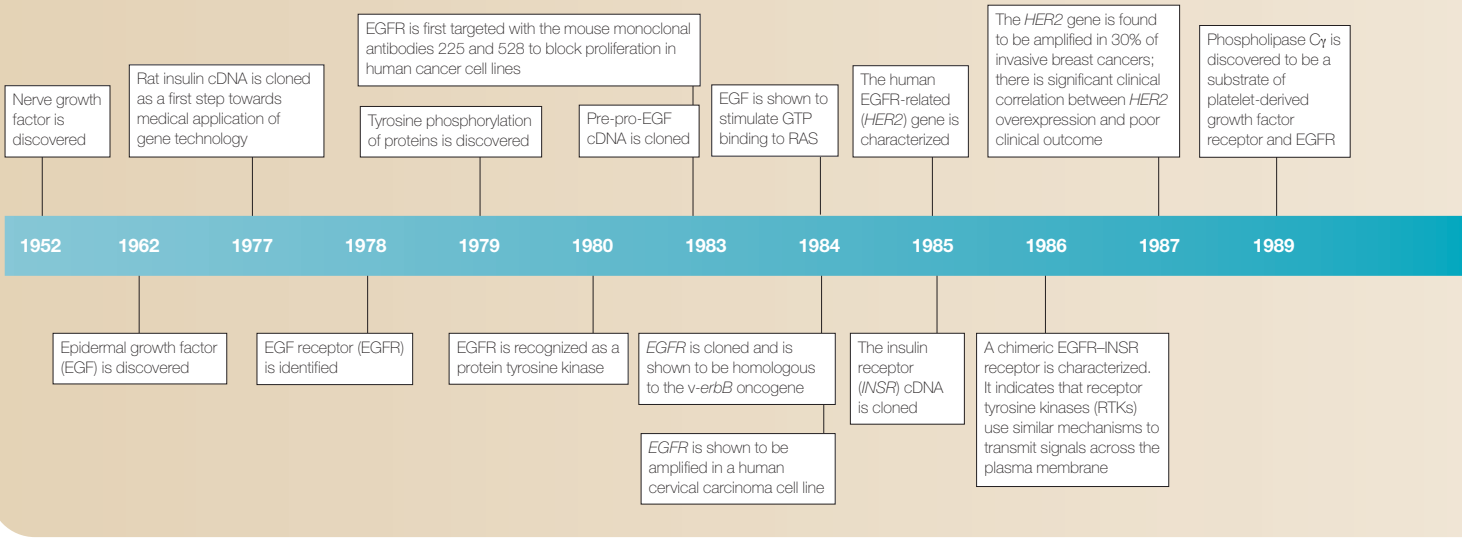
Early discoveries

The beginning of growth-factor research can be traced back to 1952, when Rita Levi-Montalcini in the laboratory of Viktor Hamburger discovered a secreted factor in mouse tumour cells that potently promoted neurite outgrowth in chicken embryos[1] (TIMELINE 1). This protein — nerve growth factor (NGF) — was purified from snake venom and mouse salivary-gland extracts[2,3] by Levi-Montalcini and Stanley Cohen in 1957. Five years later, following on from the work on NGF, Cohen isolated and characterized another salivary-gland protein that induced precocious eyelid opening and tooth eruption when injected into newborn mice[4]. This novel bioresponse-mediating substance was termed epidermal growth factor (EGF), as it stimulated the proliferation of epithelial cells[5]. In 1986, these important discoveries of NGF and EGF earned Levi-Montalcini and Cohen the Nobel Prize in Physiology or Medicine.

In 1975, using ^{125}I-labelled EGF and fibroblasts from different species, Graham Carpenter confirmed the presence of specific binding sites (receptors) for EGF on the surface of target cells (REF. 6). Three years later, Cohen and co-workers identified EGFR as a 170-kDa membrane component that showed increased ^{32}P incorporation in response to EGF treatment in A-431 epidermoid carcinoma cells[7]. It was proposed at that time that the phosphorylation of membrane components and membrane-associated proteins might be crucial events in the generation of intracellular signals that regulate proliferation. However, it was not until 1979 that the modification of proteins by phosphorylation on tyrosine residues was discovered through the study of tumour viruses[8]. Hunter and Sefton then made the important finding in 1980 that the transforming protein of Rous sarcoma tumour virus, v-SRC, has tyrosine-phosphorylation activity[9]. This indicated that deregulated protein tyrosine phosphorylation might be important in tumorigenesis. Moreover, the seminal discovery of the cellular origin of animal retroviral oncogenes by the 1989 Nobel laureates Michael Bishop and Harold Varmus raised the question as to whether oncogenes that initiate signalling pathways through tyrosine phosphorylation could also induce human cancer.

The concept of signal generation by tyrosine phosphorylation gained further experimental support in the early 1980s when three reports showed that EGFR[10], the insulin receptor (INSR)[11] and the platelet-derived growth factor receptor (PDGFR)[12] are protein tyrosine kinases that can be activated by their respective ligands. Subsequently, Hunter and co-workers showed that the stimulation of A431 cells by EGF, and that of NIH-3T3 cells by PDGF, leads to rapid tyrosine phosphorylation of intracellular proteins downstream of the activated growth-factor receptors[13,14]. Further molecular characterization of RTKs and their downstream signalling partners, however, had to wait until the mid-to-late 1980s.

