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Changing the course of oncogenesis: The development of tyrosine kinase inhibitors

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1. Introduction

Over the past 30 years a huge amount of information has been gathered about tumour biology and the carcinogenic process. It is known that cancer develops as a result of a disturbance in the critical balance between the rate of cell-cycle progression and cell growth on one hand, and apoptosis on the other. Research has increased understanding of the unique features that enable tumour cells to grow and spread. Of particular interest are the tyrosine kinases – signalling molecules involved in cell survival, motility, differentiation and mitogenesis. Genetic and structural alterations in tyrosine kinases are associated with a large number of cancers.¹ Targeted inhibition of tyrosine kinases and their signalling cascades has been shown to be a successful anticancer strategy, the most successful to date being imatinib in chronic myeloid leukaemia (CML) and gastrointestinal stromal tumours.² As understanding of critical downstream signalling pathways involved in tumorigenesis becomes more sophisticated, so will the ability to selectively inhibit these pathways, and ultimately, to treat all types of cancer.

2. Oncogenic cellular transformation

Tumour cells are cells that have lost the ability to undergo differentiation, senescence and apoptosis. Furthermore, they have lost the normal mechanistic checks that would curb over-proliferation. Therefore, tumour cells can be seen as highly specialised cells that can proliferate independently of extracellular mitotic signals. Other hallmarks of tumour development include the ability to metastasise to distant sites by migration through the extracellular matrix, induction of angiogenesis and invasion of tissue. These unique features of tumour cells are often mediated by signalling pathways that have become deregulated. Therefore, these pathways represent promising targets for anticancer therapies. However, one of the difficulties in developing successful compounds to target these signalling pathways is that they are highly complex. Both positive and negative regulatory elements are present, and there is often cross-talk with other pathways, such that selectively inhibiting oncogenic pathways while leaving normal physiological pathways intact becomes incredibly difficult. Understanding of the mutations and genetic rearrangements involved in tumorigenesis has been greatly helped by the publication of the human genome.

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Precise genetic features associated with certain tumours can now be pinpointed. For example, it is now known that tumours often overexpress certain kinases that have effects on signalling pathways, leading to abnormal proliferation and metastasis. These kinases and their signalling cascades are therefore excellent targets for anticancer therapeutics, which can be designed to specifically inhibit, moderate or interfere with their function.

3. Tyrosine kinase inhibitors as cancer treatments

The human genome includes more than 20,000 genes, of which at least 500 encode protein kinases – the ‘human kinome’ (Fig. 1).³ Protein kinases are involved in the regulation of a wide range of biological processes. Because of their regulatory function, protein kinases play a major role in human disease, particularly cancer and inflammation.⁴ Certain kinase signalling pathways are targeted much more frequently by oncogenic mutations than others. One of the most frequently implicated classes of kinases in cancer is the tyrosine kinases. Within the human kinome there are more than 90 tyrosine kinases, of which 38 are cytoplasmic (divided into 10 subfamilies) and 58 are receptor tyrosine kinases (RTKs; divided into 20 subfamilies).³

3.1. RTKs

RTKs are characterised by a catalytic cytoplasmic domain. Upon ligand-induced dimerisation, the cytoplasmic domains transphosphorylate each other. The phosphorylated tyrosines then initiate a signal cascade via recruitment of proteins with phosphotyrosine binding or protein tyrosine-binding domains (Fig. 2). These signalling cascades ultimately lead to changes in gene expression affecting mitogenesis, survival, differentiation and motility.⁵ Perturbation of RTK signalling by point mutations, genomic rearrangements or other genetic alterations can result in deregulation of activity, such that the kinase becomes a potent oncoprotein capable of causing malignant transformation. Such aberrant signalling through RTKs is implicated in a number of different cancers.¹ RTKs are therefore excellent targets for the development of anticancer therapies.

