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ErbB RECEPTOR TYROSINE KINASE INHIBITORS AS THERAPEUTIC AGENTS

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1. ABSTRACT

The ErbB family of receptor tyrosine kinases comprise four members: EGFR, ErbB2, ErbB3 and ErbB4. All are essential for normal development and participate in the functioning of normal cells. ErbB receptors, particularly EGFR and ErbB2 are commonly deregulated in certain prevalent forms of human cancer. Recently a number of small molecule inhibitors of the tyrosine kinase activity of these receptors have been developed. Some of these agents, known as TKIs, are progressing through clinical trials in patients with aberrant ErbB receptor expression in their tumors. This article provides a brief overview on the structure and biology of ErbB receptors and their ligands before discussing in detail the development and current status of ErbB receptor TKIs. These agents are shown to inhibit multiple features of cancer cells including proliferation, survival, invasion and angiogenesis. It is clear from recent studies that not all cancer cells that overexpress ErbB receptors will be sensitive to TKIs. Potential explanations for resistance to these molecules are reviewed. Finally the prospect of using TKIs in combination with existing chemotherapeutic agents is discussed.

2. INTRODUCTION

ErbB receptors belong to the type I subclass of receptors that express intrinsic tyrosine kinase activity. The family comprises four members, ErbB1 (also known as epidermal growth factor receptor (EGFR) or HER1), ErbB2 (HER2/neu), ErbB3 (HER3) and ErbB4 (HER4). As well as regulating the behaviour of normal cells these receptors are commonly deregulated in many prevalent forms of human cancer. As such they have been the subject of drug targeting strategies for a number of years. Increasing knowledge of the structure and function of ErbB receptors in cancer, together with progress in developing chemical inhibitors of these molecules, has now led to advanced clinical trials for certain classes of drug and the real prospect that ErbB receptor inhibition will become a

commonly used anti-cancer drug therapy in the near future. The purpose of this article is to review the latest advances in the development of molecules that inhibit ErbB receptor activity by targeting the tyrosine kinase catalytic center which is essential for their function. We present only a brief overview of the structural and functional aspects of the ErbB family. For more detailed reviews see (1-4).

3. STRUCTURE AND BIOLOGICAL FUNCTIONS OF ErbB RECEPTORS

All ErbB receptors are single-pass transmembrane glycoproteins that consist of an N-terminal extracellular domain, a hydrophobic membrane-spanning region and a long C-terminal tail which houses the catalytic kinase domain and various regulatory phosphorylation sites. The tyrosine kinase domains of ErbB receptors are highly homologous with an overall amino acid identity score of 53%. The least homologous member of the family is ErbB3, which lacks certain critical features in the kinase domain and, as such, is severely compromised in its catalytic activity (figure 1 and (5)). Comparison of the sequences of ErbB receptor kinase domains, excluding ErbB3, reveals 71% identity and 76% conservation.

Studies using transgenic mice, in which each of the ErbB receptors has individually been knocked out, have revealed that all members are essential for normal mammalian development. Indeed null mutations in each of the ErbB gene loci are embryonic lethal in mice (6-9). In the adult ErbB receptors are expressed widely in cells of epithelial origin where they mediate the growth, movement and survival of these cells in response to ligands presented or secreted by neighbouring mesenchymal tissue (figure 2). Tissue- and cell specific expression of ErbB receptors and their respective ligands directs biological responses (4).

4. REGULATION OF RECEPTOR ACTIVITY

4.1. ErbB Ligands

The ligands that bind to ErbB receptors all contain EGF-like motifs and are usually synthesised as transmembrane precursors (10). They can function in their anchored form in signaling between juxtaposed cells or as soluble autocrine or paracrine factors following their release by specific proteases (figure 2). Three major classes of ErbB ligand exist. The first, which bind specifically to EGFR, includes EGF, amphiregulin and TGF-alpha. The second, which bind both ErbB1 and ErbB4, include betacellulin, heparin-binding EGF (HB-EGF) and epiregulin. The third class, which bind to ErbB3 and ErbB4, constitute the heregulin family of which there are at least four members. No ErbB2-specific ligands have been identified. Indeed following a rigorous screen for ErbB2-selective ligands it has been proposed that the sole function of ErbB2 is to serve as the preferred dimerization partner for the other ErbB receptors (11).

4.2. Receptor Activation and Signaling

Binding of ligand to the extracellular portion promotes receptor dimerization, which can occur between the same receptor subtype (homodimerization) or between different receptor subtypes (heterodimerization). At least ten dimeric species are possible and their formation depends upon the respective levels of expression of each ErbB receptor subtype and the ligand presented. Different dimeric species are believed to direct distinct biological outcomes such as proliferation, differentiation, movement and survival (4).

Dimerization activates the intrinsic tyrosine kinase activity of each receptor in the pair, which in turn leads to phosphorylation of the complex on multiple tyrosine residues. These sites then serve to dock a range of key signaling molecules that trigger multiple pathways leading to alterations in gene expression and other biological responses (figure 2). High levels of receptor expression, as occurs in some human malignancies, can result in formation of active dimers in the absence of ligand (12). Each ErbB member contains a specific subset of phosphorylatable tyrosine residues that enable it to activate a unique set of signals (13). As such heterodimerization diversifies signaling and thereby biological outcome. The formation of heterodimers, either spontaneously or *via* ligand binding, also alters signaling strength and kinetics (3).