Timeline 1 | **Breakthrough discoveries on RTK signal transduction and RTK-based cancer therapy**



EGFR primary structure

The development of molecular cloning in the mid-1970s led to an important breakthrough in the field of RTK research. This technology allowed the identification of the cDNAs that encode important physiological peptide hormones and growth factors, such as insulin[15,16], EGF[17,18], insulin-like growth factor 2 (IGF2)[19], NGF[20], PDGF[21,22] and transforming growth factor-α (TGF-α)[23]. This, in turn, led to their sequencing and the determination of their amino-acid sequences. Incidentally, the ability to produce peptides such as somatostatin[24] in bacteria and, later, to manufacture medically important hormones, including insulin and growth hormone[25], on a large scale gave rise to the biotech industry in the late 1970s.

As cDNA cloning technologies improved during the early 1980s, it became feasible to clone large gene transcripts. Several laboratories took advantage of this by directing their efforts towards the identification of the cell-surface receptor that mediates the mitogenic activity of EGF. It was widely expected that this accomplishment would significantly improve the understanding of the mechanisms that regulate basic biological phenomena, such as the proliferation and differentiation of both normal and transformed cells. In 1984, a team of collaborators from the Imperial Cancer Research Fund (ICRF; now part of Cancer Research UK), Genentech and The Weizmann Institute of Science isolated and characterized the cDNA sequence of human *EGFR* — the prototypical RTK — from normal placental cells and A431 tumour cells[26].

The first peptide sequences of purified EGFR immediately caused a sensation. Julian Downward — who at that time was in Michael Waterfield's laboratory at the ICRF — searched known protein sequences for matches and hit the jackpot[27]. He found a high level of similarity between the EGFR peptides and sequences of an avian oncogene, v-*erbB*, which had been reported shortly before by Tadashi Yamamoto[28]. This discovery connected, for the first time, an animal oncogene with a human gene that encoded a cell-growth-controlling membrane protein.

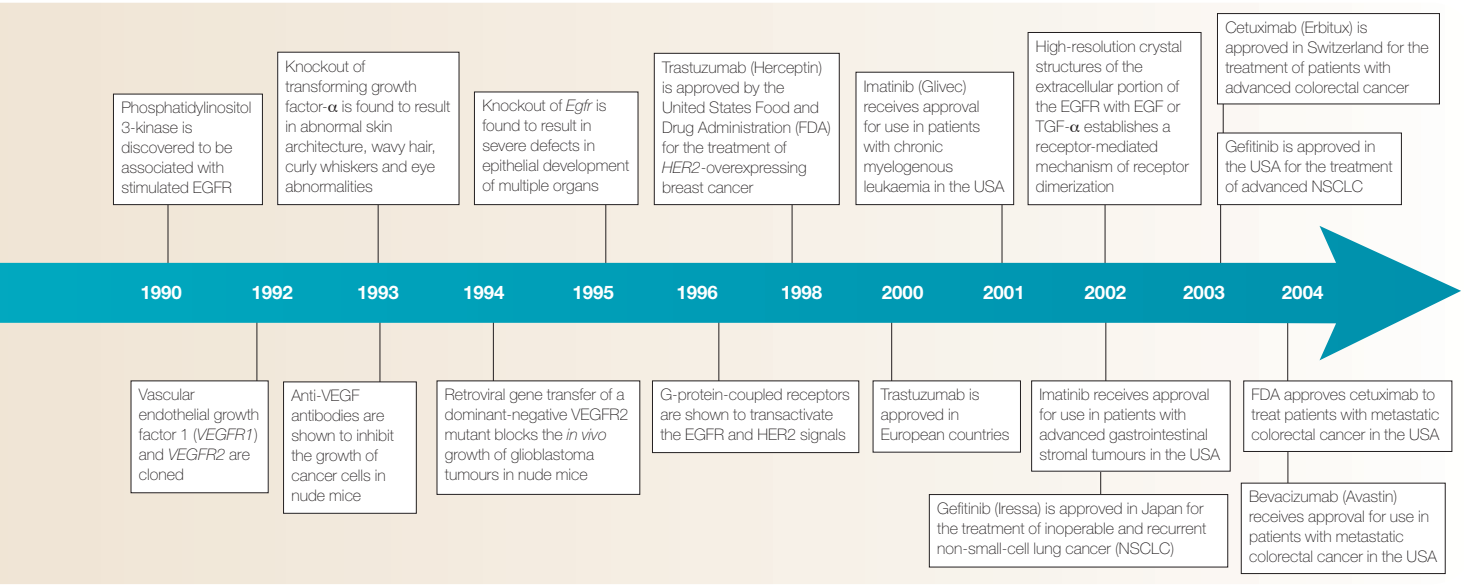
More detailed information was obtained from cloning and sequencing the complete *EGFR* cDNA[26]. Truncations, deletions and mutations in the v-*erbB* oncogene were identified that, as it was speculated at the time, were found to be the genetic basis of the conversion of a proto-oncogene into an oncogene that can cause malignant cancer in chickens infected with avian erythroblastosis. Moreover, the characterization of the *EGFR* cDNA provided the first complete amino-acid sequence of a cell-surface receptor that had signal-generating ability and provided detailed insights into its molecular architecture.

Human EGFR was found to be a large glycoprotein with a modular structure: it contains an extracellular ligand-binding domain, a transmembrane region and a cytoplasmic tyrosine-kinase region that is flanked by non-catalytic regulatory regions. The *EGFR* cDNA-cloning project[26] yielded two other important discoveries. First, Southern blot analysis with an *EGFR* cDNA probe showed a 25-fold amplification of *EGFR* in human A431 epidermal carcinoma cells — a prototypical genetic abnormality that should prove to be highly relevant to future developments. Second, the screening of cDNA libraries yielded sequences that were related to *EGFR* but clearly distinct, which gained importance in subsequent studies. The EGFR family is now known to comprise four members: EGFR, human EGFR-related 2 (HER2; also known as neu/ERBB2), the kinase-impaired HER3 and HER4.