3.1.1. The EGFR receptor (ErbB) family

One of the most important RTK subfamilies in cancer is the epidermal growth factor receptor (EGFR) family, also known as the ErbB family. Studies in the late 1980s using murine NIH 3T3 fibroblasts demonstrated that hyperstimulation of the EGFR signalling pathway by overexpression of human EGFR was sufficient for oncogenic transformation.⁶

The EGFR family comprises four membrane-bound tyrosine kinases (Fig. 3): EGFR (Her1/ErbB1), Her2/neu (ErbB2), Her3 (ErbB3) and Her4 (ErbB4). Each receptor has typical RTK topology – an extracellular ligand-binding domain, a transmembrane domain, and a cytoplasmic domain with intrinsic kinase activity (except Her3, which requires dimerisation with other members for kinase activity).⁷ Within the family there is some sequence homology, but members differ in terms of their binding affinity for different EGF-like growth

factors and degree of kinase activity.⁸ A large number of molecules – known as EGF-like growth factors – are able to activate the EGFR family members, including transforming growth factor (TGF)- α , neuregulins, amphiregulin, epiregulin, heparin-binding EGF and EGF itself.⁹ Following binding, both homo- and heterodimers can form, with each dimer combination activating a different signalling pathway. For example, EGFR–Her2 heterodimers are associated with a more intense and sustained proliferative signal than EGFR–EGFR homodimers.^{10,11} Factors that influence the formation of the different dimer combinations include the ligands bound to the receptors, as well as the relative abundance of each receptor. The number of possible ligands and dimer combinations results in a high level of complexity and diversity to the downstream signalling cascades induced. Depending on the particular ligands and receptors involved, outputs of EGFR signalling can affect apoptosis; angiogenesis; cellular migration, invasion and metastasis; differentiation; adhesion; or proliferation (Fig. 4). Important signalling pathways induced by EGFR activation include the mitogen-activated protein kinase (MAPK) pathway, which mediates cellular proliferation, phosphoinositide 3-kinase and the anti-apoptotic Akt/protein kinase B (PKB) pathway.² Overstimulation of EGFR signalling pathways in cancer may be due to increased production of ligands, receptor gene amplification occurring as a result of mutations or genetic rearrangements, or overexpression of the receptors. Furthermore, overexpression of EGFR is associated with a poor prognosis and the development of resistance to chemotherapy, radiotherapy and hormone therapy in breast, ovarian, colorectal, head and neck, and non-small-cell lung cancer (NSCLC).^{13–23}

Several EGFR inhibitors have been developed as anticancer therapies: gefitinib, erlotinib and cetuximab against EGFR, and trastuzumab against Her2. Both gefitinib and erlotinib are small molecule inhibitors that compete for the adenosine triphosphate (ATP)-binding site in the cytoplasmic tail of the EGFR tyrosine kinase. Gefitinib initially received conditional approval for NSCLC from the Food and Drug Administration (FDA) in May 2003, based on response rates in uncontrolled phase II studies. However, in phase III trials, although gefitinib showed a statistically significant tumour shrinkage, it failed to show a survival advantage over placebo.²⁴ Better results have been achieved with erlotinib. In a phase III clinical trial of patients with advanced-stage (Stage IIIB or IV) NSCLC and 1 or 2 prior chemotherapy regimens, erlotinib was associated with a 2-month benefit in terms of overall survival compared with placebo (6.7 versus 4.7 months; 95% CI [0.58–0.85]; $P < 0.001$).²⁵

Cetuximab is a monoclonal human–murine chimeric antibody that competes with the ligand-binding site of EGFR. Cetuximab has shown significant anti-tumour activity in phase III studies on head and neck cancer and colorectal cancer when added to traditional radiation or chemotherapy. In a study of irinotecan-refractory metastatic colorectal cancer, patients receiving cetuximab in combination with irinotecan chemotherapy showed a median time to progression of 4.1 months compared with just 1.5 months in patients receiving cetuximab alone ($P < 0.001$).²⁶ Combination therapy was also associated with a greater median survival