Mapping of the individual tyrosine residues phosphorylated as a result of receptor activation indicates that ErbB receptors stimulate multiple but overlapping signals (4). Nevertheless significant differences exist. For example, although all four ErbB receptors are capable of stimulating the phosphoinositide 3-OH kinase (PI3K) pathway, ErbB3 is an extremely efficient activator of this pathway as a result of its battery of seven tyrosine residues that, when phosphorylated, provide docking sites for the p85 regulatory subunit of PI3K. Despite this, ErbB3 is catalytically incompetent and therefore requires activation by another ErbB receptor within a heterodimer in order to activate PI3K.

4.3. Receptor transactivation

As well as acting as a receptor for the range of ErbB-directed ligands summarized above, there exists a wealth of evidence that ErbB receptors also participate in signal transduction mechanisms elicited by a variety of other growth factors and hormones. This process, termed transactivation, is particularly well characterized for a number of G protein coupled receptors (GPCRs), which have been shown to stimulate the Ras/ERK pathway, through activation of the EGFR (reviewed in (14, 15) and see figure 2). Transactivation of ErbB2 by GPCRs has also been reported (16). The mechanisms through which transactivation take place are still unclear and may vary from cell type to cell type. For example, a number of G protein coupled receptors activate the EGFR by stimulating cell surface matrix metalloproteinases (MMPs), particularly MMP3 and MMP9, which then cleave membrane anchored ErbB receptor ligands, such as HB-EGF (see figure 2). Alternative, but not necessarily mutually exclusive, mechanisms involve Ca^{2+} signaling, protein kinase C and the tyrosine kinase c-src. Regardless of the mechanism, transactivation leads to activation of the EGFR tyrosine