In the years following the elucidation of the primary structure of EGFR, the nucleotide sequences and deduced primary amino-acid sequences of several other RTKs were reported by the Genentech laboratory and its collaborators. These included INSR[29,30], the IGF1 receptor (IGF1R)[31] and PDGFR[32], as well as those that are encoded by the proto-oncogenes *KIT*[33] and *FMS*[34]. This series of studies confirmed that, in spite of their unique biological roles, RTKs are highly related in structure and share a domain arrangement that is very similar to that of EGFR. The RTK class of cell-surface receptors now comprises 58 known members that are distributed among 20 subfamilies. More than half of these have been found to be overexpressed or mutated in human hyperproliferative or hypoproliferative diseases and are therefore considered to be targets for cancer therapy[35].

Signal transduction through RTKs

Mechanisms of RTK activation. An important challenge after the cDNA cloning of several RTKs was the identification of the molecular mechanisms by which these receptors transmit signals across the plasma membrane. In a landmark paper published in 1986, Ullrich and co-workers reported that



they had designed a chimeric receptor molecule that comprised the extracellular region of INSR joined to the transmembrane and intracellular domains of EGFR[36]. Remarkably, they found that the EGFR kinase domain of the chimeric protein was activated by insulin binding, indicating that individual RTKs use closely related mechanisms for signal transduction across the plasma membrane. This, and several other later studies, established that ligand binding to RTKs results in their dimerization and the autophosphorylation of key tyrosine residues in the activation loops of their catalytic kinase domains, resulting in stimulation of tyrosine-kinase activity[37].

Crystallographic studies in the 1990s provided more detailed structural information as to how the dimerization of RTKs is regulated by their growth-factor ligands. For extracellular regions of the vascular endothelial growth factor (VEGF) receptor FLT1 (REF. 38), and the NGF receptor TRKA[39], a bivalent ligand was located at the receptor–receptor interface, where it was shown to directly mediate dimerization. Surprisingly, a different arrangement was found in the case of EGFR, for which two monomeric ligand molecules are bound to an EGF-induced dimer of the EGFR extracellular region[40]. Consistent with this — in two recent publications — Ogiso and colleagues[41], as well as Garrett and colleagues[42], reported that EGFR dimerization is mediated by a unique 'dimerization loop', which becomes exposed after growth-factor-induced domain rearrangement. These data showed that EGFR dimerization is mediated entirely by receptor–receptor interactions (FIG. 1a).

Downstream signalling. In the early 1980s, how the tyrosine-kinase activity of activated RTKs leads to intracellular signal generation and cellular responses was still unknown. A first step towards answering this key question was provided in 1984 when Kamata and Feramisco showed that EGF stimulates the RAS oncoprotein to switch from its inactive GDP-bound form to its active GTP-bound form[43]. Two years later, Stacey and co-workers showed that RAS is essential for cell transformation by RTK-derived oncoproteins[44], indicating that RAS is a downstream mediator of activated RTKs.

In 1989, phospholipase Cγ1 (PLCγ1) was identified as the first downstream substrate that physically interacts with activated EGFR, demonstrating a connection between EGFR stimulation, phosphatidylinositol turnover, intracellular Ca^{2+} mobilization and activation of protein kinase C (PKC)[45,46] (FIG. 1b). The determination of the structure of PLCγ1 led to the identification of SRC-homology 2 (SH2) domains and led to some key discoveries by the groups of Pawson and Hanafus. They found that SH2 domains bind tyrosine-phosphorylated peptides *in vitro*[47,48] and that phosphotyrosine residues in the cytoplasmic regions of RTKs are recognized as docking sites by signalling factors such as PLCγ1 through their SH2 domains.

It was not until 1990 that Julian Downward, as well as Wolfman and Macara, identified one of the missing links between RTKs and RAS. They identified several mammalian cytosolic factors that catalyse the exchange of RAS-bound GDP for GTP — the RAS guanine-nucleotide-exchange factors (GEFs)[49,50], or SOS proteins. The SH3-domain-containing adaptor molecule GRB2 (also known as ASH) was discovered two years later[51–53] and completed the now classical RTK– GRB2–SOS–RAS signal-transduction cascade[54]. These observations, together with a series of publications that linked RAS to RAF and MEK, provided important insights into how RTKs regulate extracellular-signal-regulated kinases (ERKs) and the transcription of genes that are required for proliferation and other important cellular responses[55].

An important advance in the following years was the identification of additional downstream signalling pathways of activated RTKs. Phosphatidylinositol 3-kinase (PI3K)[56], the survival mediator AKT (also known as protein kinase B)[57] and the signal transducer and activator of transcription (STAT) proteins[58] were found to be activated by RTKs and to be involved in cellular responses such as anti-apoptotic signalling, motility and invasiveness.