The Human Protein Kinases

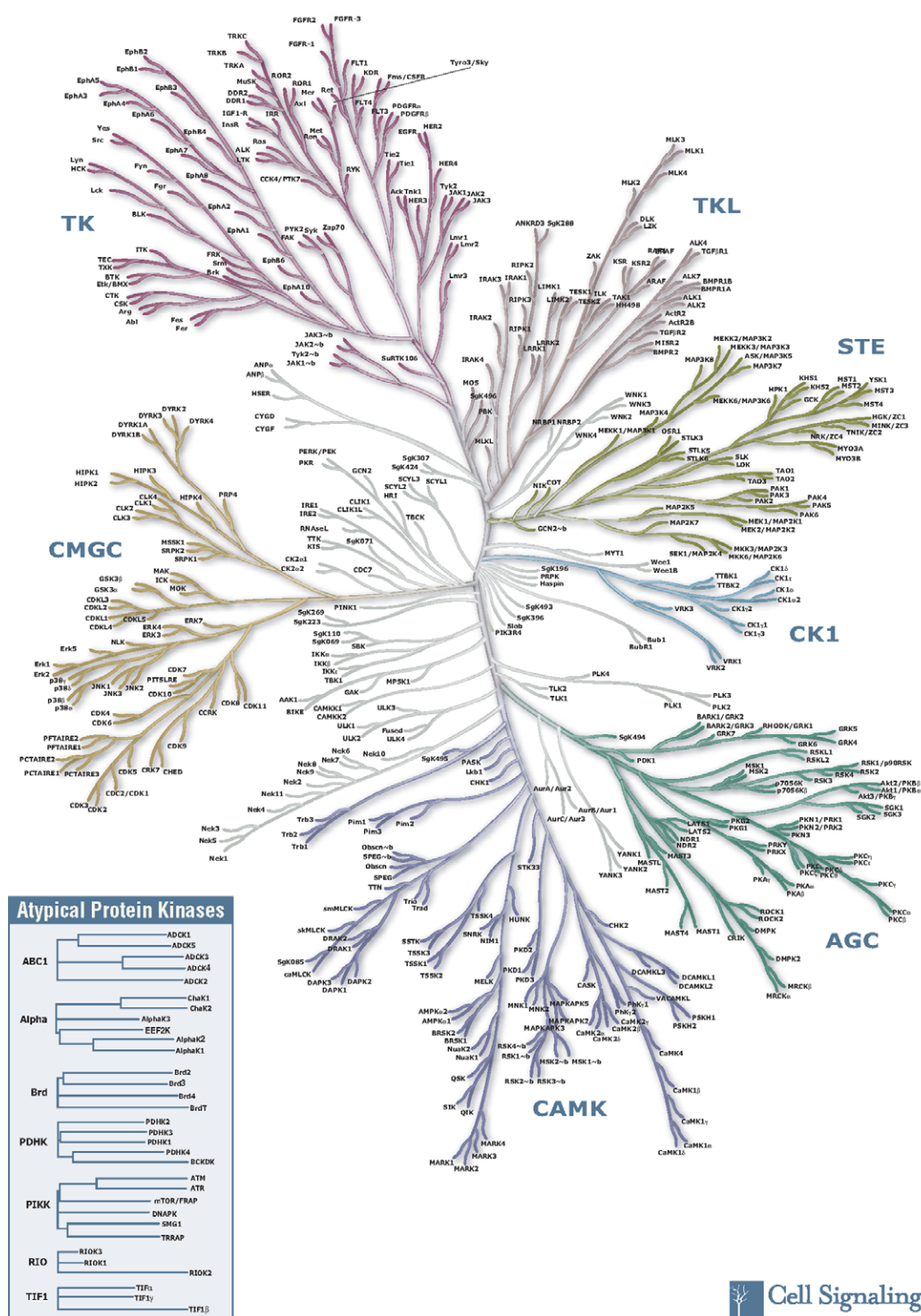


Fig. 1 – The human kinome. Kinome dendrogram figure courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

time (8.6 versus 6.9 months; $P = 0.48$). Furthermore, a clinical trial in head and neck squamous cell carcinoma demonstrated that cetuximab plus radiation therapy was associated with a significantly greater median survival at 2 years compared with radiation alone (54 months versus 28 months; $P = 0.02$).²⁷

Excellent results have been obtained using trastuzumab in the adjuvant treatment of Her2-positive breast cancer. Trastuzumab is a humanised monoclonal antibody that binds to the extracellular domain of Her2. Results from the combined studies by the National Surgical Adjuvant Breast and Bowel Project trial (B-31), the North Central Cancer Treatment Group