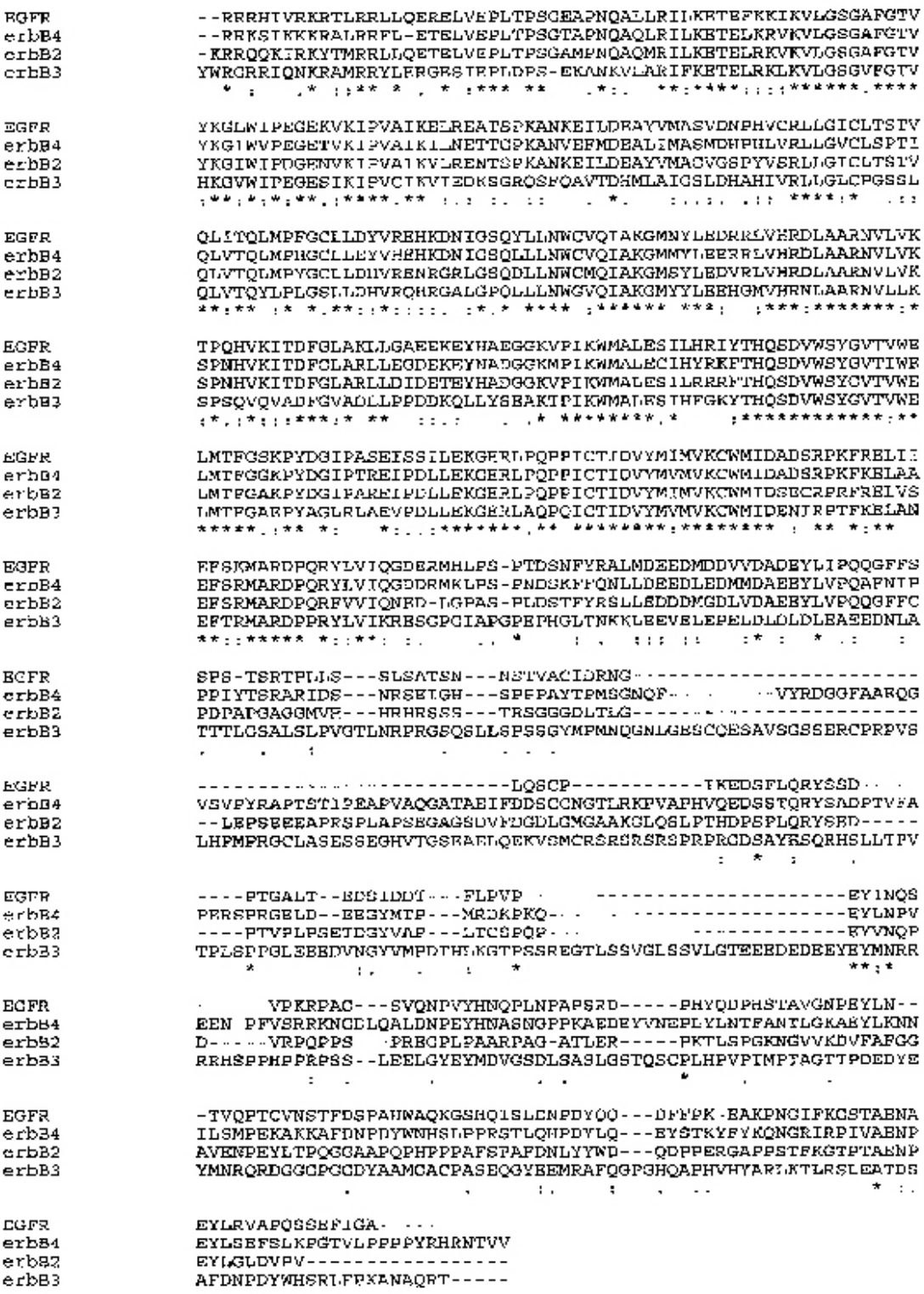

Figure 1. Alignment of ErbB receptor intracellular domain amino acid sequences. The sequences were aligned using the program ClustalW. Residues identical in all four proteins are marked with asterisks (*), conserved residues are marked with colons (:) and variable residues with periods (.). The predicted tyrosine kinase catalytic regions are boxed. Amino acids within kinase domains conserved among all protein kinases (36) are depicted by black bars, and function as follows: 1 and 3, ATP binding; 2, 4 and 5, phosphotransferase reaction. Substitutions in ErbB3 within these critical residues are circled. The arrow indicates the cysteinyl residue targeted for modification by two recently developed irreversible EGFR TKIs (further details are given in the text and in Table 2).

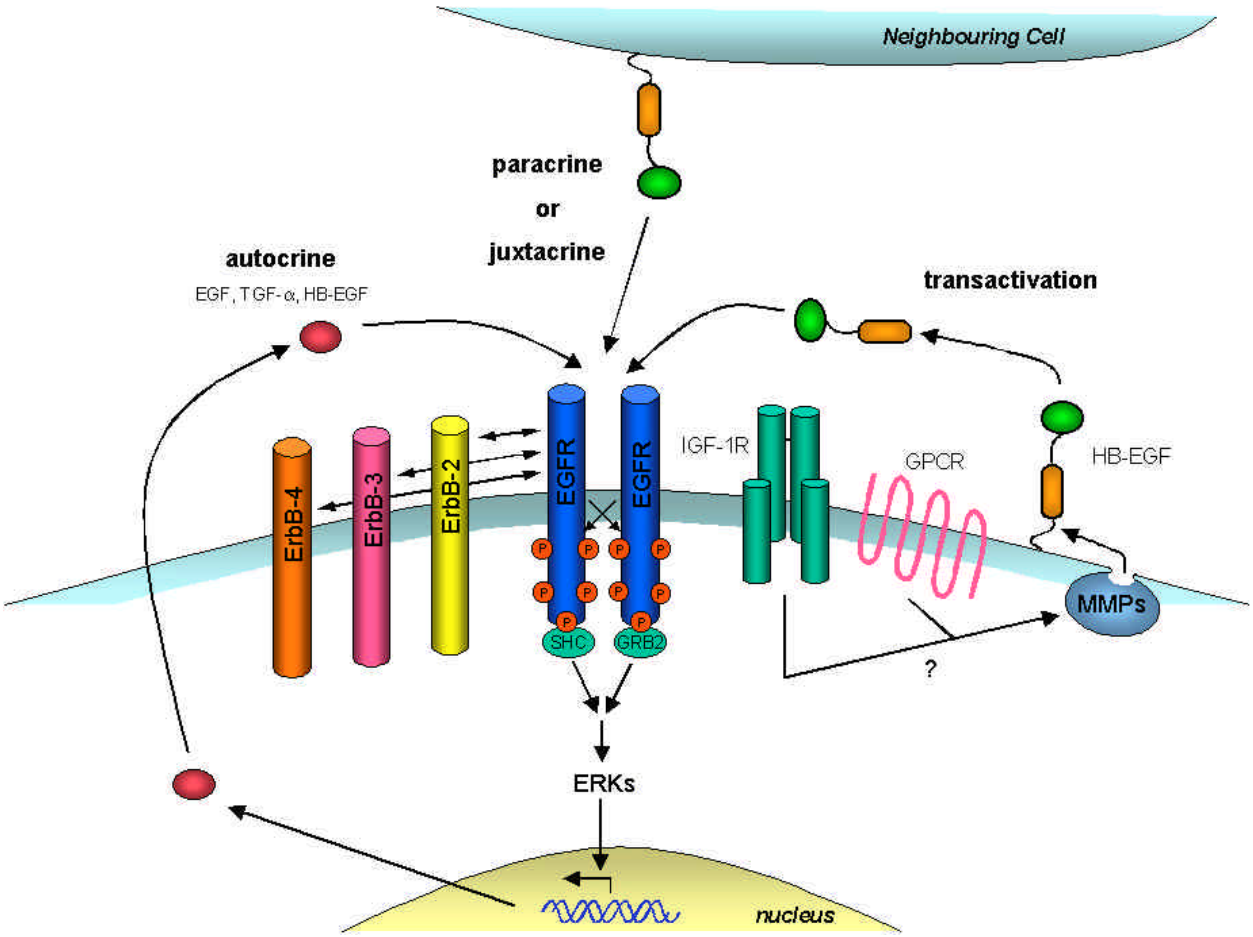

Figure 2. The EGFR is activated via multiple mechanisms. The EGFR receptor is activated by ligands that are derived from within the same cell (autocrine), from neighbouring cells (paracrine/juxtacrine) or as a result of the activation of heterologous receptors (transactivation). Receptor ligation results in dimerization with other EGFR monomers (homodimerization) or with other ErbB receptors (heterodimerization), receptor kinase activation and phosphorylation on multiple tyrosyl residues in the C-terminal region. These sites recruit phosphotyrosine-binding proteins such as GRB2 and SHC which trigger signaling pathways such as the ERK pathway. Only the ERK activation pathway is shown for clarity. Abbreviations: IGF1-R, insulin-like growth factor-1 receptor; GPCR, G protein-coupled receptor; MMP, matrix metalloproteinase; HB-EGF, heparin-binding epidermal growth factor.

kinase and autophosphorylation in a manner that appears qualitatively indistinct from that induced by ErbB ligands.