In recent years, EGFR and other RTKs have been shown to be stimulated by heterologous signals that arise from cytokine receptors[59], integrins[60], membrane depolarization[61], cellular stress[62] and G-protein-coupled receptors (GPCRs)[63] (FIG. 1c). In several normal and transformed cell types, EGFR signal transactivation by GPCRs has been shown to rely on cell-surface metalloproteases that mediate the processing of EGF-like-growth-factor precursors[64,65]. Recently, EGFR signal transactivation pathways have been implicated in the pathogenesis of hyperproliferative diseases, such as cancer[64,65] and cardiac hypertrophy[66], as well as in *Staphylococcus aureus*[67] and *Helicobacter pylori*[68] infections. Taken together, these findings established that EGFR functions as a

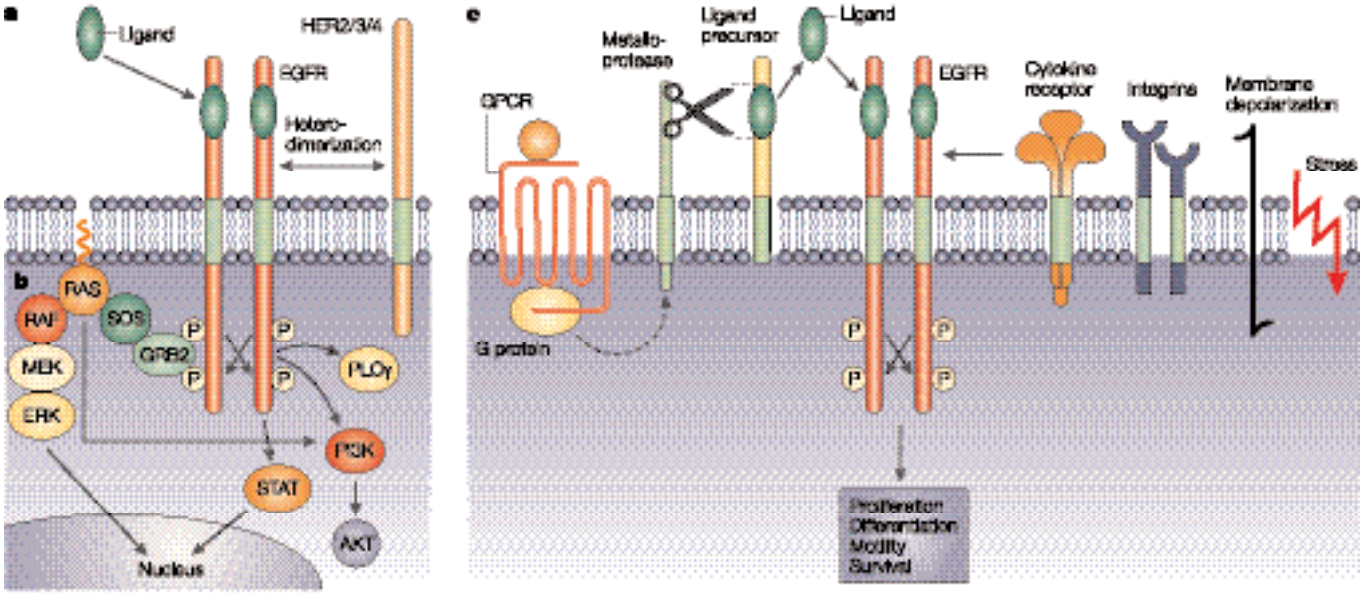


Figure 1 | **The epidermal growth factor receptor signalling network. a** | Ligand binding to the epidermal growth factor receptor (EGFR) induces dimerization through a receptor-mediated mechanism. Signal diversification is generated by the presence of multiple EGF-like ligands and the formation of different dimeric receptor combinations. **b** | Receptor dimerization results in cross-autophosphorylation of key tyrosine residues in the cytoplasmic domain, which function as docking sites for downstream signal transducers. EGFR stimulation results in activation of signalling cascades that include the RTK–GRB2–SOS–RAS–RAF–MEK–ERK, PI3K–AKT, PLCγ and STAT pathways. EGFR can activate PI3K through RAS-GTP in some cell types. **c** | EGFR acts as a point of convergence for heterologous signals from G-protein-coupled receptors (GPCRs; metalloprotease-mediated EGFR signal transactivation), cytokine receptors, integrins, membrane depolarization and agents that are induced by cellular stress. The EGFR thereby defines crucial cellular responses, such as proliferation, differentiation, motility and survival. ERK, extracellular-signal-regulated kinase; GEF, guanine-nucleotide-exchange factor; PI3K, phosphatidylinositol 3-kinase; PLCγ, phospholipase Cγ; STAT, signal transducer and activator of transcription.

point of convergence for diverse signalling pathways and defines key biological outcomes, such as cell proliferation, differentiation, motility and survival, in response to a wide range of physiological stimuli.

In vivo functions of RTKs

The use of gene-targeted mouse models has led to important findings regarding the role of EGFR and other RTKs, as well as their ligands, in mammalian development (TABLE 1). By 1995, inactivation of *Egfr* was reported by three groups. *Egfr* knockout in mice resulted in embryonic lethality[69] or severe failure of epithelial development in several organs — including skin, lung and gastrointestinal tract[70,71]. The multiple abnormalities associated with *Egfr* deficiency confirmed that this receptor is required for a wide range of cellular activities and for epithelial development *in vivo*. Subsequently, the targeted disruption of the other EGFR family members — Her2, Her3 and Her4 (REFS 72–74) — were shown to cause defects in neural and cardiac development. In contrast to *Egfr* knockout, the tissue-restricted or mild phenotypes of knockouts for several Egfr ligands — such as Egf[75], Tgf-α[76,77], amphiregulin[75] and heparin-binding Egf (HB-Egf)[78,79] — indicated considerable redundancy in the functions of these growth factors. In 1998, Roy Black and co-workers reported that the targeted disruption of the metalloproteinase tumour-necrosis factor-α-converting enzyme (Tace)[80] results in a similar phenotype to that found in *Egfr*-null mice — it is characterized by multiple epithelial defects[81]. Tace was found to be required for ectodomain cleavage and solubilization of Tgf-α, and there is evidence to indicate that Tace has an even broader role in regulating the availability of other Egf-like growth factors[65,82].