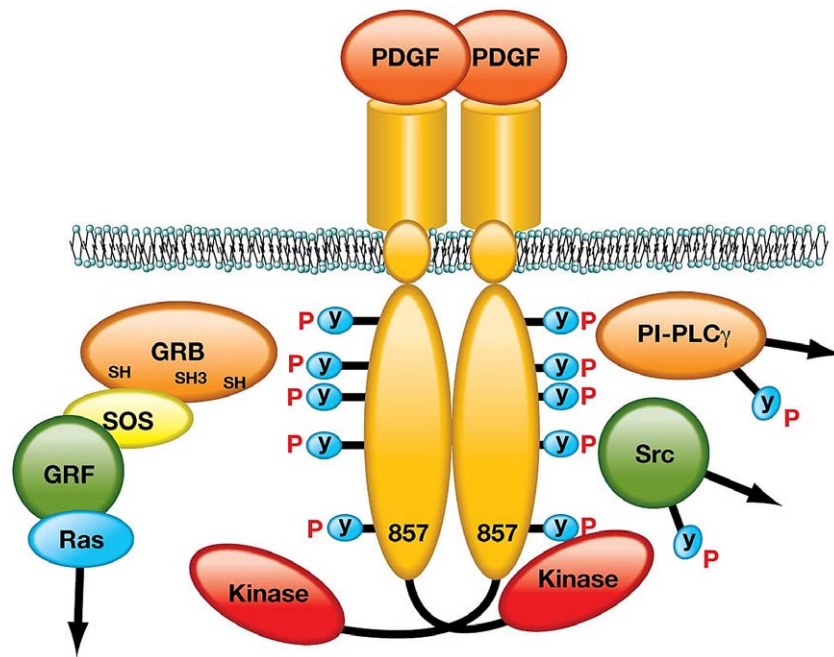


Fig. 2 – Tyrosine kinase receptors. A tyrosine kinase receptor complex is activated by ligand binding (in this case, platelet-derived growth factor [PDGF]), triggering multiple signal transduction pathways within the cell.

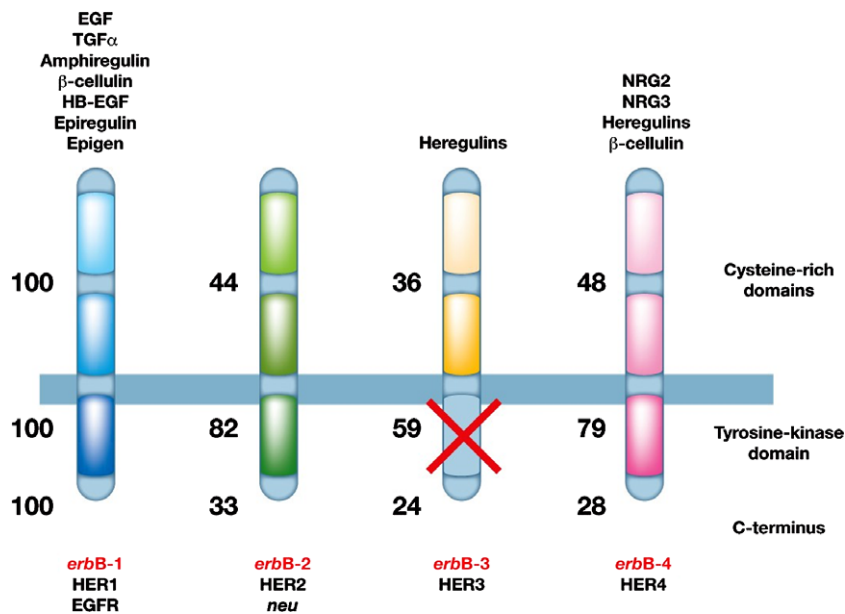
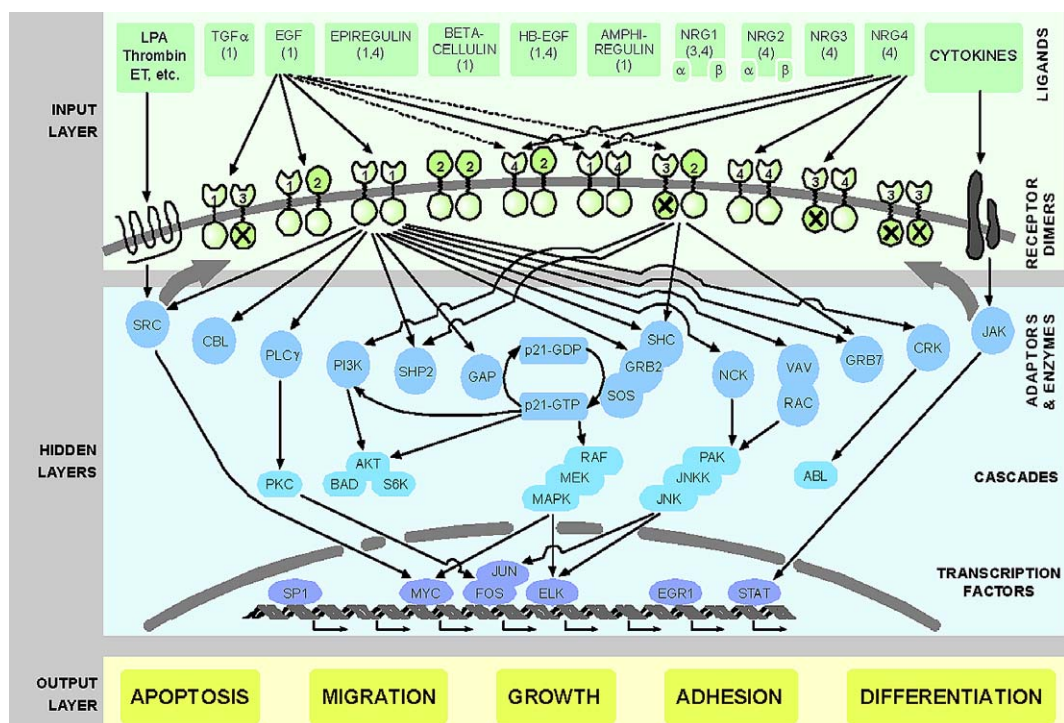