Other tyrosine kinase receptors such as the insulin-like growth factor-1 receptor (17) and the platelet-derived growth factor receptor (18, 19) have also been shown to transactivate the EGFR. Again the mechanisms are unclear, but probably involve those outlined above as well as the direct functional interactions between the receptor subtypes (20).

5. ErbB RECEPTORS AND CANCER

Overexpression of ErbB receptors and/or their respective ligands is a frequent occurrence in human malignancy. Overexpression of EGFR and ErbB2 in particular appears to have direct consequences in terms of poor patient prognosis (see (21) and (22) for recent reviews). Table 1 summarizes the available data on frequency of expression of ErbB receptor proteins in several common human cancers. In general overexpression of EGFR and ErbB2 is associated with tumor invasion and metastasis, late stage disease, resistance to chemotherapy and poor outcome. By contrast less data is available on the significance of ErbB3 or ErbB4 expression and cancer progression.

The overexpression of both EGFR and ErbB2 usually occurs as a result of amplifications of each respective gene. In addition gene rearrangements give rise to at least three EGFR variants, known as EGFRvI, vII and vIII. The three variants contain various deletions within their extracellular domains. The best characterized of these is EGFRvIII (23). This variant, which does not bind ligand and expresses constitutively active tyrosine kinase activity in the absence of dimerization, can induce transformation of fibroblasts and is highly expressed in a number of human cancers (23-25).

Table 1. Expression of ErbB receptors in common human cancers

Tissue	Expression (%)				References
	EGFR	ErbB2	ErbB3	ErbB4	
Bladder	31-72	9-36	30-56	30	102, 103
Breast	14-91	25-30	22-52		104, 105
Colon	50-80	26-90	89		106
Stomach	40-81	26-56	35		107-110
Head and Neck	80-100				
Non Small Cell Lung	40-80	18-37			111, 112
Ovary	35-70	28-65		90	113-116
Pancreas	30-50				

Figures quoted are from studies giving the percentage of tumors staining positively for each receptor by immunohistochemistry. Where shown ranges encompass the lowest and highest values reported from different studies.

Although the elevated expression of either EGFR or ErbB2 by themselves are associated with cancer progression, it is most likely that co-expression of these proteins with other ErbB receptors or with ErbB ligands is of primary importance in this regard. For example overexpression of the EGFR in tumor tissue is often associated with increased expression of the EGFR ligand TGF-alpha (26). Indeed it appears that EGFR ligand overexpression may be necessary for cellular transformation (27). Moreover recent data indicate that co-expression of TGF-alpha and EGFR predicts a worse prognosis in breast cancer patients (28). Overexpression of one ErbB receptor subtype may indirectly influence the activation of another, for example by modifying the actions of autocrine ligands. In this regard, ErbB2 overexpression is linked to increased TGF-alpha signaling through EGFR (29).

It is also well documented that cellular transformation induced by ErbB ligands requires the presence of multiple ErbB receptors. For example overexpression of ErbB2 greatly enhances EGF-induced transformation of fibroblasts (30, 31). More recent studies suggest that the presence of functioning receptor heterodimeric complexes is more important than the expression levels of individual ErbB receptors. For example ErbB3 is frequently co-expressed with ErbB2 in tumor tissues and ErbB2/ErbB3 expressing tumor cells show particularly aggressive behaviour (32, 33).

6. ErbB RECEPTOR TYROSINE KINASE INHIBITORS (TKIs)

The last few years have witnessed major advances in the development of low molecular weight selective inhibitors of ErbB tyrosine kinases. We provide a brief overview of the development of ErbB TKIs from a historical perspective. For an excellent comprehensive review on this subject and detailed consideration of structural aspects of TKI design see (34).

6.1. History

Naturally occurring protein kinase inhibitors have been known for decades. Amongst these, a number of fungal metabolites such as quercetin, genistein, lavendustin A and erbstatin were discovered to possess inhibitory action against tyrosine kinases (35). In general these compounds exert broad inhibitory action not only against many tyrosine kinases but also against some serine/threonine kinases. Their action is primarily competitive with ATP in the phosphotransferase reaction and their broad specificity derives from the conserved nature of protein tyrosine kinase domains (36). Nevertheless these compounds have been invaluable in providing templates from which more selective molecules have been derived. Most notably the erbstatin structure was used by Levitzki and co-workers as a template to develop a series of novel TKIs termed tyrphostins (37-42). Although initial reports suggested that erbstatin's action was competitive with respect to substrate, subsequent studies indicated a mixed competitive inhibition with respect to ATP and substrate (34). A number of these agents have shown selectivity for individual tyrosine kinases including the EGFR (AG1478) and ErbB2 (AG825).

A major advance in the field was the development of a novel class of quinazoline based TKIs by Fry and colleagues at Parke-Davis in 1994. One of these compounds PD153035 showed exquisite selectivity for the EGFR and extremely high potency with a K_i in the picomolar range (43). This discovery provided the impetus for many groups to develop quinazoline-based moities and a number of such molecules are currently undergoing clinical evaluation (see below).

Various pyrimidine-based molecules such as the pyrollopyrimidines (44) have also been developed and demonstrated to possess selectivity for ErbB receptor tyrosine kinases as well as the desired potency.