Deregulation of EGFR in human cancer

In the 1980s, numerous reports described the overexpression of EGFR in various epithelial tumours and substantiated the view that deregulated EGFR signalling has an important role in human cancers. Following these observations, increased stimulation of EGFR through autocrine growth-factor loops, in particular through TGF-α[83], was identified as a common mechanism of RTK deregulation. Moreover, many laboratories embarked on a massive search for *EGFR* mutations in human cancers and several deletions and point mutations were described that result in increased catalytic tyrosine-kinase activity of the receptor[84]. The most prevalent of these mutations in tumours was found to be *EGFR*vIII, an *EGFR* deletion mutant that lacks exons 2–7, which can arise from gene rearrangement or alternative mRNA splicing[85]. More recently, impaired receptor downregulation has been recognized as another mechanism of RTK deregulation[86]. In particular, oncogenic forms of the ubiquitin ligase CBL were shown to function as dominant-negative mutants that prevent CBL from negatively regulating RTKs[87,88].

Discovery of HER2/*neu*

In 1985, as a by-product of the *EGFR* cloning project, Axel Ullrich's group at Genentech described the complete primary structure of a putative RTK that showed a high level of homology to human EGFR and was therefore named human EGFR-related 2 (HER2)[89]. Other laboratories independently identified this new EGFR relative with unknown function and named it ERBB2 (REF. 90). Interestingly, the chromosomal localization of *HER2* is identical to that of the rat *neu* oncogene[91] — as was established by a collaboration between the Weinberg and Ullrich laboratories and in the laboratory of Uta Franke — and this provided another connection between an RTK and cancer development in animals. The oncogenic significance of *neu* was further substantiated when Robert Weinberg and co-workers showed that monoclonal antibodies (mAbs) against the *neu* oncogene reverted its transforming effects in

Table 1 | **Use of gene knockouts to study the functions of receptor tyrosine kinases and their ligands**

Mouse model	Description	Phenotype	References
Egfr$^{-/-}$	*Egfr* knockout	Impaired epithelial development in several organs, including skin, heart, lung and gastrointestinal tract; survival for up to 3 weeks after birth	69–71
Her2$^{-/-}$	*Her2* knockout	Defects in neural and cardiac development; embryonic lethality	72
Her3$^{-/-}$	*Her3* knockout	Defects in cerebellar and cardiac development; embryonic lethality	73
Her4$^{-/-}$	*Her4* knockout	Defects in neural and cardiac development; embryonic lethality	74
Tgfα$^{-/-}$	*Tgf-α* knockout	Abnormal skin architecture, wavy hair, curly whiskers and corneal inflammation	76,77
HB-Egf$^{-/-}$	*HB-Egf* knockout	Heart failure with enlarged ventricular chambers and cardiac valves; lethality in first postnatal week	78
Egf$^{-/-}$	*Egf* knockout	No overt phenotype	75
Egf$^{-/-}$, *Ar*$^{-/-}$	*Egf/Ar* double-knockout	Defects in mammary-gland development	75
Tace$^{\Delta Zn/\Delta Zn}$	Disruption of essential Zn^{2+}-binding motif	Multiple epithelial defects affecting eyes, hair and skin; corneal inflammation; lethality between E17.5 and first day after birth	81
Pdgfrα$^{-/-}$	*Pdgfrα* knockout	Defects in neural-crest-cell development and somite patterning; embryonic lethality	133
Pdgfrβ$^{-/-}$	*Pdgfrβ* knockout	Defects in kidney development; haematological disorders; embryonic lethality	134
Vegfr1$^{-/-}$	*Vegfr1* knockout	Defects in organization of embryonic vasculature; lethality *in utero* at mid-somite stage	119
Vegfr2$^{-/-}$	*Vegfr2* knockout	Defects in development of haematopoietic and endothelial cells; lethality *in utero* 8.5–9.5 days post coitum	120
Vegf$^{-/+}$	Heterozygous *Vegf* knockout	Defects in angiogenesis and blood-island formation; embryonic lethality	135,136

Ar, amphiregulin; *Egf*, epidermal growth factor; *Egfr*, Egf receptor; *HB-Egf*, heparin-binding Egf; *Her*, human Egfr-related; *Pdgfr*, platelet-derived growth factor receptor; *Tace*, tumor-necrosis factor-α-converting enzyme; *Tgfα*, transforming growth factor-α; *Vegf*, vascular endothelial growth factor; *Vegfr*, Vegf receptor.

NIH-3T3 cells[92]. The crucial next step, which addressed the key question of whether genetic abnormalities in the EGFR or HER2 systems could be identified in human tumours, was made through a collaboration formed in 1985 by the Ullrich laboratory and Dennis Slamon, an oncologist at the University of California, Los Angeles. Slamon had assembled a collection of primary breast tumours and was ready to use Ullrich's gene probes to search for abnormalities in tumour DNA. Two years later, this collaborative team reported that the *HER2* gene is amplified in 30% of invasive breast cancers and, for the first time, showed a significant correlation between *HER2* overexpression in tumours and reduced patient survival and time to relapse[93]. These findings established *HER2* as a prognostic factor and indicated a crucial role of *HER2* overexpression in the pathogenesis of breast and ovarian cancers[94].

Given that a specific ligand for HER2 homodimers had, and has still, not been identified, the role of HER2 within the cellular signalling network was largely unclear during the years following its discovery. The first clue to this was provided in 1988, when Stern and Kamps showed that EGFR activation induces transphosphorylation of HER2 through heterodimerization[95]. This was subsequently confirmed by King and colleagues[96] and extended by Nancy Hynes and co-workers, who showed that HER2 is the preferred heterodimerization partner for EGFR, HER3 and HER4, and that HER2 thereby provides an additional mechanism for the recruitment of diverse intracellular signalling pathways[97]. This and other studies established that the existence of multiple ligands and receptors provides the EGFR signalling network with the ability to regulate a wide range of cellular responses.