Fig. 3 – The EGFR (ErbB) family and their ligands.

Trial (N9831) and the Herceptin Adjuvant (HERA; Breast International Group [BIG] 01-01) trial all showed highly significant reductions in the risk of recurrence.^{28,29} The absolute benefit in terms of disease-free survival in the HERA trial was 8.4% (95% CI [2.1–14.8]) at a 2-year follow-up.³⁰ The joint analysis of B-31 and N9831 trials showed an absolute benefit at four years post-follow-up of 18.2% (95% CI [8.1–15.4]), exceeding all previously reported benefits in breast cancer.^{28,29} Furthermore, there is growing evidence that trastuzumab-combined chemotherapy elicits high levels of pathological complete response in the neoadjuvant setting.³¹

3.2. Cytoplasmic tyrosine kinases

Although there are many more RTK inhibitors in development compared with cytoplasmic tyrosine kinase inhibitors, the first clinically successful tyrosine kinase inhibitor was against a cytoplasmic kinase; the Bcr-Abl tyrosine kinase is expressed in more than 95% of patients with CML and around 25–30% of adults with acute lymphoblastic leukaemia (ALL).^{2,32} The Bcr-Abl gene is a hybrid resulting from a translocation between chromosomes 9 and 22.³³ Whereas the activity of the normal Abl tyrosine kinase is tightly regulated, the



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Fig. 4 – The EGFR/ErbB complex signalling network. Reproduced with permission from *Nature Reviews Molecular Cell Biology*.¹²

Bcr-Abl kinase is constitutively activated. The Bcr-Abl kinase activates oncogenic signalling pathways, and has been shown to induce leukaemia in both experimental animals and humans.^{33–35} Bcr-Abl induces phosphorylation in a number of target proteins, including Crkl, Shc, Syp, Fes, Vav and paxillin.^{36–40} Bcr-Abl is also thought to interact with the Ras signalling pathway via binding to GRB2 (growth factor receptor bound protein 2) and SOS (son of sevenless),⁴¹ and the JAK/STAT (Janus kinase/signal transducers and activators of transcription) signalling pathway.⁴² The activation of these signalling pathways by Bcr-Abl results in increased cell adhesion, inhibition of apoptosis, and promotion of anti-apoptotic proteins, upregulation of cell survival genes and growth-factor independent growth – all the hallmarks of tumourigenesis. The degree of transforming activity of Bcr-Abl appears to correlate with the degree of tyrosine kinase activity.³⁵

The small molecule, imatinib, was designed to block the ATP-binding site of Bcr-Abl, preventing phosphorylation and thus inhibiting kinase activity. Imatinib blocks proliferation and induces apoptosis of Bcr-Abl-expressing cells. Following treatment, response to imatinib is rapid, with most patients achieving a complete haematological response within the first 4–6 weeks of therapy. Furthermore, more than 50% of patients achieved a complete cytogenetic response within 3 months of initiating treatment.² Indeed, patients treated with imatinib showed significantly better results than patients treated with conventional interferon (IFN)- α therapy with regard to haematological and cytogenetic response, progression to accelerated phase or blast crisis and discontinuation of treatment due to intolerance.⁴³