6.2. Current status

At the time of writing a number of ErbB TKI agents have reached various stages of clinical trials (see table 2 and figure 3). The majority of these agents have quinazoline-based structures and are competitive inhibitors; some such as ZD1839 (Iressa™) are relatively selective for EGFR, whereas others such as GW2016 are effective against EGFR and ErbB2 (45). Models of the molecular interactions between competitive quinazoline-type TKIs and the ATP binding pocket within the EGFR have been proposed (46). There are also at least two compounds whose mechanism of action is irreversible, involving modification of a critical cysteinyl residue at the catalytic site (see table 2 and figure 1).

6.3. Modes of action of inhibitors

From table 2 it can be seen that ErbB receptor TKIs that have progressed furthest in clinical trial fall into three basic categories: reversible competitive inhibitors with selectivity for EGFR, reversible competitive inhibitors

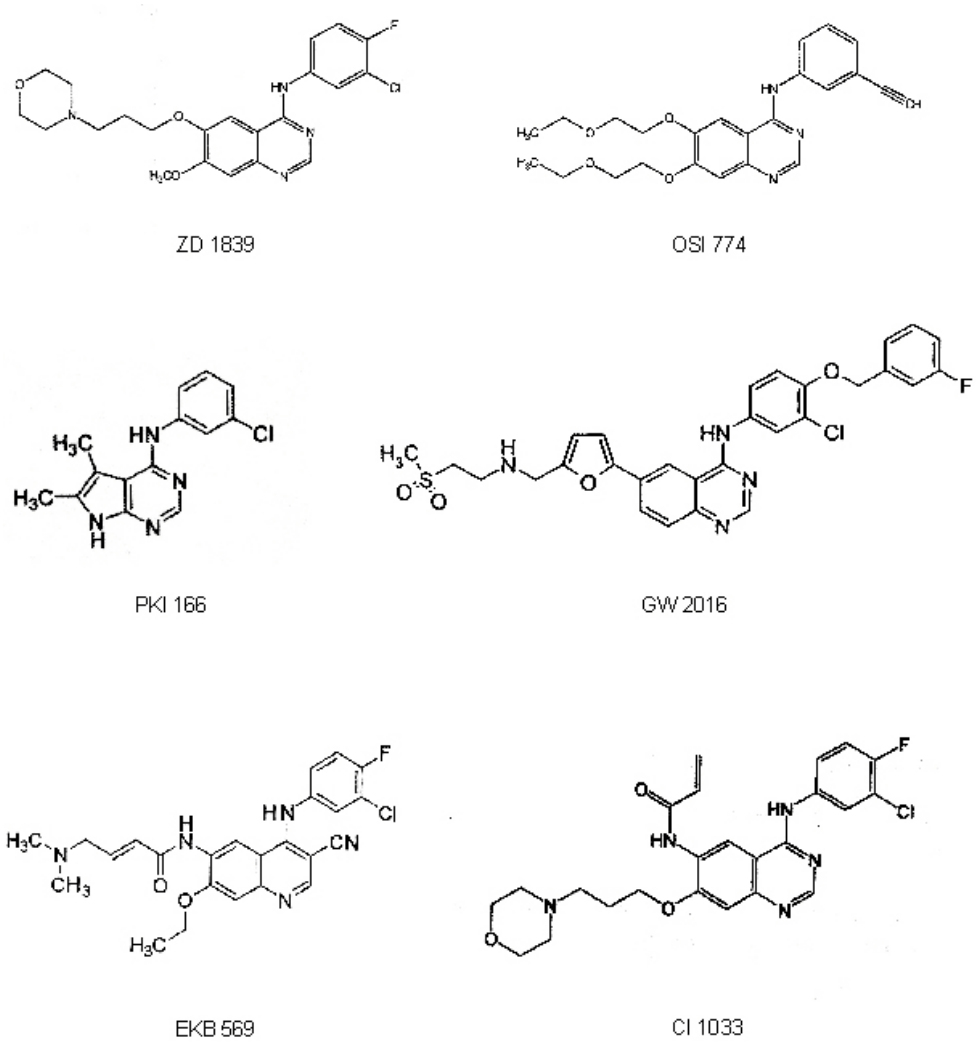

Figure 3. Chemical structures of ErbB receptor TKIs in clinical development.

with pan-ErbB receptor selectivity and irreversible inhibitors. Each of these classes appears in principle to offer both advantages and disadvantages in comparison with the others.

With competitive inhibitors it has been argued that intracellular ATP concentrations, which are usually within the range 5-15 mM, may limit the efficiency of these compounds in tumor cells (47). However this is not borne out by the available data. Some of the ErbB receptor inhibitors with competitive action show half maximal inhibitory concentrations in cells in the nanomolar range, and indeed have been developed because of this high potency. One possible reason for the extraordinary potency of quinazoline-based EGFR TKIs is their ability, not only to compete with ATP, but also to induce the formation of non-phosphorylated, signaling-inactive receptor dimers (48). Furthermore, quinazolines may also induce the formation of EGFR/ErbB2 heterodimers, potentially sequestering ErbB2 and preventing it from forming active dimers either with itself or with other ErbB receptors. A more recent study showed, in addition, that the quinazolines PD153035 and ZD1839 promote sequestration of the EGFR and its ligand into inactive complexes (49).