RTK-based cancer therapies

Monoclonal antibodies. The discovery of *HER2* gene amplification in breast and ovarian cancer provided an important opportunity to evaluate the concept of target-specific cancer therapy. The Genentech group set out to develop HER2-specific mAbs and to assess their anti-oncogenic potential in cell-culture and animal-model systems[98,99]. This provided the basis for the subsequent humanization of mAb 4D5 and the development of the therapeutic antibody trastuzumab (Herceptin, Genentech, Inc.) as the first targeted anti-kinase therapeutic agent based on genomic research (FIG. 2). Trastuzumab was approved by the United States Food and Drug Administration (FDA) for the treatment of *HER2*-overexpressing metastatic breast cancer in 1998 (see TABLE 2 for a selection of

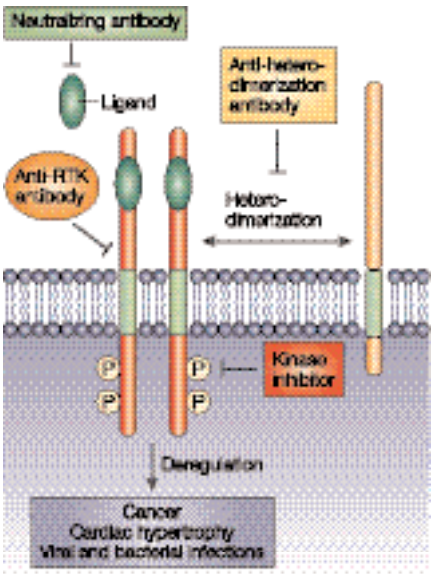


Figure 2 | **Receptor tyrosine kinases: sites of therapeutic intervention.** Deregulation of the receptor tyrosine kinase (RTK) signalling network is crucial for the development and progression of hyperproliferative diseases (for example, cancer and cardiac hypertrophy) and infectious diseases (for example, bacterial and viral infections). Neutralizing antibodies, which block the bioactivity of RTK ligands, RTK-targeted antibodies, which either target overexpressed receptors or receptor heterodimerization, and small-molecule inhibitors of RTK kinase activity have been developed to interfere with RTK signal transduction.

Table 2 | **Cancer therapies targeted to receptor tyrosine kinases**

Names	Targets	Status	Description	Company
Trastuzumab, Herceptin	HER2	Approved for metastatic breast cancer	Humanized anti-HER2 IgG1κ	Genentech
Imatinib, Glivec, STI571	BCR–ABL, KIT, PDGFR	Approved for CML and GIST	2-Phenylaminopyrimidine	Novartis
Gefitinib, Iressa, ZD1839	EGFR	Approved for NSCLC	Quinazoline	AstraZeneca
Cetuximab, Erbitux	EGFR	Approved for colorectal cancer	Chimeric anti-EGFR IgG1	ImClone/Merck
Bevacizumab, Avastin	VEGF	Approved for colorectal cancer	Humanized anti-VEGF (rhu mAb-VEGF)	Genentech
OSI-774, Tarceva	EGFR	Clinical development	Quinazoline	Genentech/ Roche/OSI
CI-1033	EGFR, HER2	Clinical development	4-Anilinoquinazoline, irreversible inhibitor	Pfizer
EKB-569	EGFR, HER2	Clinical development	4-Anilinoquinoline-3-carbonitrile, irreversible inhibitor	Wyeth
CDP860	PDGFR	Clinical development	Anti-PDGFβ-receptor antibody fragment	Celltech
Pertuzumab, Omnitarg, 2C4	HER2	Clinical development	Humanized anti-HER2 (heterodimerization inhibitor)	Genentech
SU6668	VEGFR2, PDGFR, FGFR	Clinical development	Indoline-2-one	Sugen/Pfizer
SU11248	VEGFR2, KIT, PDGFR, FLT3	Clinical development	Indoline-2-one	Sugen/Pfizer
ZD6474	VEGFR2	Clinical development	Quinazoline	AstraZeneca
PTK-787/ZK222584	VEGFR1/2, PDGFR	Clinical development	Anilinophthalazine	Novartis/Schering
AG013736	VEGFR2, PDGFR	Clinical development	–	Pfizer
CP549, 632	VEGFR2, FGFR1, TIE2	Clinical development	–	Pfizer
PKC-412, midostaurin	PKC, VEGFR2, PDGFR, FLT3, KIT	Clinical development	*N*-Benzoylstaurosporine	Novartis
CEP-701	FLT3, TRK kinases	Clinical development	Indolocarbazole alkaloid	Cephalon
MLN-518, CT53518	PDGFR, KIT, FLT3	Clinical development	Quinazoline	Millennium

CML, chronic myelogenous leukaemia; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; FLT, FMS-related tyrosine kinase; GIST, gastrointestinal stromal tumour; HER, human EGFR-related; Ig, immunoglobulin; NSCLC, non-small-cell lung carcinoma; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