Other cytoplasmic tyrosine kinases implicated in cancer include the Src family of kinases. Src kinases are overexpressed in a range of human cancers including colon, breast, pancreas, ovarian, prostate and lung cancer.^{44–49} Src (encoded by the *c-src* gene) is normally maintained in an inactive state within the cell via phosphorylation of a C-terminal tyrosine residue (Tyr527 in humans). During normal cell growth, Src can be transiently activated during mitosis. However, in tumour cells, Src is de-repressed as a result of disruption to the regulatory progresses that would normally suppress Src activity.⁴⁴ This is thought to be mediated via enhanced levels of protein-tyrosine phosphatase, which de-phosphorylate the C-terminal Tyr527, or mutations that prevent establishment of the inactive phosphorylated Tyr527 conformation.^{44,50}

4. Resistance to targeted therapy

A key hurdle to overcome in order to realise the full potential of targeted kinase inhibitors for the treatment of cancer will be the emergence of drug resistance in treated patients. Already, mutant forms of Bcr-Abl, Kit and EGFR have been found that confer resistance to imatinib, gefitinib and erlotinib. Resistance to imatinib is an increasing problem, with as many as 80–90% of patients with late-phase CML (blast crisis) developing imatinib resistance. Several mechanisms of resistance have been identified, including gene amplification and mutations that interfere with imatinib binding to Bcr-Abl.^{51–53} To overcome imatinib resistance, molecules have been developed that have increased binding affinity for Bcr-Abl. One of these molecules is dasatinib (BMS-354825), a novel, orally available, dual Src/Abl kinase inhibitor that has demonstrated significant potential

in treating imatinib-resistant CML. It has more than 300-fold greater potency than imatinib and has preclinical activity against 14 out of 15 imatinib-resistant Bcr-Abl mutants.⁵⁴

Currently undergoing Phase II/III trials, dasatinib has been shown to be effective in patients with CML who have progressed on imatinib. Data presented at the annual meeting of the American Society of Hematologists (ASH) in 2004 found that 86% of patients who received dasatinib achieved complete haematological response within 9 months. These results were further supported by data presented at the annual meeting of the American Society of Clinical Oncology (ASCO) 2004, with dasatinib producing a complete haematological response in 50% of patients who had accelerated disease and 28% of those who were in blast crisis.

5. The future of targeted therapy

One of the great advantages of targeted anticancer therapies is that they generally have very acceptable toxicity profiles compared with some of the older cytotoxic agents. This feature, combined with an excellent clinical efficacy, will make them attractive agents to use as first-line treatments. However, given the nature of their mode of action, targeted therapies will be of greatest benefit to those patients whose tumours are expressing the particular molecule being targeted. Therefore, it will become necessary to perform diagnostics to ensure patients receive the appropriate targeted therapy for the molecular profile of their tumour.

6. Conclusions

Aberrant, particularly constitutively active, signalling through cytoplasmic and receptor tyrosine kinases has been implicated in a number of cancers, providing a clear rationale for targeting these molecules in anticancer therapy. Following the great success of imatinib, several tyrosine kinase inhibitors have been developed as anticancer therapies, including gefitinib, erlotinib, trastuzumab and cetuximab. Despite advances in the understanding of oncogenic signalling pathways, results from clinical trials have been mixed. This may be in part due to the complexity and indeed redundancy in such pathways, but also due to problems in progressing from animal models to humans. Animal models are limited in their ability to simulate human cancer and better preclinical models are required. Furthermore, better surrogate markers that predict anti-tumour effects, as well as recognition of appropriate clinical endpoints, will enable targeted therapies that are most likely to be successful in specific patients to be quickly and easily identified.

Emerging resistance to targeted therapies will need to be addressed as the fight against cancer moves forward. A better understanding of the molecular basis behind the development of resistance will enable new compounds to be developed and existing agents intelligently selected against drug-resistant mutants. In summary, as understanding of the pathways involved in tumour proliferation and growth becomes clearer, and methods of screening compounds for clinical activity become more sophisticated, it will only be a question of time before a specific strategy for each kind of tumour is found.

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