Given the highly conserved nature of ErbB receptor kinase domains (see figure 1 and section 3), it is perhaps surprising that selective inhibitors of the EGFR have been developed that apparently show little activity versus other ErbB receptors (table 2). This may be advantageous in terms of selectivity but on the other hand TKIs that inhibit more than one ErbB receptor have the potential to be more effective anti-cancer agents. EGFR and ErbB2 are both strongly associated with cancer progression, are commonly expressed together in human tumors and cooperate to induce transformation in cell lines (see Section 5). Simultaneous blockade of both receptors could therefore provide more beneficial clinical responses than inhibition of either receptor alone. Direct evidence supporting this idea is lacking at this stage and it is worth noting that EGFR-selective TKIs have been shown to block EGF-dependent tyrosine phosphorylation of ErbB2 and

Table 2. Some ErbB receptor TKIs in clinical development

Name	Chemical Type	Target	Action	Specificity (IC_{50}, nM)		Company	Clinical Trial status
				EGFR	ErbB2		
ZD1839 (Iressa™)	quinazoline	EGFR	competitive ATP	50	2500	AstraZeneca	III
OSI774 (Tarceva™)	quinazoline	EGFR	competitive ATP	2	_	OSI Pharmaceuticals	II
GW2016	quinazoline	EGFR/ErbB2	competitive ATP	8	8	GlaxoSmithKline	I
PKI166	pyrollo-pyrimidine	EGFR/ErbB2	competitive ATP	0.7	10	Novartis	I
EKB569	cyano-quinazoline	EGFR	irreversible (Cys773)	38	1255	Wyeth-Ayerst	I
CI1033	quinazoline	pan-ErbB	irreversible (Cys773)	40	_	Pfizer	II

IC_{50} values quoted are for inhibition of receptor kinase activity *in vitro*

also to slow the growth of tumor xenografts established from cell lines overexpressing ErbB2 (50). These effects occur in the absence of direct inhibition of ErbB2 and are therefore most likely to occur as a result of blocking the EGF-stimulated phosphorylation of ErbB2 within EGFR/ErbB2 heterodimers. Therefore it may not be essential to inhibit directly both EGFR and ErbB2 in tumors that express both receptors.

Irreversible inhibitors of ErbB receptors have been developed with the idea that they may be more effective than reversible agents with a competitive mode of action. An irreversible mechanism of action should, it is argued, lead to more prolonged inhibition of receptor function compared with reversible inhibitors. Studies directly comparing the properties of reversible and irreversible EGFR inhibitors have provided compelling data to support this idea (51). It is also worth remembering that although a number of competitive ErbB receptor inhibitors have shown tumor regressing properties in xenograft studies, tumor regrowth usually occurs following withdrawal of treatment. On the other hand irreversible inhibitors may require a higher degree of selectivity for their target to obviate toxicity problems. With this in mind molecules in this class have been developed to interact with the ATP binding site and to target certain critical cysteinyl residues within this region in order to achieve the required selectivity (51).

6.4. Cancer cell processes affected by ErbB receptor TKIs

Cancer progression involves the accumulation of gene mutations and rearrangements that enable the cell to proliferate and survive in the absence of exogenous growth factors, to promote angiogenesis and finally to invade the surrounding tissue prior to metastasis to secondary sites (52). Numerous studies indicate that ErbB receptors are critically involved in all of these processes. Thus targeting these receptors may be beneficial regardless of the stage of disease.

6.4.1. Proliferation

EGF and related ligands are known to stimulate mitogenic signaling pathways, notably the Ras/ERK cascade (see above), and to increase the proliferation rate of a wide range of cancer cells (53). Not surprisingly therefore anti-proliferative effects of ErbB receptor TKIs were reported at an early stage in their development (37, 43, 54) and have since been documented extensively. More recent work examining the effects of TKIs selected for clinical development have shown similar anti-proliferative actions of these inhibitors on cells grown as xenograft tumors in athymic mice (50, 55-58) and, very recently, in patients undergoing phase I clinical trials (59). Thus ErbB TKIs appear to exhibit a common capability in slowing the proliferation rate of tumor cells. Some studies have adressed the potential mechanisms underlying the antiproliferative actions of ErbB TKIs. ZD1839, an EGFR-selective TKI has been shown to induce the expression of $p21^{CIP}$ and $p27^{KIP}$ (59, 60), suggesting that ErbB receptor inhibition prolongs or delays cell cycle progression through G1/S.

6.4.2. Apoptosis/Survival

Although EGF and related ligands may act as survival factors in normal cells, these actions may be restricted to certain cell types (61). Nevertheless evidence has been documented that certain cancer cells overexpressing the EGFR are dependent on activation of this receptor for survival (62). In light of this ErbB receptor inhibition might be expected to promote apoptosis in susceptible cell types. While some studies support this (63, 64), anti-apoptotic effects of ErbB TKIs are not universal as is the case for their anti-proliferative actions. On the other hand several reports indicate that ErbB receptor blockade enhances the pro-apoptotic actions of other chemotherapeutic agents (55, 65-67). It appears that ErbB receptor blockade may lower the threshold at which cytotoxic agents are effective, perhaps by reducing the autocrine production of survival factors. EGFR inhibition also appears to sensitize tumor cells to radiation-induced apoptosis (see section 7.2).