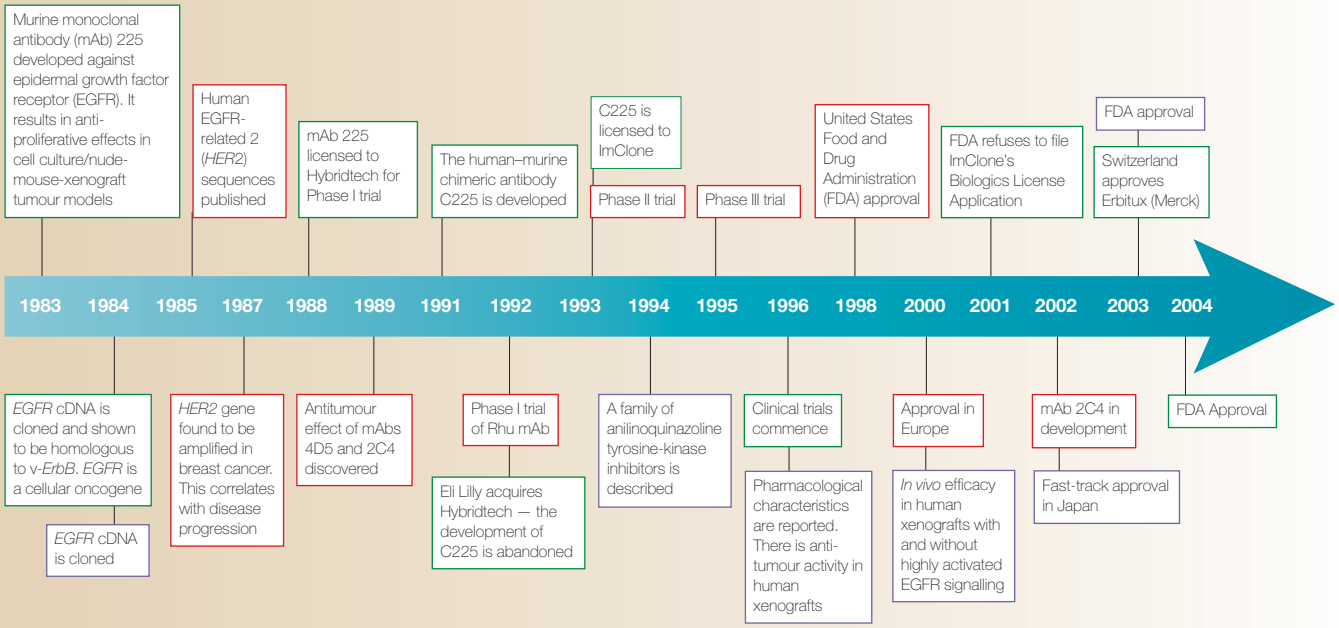
RTK-targeted therapies that have been approved or are in clinical development). Trastuzumab binds HER2 on the surface of tumour cells[98] and induces receptor internalization, inhibition of cell-cycle progression and recruitment of immune-effector cells. Demonstration of the antitumour activity of trastuzumab in breast cancer patients provided the proof of principle that therapeutic agents targeted against a human oncoprotein in a major cancer indication could be successful.

Another anti-HER2 antibody, 2C4 — known as pertuzumab in its humanized form (Omnitarg (rhu mAb-2C4), Genentech) — was first described by Hudziak and colleagues in 1989 (REF. 98). It is now in Phase II clinical trials and represents a second generation of anti-HER2 monoclonal antibodies that interfere with the mechanism of oncogenic signal generation by HER2–HER3 heterodimers[100]. It therefore complements the molecular armamentarium that is available for treating cancers that do not show HER2 expression.

A visionary effort and landmark accomplishment in EGFR-targeted cancer therapy was the design of the mouse mAbs 225 and 528 to extracellular epitopes of the receptor[101,102] by Mendelsohn and colleagues in the early 1980s. On the basis of its promising antitumour activity in cultured human tumour cell lines and rodent models, the 225 antibody was selected for clinical development. In 2003, the Swiss Agency for Therapeutic Products (Swissmedic) approved the use of the chimeric human–mouse anti-EGFR antibody cetuximab (IMC-C225 (Erbitux), ImClone Systems/ Merck KGaA) for the treatment of patients with colorectal cancer who no longer respond to standard chemotherapy treatment with irinotecan. In early 2004, the FDA approved cetuximab to treat patients in the USA with advanced, metastatic colorectal cancer. The fact that it took 20 years to develop cetuximab as a therapeutic exemplifies the many pitfalls that can significantly affect the realization of a novel concept in clinical application (TIMELINE 2).

Small-molecule inhibitors. After EGFR and other tyrosine kinases had been validated as suitable pharmacological targets for anticancer drugs, one of the hottest races in pharmaceutical development began — to identify rationally designed, small-molecule anticancer drugs. Levitzki's group at the Hebrew University in Jerusalem was at the forefront of the development of tyrosine-kinase inhibitors that were targeted to RTKs and demonstrated their potential use as antiproliferative agents in the late 1980s[45,103]. The therapeutic approach to targeting the EGFR with small-molecule inhibitors is based on the early observations by Honegger and co-workers in 1987 that mutations in the ATP-binding pocket of EGFR abrogate its tyrosine-kinase function[104] and interfere with its oncogenic signalling[105,106]. In 1994, the tyrosine-kinase inhibitory activities of quinazolines were first described[107,108], and two years later Wakeling and co-workers reported the pharmacological characteristics of gefitinib (Iressa (ZD1839), AstraZeneca) as a potent and selective inhibitor of EGFR tyrosine-kinase activity[109].

Timeline 2 | **Development of selected RTK inhibitors as anticancer therapeutics**



Stages in the development of different receptor tyrosine kinase inhibitors are shown as follows: green boxes, cetuximab; purple boxes, gefitinib; red boxes, trastuzumab.

In 2002, gefitinib was approved in Japan for the treatment of inoperable and recurrent non-small-cell lung carcinoma (NSCLC) and was also approved a year later in the USA.