6.4.3. Invasion and Metastasis

Overexpression of the EGFR (26) or ErbB2 (68) is strongly associated with increased metastatic potential in cancer patients. ErbB receptor activation induces invasion in cancer cells by increasing motility, disrupting cell-substrate and cell-cell interactions and by promoting the secretion of matrix-degrading proteases (see (69) for review). Interestingly promotion of invasion by EGF ligands appears to require ErbB2 (70) suggesting that blocking invasiveness of tumors with ErbB TKIs may require that ErbB2 is inhibited.

6.4.4. Angiogenesis

There is well documented evidence that EGFR promotes angiogenesis by increasing the production of pro-angiogenic factors such as VEGF, IL-8 and bFGF by tumor cells (71-73). Similarly overexpression or activation of

ErbB2 is strongly associated with VEGF expression and angiogenesis (74). The downstream signaling targets underpinning these effects include the HIF-1alpha family of transcription factors which transactivate pro-angiogenic genes (75). A number of studies have shown that inhibition of ErbB receptors using monoclonal antibodies reduces angiogenic factor production (76, 77). Recently the impact of ErbB receptor TKIs on tumor angiogenesis have been examined. In human pancreatic carcinoma cells, established as xenografts in nude mice, PKI166 (an EGFR TKI) reduced the production of VEGF and IL-8 and decreased microvessel density within the tumor (65). Studies using ZD1839 demonstrated similar anti-angiogenic effects against colon cancer cell xenografts (78). More recently the anti-angiogenic actions of ZD1839 were shown to occur through a dual mechanism resulting in reduced production of pro-angiogenic factors by the tumor cells together with direct effects on endothelial cell migration (79).

7. CONSIDERATIONS IN THE USE OF ErbB RECEPTOR TKIs AS THERAPEUTIC AGENTS

Although some ErbB receptor TKIs that have advanced rapidly through clinical trials are very likely to obtain approval for clinical use in the near future, there remain at least two key questions surounding the predictibility of the effectiveness of these agents. Firstly, predicting which patients will respond to ErbB receptor inhibition is likely to require more information than a reliable measure of target receptor expression level. Secondly, although *in vitro* studies have provided initial data on which existing chemotherapeutic agents will combine most effectively with ErbB receptor TKIs, it will be some time before optimally effective treatment regimens are established.

7.1. EGFR expression level does not predict responsiveness to anti-EGFR drugs

Clearly the potential use of ErbB TKIs as anti-cancer therapies is being targeted to patients with cancers that overexpress EGFR and/or ErbB2. The hope is that routine screening of patients tumors for ErbB receptor status will identify patients most likely to benefit from these new agents. Emerging data indicates that predicting response may be a far more complex issue than initially anticipated. It is already known that, although clinical responses to the ErbB2 receptor monoclonal antibody herceptin (trastuzumab^{TM}) occur in patients with tumors having high levels of receptor expression, a substantial proportion of patients with elevated receptor levels are resistant to treatment (80).

Recent *in vitro* studies, using a number of the novel ErbB receptor TKIs discussed in this article, also indicate that determining ErbB receptor levels alone is unlikely to be sufficient to predict likely responsiveness *in vivo*. For example the EGFR TKI ZD1839 inhibits the *in vitro* proliferation of a range of cancer cell lines expressing widely differing levels of EGFR (50). This lack of correlation has been explored in more detail in a recent study (81) using AG1478 and a series of EGFR-selective quinazoline TKIs. This work showed that although, as predicted, EGFR inhibitors are generally more effective in lines that overexpress EGFR, this association only stands when the cell responds to EGF mitogenically. Furthermore EGFR-specific inhibitors were shown universally to block EGFR tyrosine phosphorylation and signaling as far as ERK MAP kinase in all lines tested, suggesting that insensitive lines utilize signals that either lie downstream of ERKs or on ERK-independent pathways.

The lack of correlation between ErbB receptor expression level and responsiveness to drugs targeted to these receptors is perhaps not surprising given the propensity of ErbB receptors to form heterodimers and to respond to a wide variety of paracrine and autocrine ligands. A recent report suggests that high levels of ErbB2 limit the effectiveness of drugs targeted to the EGFR (82). On the other hand several studies have demonstrated the effectiveness of ZD1839 in slowing the proliferation of ErbB2-overexpressing EGFR-positive tumor cell lines (50, 56, 83).

Co-expression of other oncogenic signaling molecules may also limit the effectiveness of ErbB targeted therapies. For example sensitivity to AG1478 glioblastoma multiforme cell lines depended upon IGF-1 receptor induction; in resistant cells, but not sensitive cells, AG1478 treatment induced expression of the IGF-1 receptor, which in turn upregulated signaling via the PI 3-kinase pathway (84). Interestingly enhanced IGF-1 signaling also causes resistance to herceptin in breast cancer cells (85).

These studies illustrate the likely importance in defining reliable surrogate markers of responsiveness in patients. The mere demonstration that EGFR kinase activity is blocked (for example, by using phospho-EGFR antibodies) would appear insufficient for predicting response to drug treatment.