Several pharmaceutical companies and academic laboratories have successfully developed small-molecule tyrosine-kinase inhibitors. Imatinib (Glivec (STI571), Novartis; known as Gleevec in the USA) was originally developed as a derivative of a PKC inhibitor by Ciba scientists led by Alex Matter. It has provided the proof of concept for the clinical efficacy and tolerability of this class of compound and was the first selective inhibitor to be approved by the FDA for the treatment of cancer. Imatinib was first described in 1996 by Druker and colleagues as having potent activity against the BCR–ABL oncoprotein[110]. This constitutively active non-receptor tyrosine kinase is expressed in chronic myelogenous leukaemia (CML) cells that express the Philadelphia chromosome — a reciprocal translocation between chromosomes 9 and 22 that replaces the first exon of *ABL* with sequences from the *BCR* gene. This translocation represents the key oncogenic event in 95% of patients with CML. In addition to ABL and BCR–ABL, the RTKs PDGFR and KIT were also found to be potently inhibited by imatinib[111]. As KIT is thought to have an important role in the pathogenesis of gastrointestinal stromal tumours (GISTs), clinical studies with imatinib were successfully extended to this tumour type[112]. Imatinib received approval by the FDA for use in patients with CML in 2001 and for advanced GIST in 2002.

RTKs and anti-angiogenic therapy

VEGF and its receptors as targets. VEGF and its receptors are known to have important functions in the regulation of tumour angiogenesis[113,114]. In 1992, DeFries discovered that FMS-like-tyrosine kinase 1 (FLT1) is a receptor for VEGF[115]; a second VEGF receptor, VEGFR2 (also known as FLK1 or KDR), was subsequently described[116–118]. A crucial role for both of these RTKs in angiogenesis was shown in knockout mice[119,120]. Proof that VEGF and VEGFR signalling are required for tumour angiogenesis was presented in two seminal studies in the mid-1990s. Napoleone Ferrara and his associates showed that anti-Vegf antibodies abrogate the growth of tumour xenografts in nude mice[121], and Birgit Millauer and colleagues showed that a dominant-negative Vegfr2 mutant blocks the subcutaneous growth of experimentally induced glioblastomas in the same model[122]. The broad relevance of this discovery was later substantiated by data that were obtained from various other tumour types[123]. The use of retroviruses encoding dominant-interfering mutants of RTKs in this series of experiments indicated a therapeutic application of retroviral gene therapies in the treatment of human cancers. More importantly, however, the experimental results of Millauer and Ferrara demonstrated the clinical potential of anti-angiogenic therapy by targeting either the ligand or the corresponding receptor as crucial elements of a biological signalling system.

Therapeutic applications. On the basis of these findings, VEGF and VEGFRs became established as important targets for therapeutic intervention in tumour growth. VEGF was targeted by monoclonal neutralizing anti-bodies and VEGFR by small chemical compounds. Bevacizumab (Avastin, Genentech) is a humanized antibody against VEGF[124] that has recently been approved by the FDA for the treatment of colorectal cancer in the USA. Bevacizumab is the first FDA-approved therapy that is designed to inhibit angiogenesis.

The first small-molecule VEGFR antagonist to enter clinical trials was SU5416 (Sugen/ Pfizer), which was later followed by SU6668. These compounds competitively block ATP binding to the tyrosine-kinase domain of the receptor, thereby inhibiting tumour angiogenesis *in vivo* and inhibiting the growth of xenografts that are established from various human cancers[125,126]. The related compound SU11248 targets multiple receptor tyrosine kinases[127], including KIT, PDGFR, FLT3 and VEGFR2, and is now being evaluated in Phase II clinical trials for the treatment of patients with various

cancers. The angiogenesis inhibitors ZD6474 (AstraZeneca)[128] and PTK-787 (Novartis/Schering)[129] are other promising compounds that have progressed to Phase II and III clinical trials, respectively.

Conclusions

In the 20 years since the cloning of the first cDNA encoding an RTK — EGFR — much progress has been made in our understanding of the fundamental signalling mechanisms of RTKs, their biology and the pathological consequences of their deregulation. Although a complete understanding of RTK function and dysfunction in diverse tissues and multiple biological processes is still to be achieved, studies of members of this family have already had a significant impact on cancer therapy. Trastuzumab, imatinib, gefitinib, cetuximab and bevacizumab have demonstrated the potential of molecularly targeted cancer therapeutics. Several other RTK-based, experimental anticancer strategies are now undergoing clinical evaluation — for example, drugs that target FLT3 in acute myeloid leukaemia (AML) — or are in preclinical development, such as modulators of IGF1R, MET, VEGFR3, TIE2 and TRK receptor signalling (TABLE 2). Important questions — such as the definition of the optimal dose and schedule of drug administration and the issue of drug resistance that has been seen in imatinib-treated CML[130] and GIST[131] patients — remain to be addressed.

Both academic and industrial research will further focus on evaluating RTKs as promising molecular targets for cancer treatment. An impressive example is a recent study in which high-throughput sequencing technologies, combined with bioinformatics, were used to systematically analyse the tyrosine kinome in colorectal cancer[132]. This large-scale sequencing approach identified several previously unknown mutations in tyrosine-kinase genes that could be targeted for therapeutic intervention in the future. Due to the extensive complexity of pathogenic alterations in the cancer-cell signalling network, genomics-based diagnostic techniques, such as gene-array, tissue-array and single-nucleotide-polymorphism analysis, will help to identify patients who are likely to respond favourably to a particular drug that is targeted to a signalling molecule. Ultimately, because of the plasticity of the cancer-cell genome, it will be essential to develop combination therapies involving small-molecule and antibody cocktails that function through distinct and complementary mechanisms of action in order to achieve the rapid and complete eradication of tumours.

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Competing interests statement
The authors declare that they have no competing financial interests.

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