7.2. ErbB receptor TKIs in combination with other anticancer drugs

Because their mechanism of action differs from that of conventional chemotherapies, small molecule ErbB TKIs have great potential to be used in combination with existing cytotoxic drugs. Moreover because tumor cells often exhibit multiple defects in signal transduction, combining two or more drugs that target more than one pathway may be more beneficial than using inhibitors in isolation. Clinical studies with herceptin have shown the beneficial effects of combining ErbB receptor inhibitors with conventional chemotherapy (86). Preclinical studies with ErbB TKIs suggest that similar benefits may accrue by combining these drugs with standard chemotherapy. For example combination of the EGFR-TKI PKI166 with gemcitabine leads to enhanced anti-cancer effects *in vivo* compared with either agent alone (87). ZD1839 has also been shown to potentiate the activity of a wide range of cytotoxic drugs, each with differing mechanisms of action, against colon cancer cell xenografts (55). In this study ZD1839, when applied alone, was effective at reducing proliferation but had little effect on cell survival. However in combination with cytotoxic agents, ZD1839 induced a marked potentiation of apoptotic cell death. The same

group also found that ZD1839 potentiated the action of taxanes against multidrug resistant breast cancer cells (88). The pan-ErbB TK inhibitor CI1033 also enhances the cytotoxicity of topoisomerase inhibitors against breast cancer cells (67). Interestingly the mechanism involved modulation of the uptake and accumulation of the cytotoxic agent by CI1033. Another recent study using CI1033 and gemcitabine identified molecular responses to drug treatment that explained the synergistic actions of the two agents on apoptosis in breast cancer cell lines (89). Specifically CI1033 treatment led to inactivation of PKB/Akt and ERKs whereas gemcitabine induced activation of p38 MAP kinase. The combined effects of these alterations were shown to lead to accelerated rates of apoptotic cell death.

Other studies have demonstrated the potential utility in blocking multiple signaling targets. Combined treatment regimens involving ZD1839 with a protein kinase A blocker (90) or with herceptin (91) led to increased anti-cancer effects compared with single agent treatments.

Finally, recent studies indicate that ErbB receptor blockade can also enhance the effectiveness of radiotherapy. The basis for this interaction appears to lie in the ability of radiation to activate the EGFR (through an ill-defined mechanism), which then leads to increased proliferation in cells that survive radiation treatment (92). A number of studies have now shown that inhibition of EGFR increases the effectiveness of radiotherapy in cell line xenografts (93, 94).

7.3. Comparisons with other ErbB receptor-targeted therapies

In parallel with the recent advances made in the development of TKIs for clinical use, other strategies, also aimed at blocking ErbB receptor expression or function in tumors, have been developed. These include monoclonal antibodies (mAbs), antisense oligonucleotides and receptor-targeted cytotoxins. MAbs have progressed furthest in clinical trials and herceptin, a mAb that targets ErbB2, was recently approved for the treatment of advanced breast cancers that overexpress this receptor. In addition, EGFR-specific mAbs such as C225 (cetuximab^{TM}) are currently undergoing clinical evaluation.

MAbs such as herceptin differ from TKIs in several respects. Most obviously the basic mechanism of action is different. TKIs act as inhibitors of receptor kinase activity and thereby block downstream signaling events, whereas herceptin binds to the the extracellular domain of the receptor and causes its down-modulation from the cell surface (95). The mechanisms involved remain unclear but possibilities include antibody-mediated induction of receptor homodimers that phosphorylate but do not signal downstream or the disruption of ErbB2 heterodimer formation with other ErbB receptors. As noted above ErbB2-containing heterodimers are potent signal transducers and may be necessary for transformation. Like TKIs, the anti-tumor activity of herceptin appears to be cytostatic rather than cytoxic (96, 97). Part of the cytostatic effect appears to be mediated via herceptin-induced accumulation of the cell cycle inhibitory protein $p27^{KIP}$ (98). Herceptin and other mAbs may also induce host immune responses leading to killer cell-mediated cytotoxicity and complement fixation (97).

The contrasts in the basic mechanisms of action of mAbs versus TKIs extend to other issues that are likely to affect their clinical utility and efficacy. As mentioned above herceptin is already in clinical use but, while some TKIs may also soon receive regulatory approval, it will be some time before the relative benefits of TKIs and mAbs can be evaluated. One perceived advantage of small molecule TKIs over mAbs is their route of administration. TKIs are orally active and likely to be taken in the form of a pill once daily whereas herceptin must be administered intravenously on a weekly schedule. Another issue relates to tissue accessibility. MAbs may be less able to access certain tissues, such as the intestinal epithelium (99). This appears to give mAbs an advantage over TKIs in terms of side effects. Diarrhea is one of the main side effects observed with TKIs and limits the doses of these drugs that can be used. No such effects have been observed with mAbs. On the other hand the large structure of mAbs compared to small molecule TKIs has been suggested to underlie the inability of herceptin to penetrate and inhibit the growth of ErbB2 positive ductal carcinoma *in situ* tumor xenografts (100). One potential advantage of mAbs lies in their complete specificity for their target whereas the specificity of TKIs is only relative. Given the conserved nature of the catalytic domains of tyrosine kinases, the region to which many TKIs are targeted, it is possible that TKIs may inhibit cellular tyrosine kinases in addition to their primary target. Instinctively this may be perceived as limiting the utility of TKIs due to a greater potential to generate side effects but it is also possible that an ability to inhibit more than one cellular target could give rise to more effective anti-cancer drugs. For a more detailed appraisal of the relative merits of mAbs versus TKIs see (99, 101)

8. PERSPECTIVE

In this review we have attempted to highlight the importance of ErbB receptors in human cancer and describe the latest developments in the attempts to synthesize low molecular weight compounds that inhibit the tyrosine kinase activity of these receptors. In the last few years some of these molecules have progressed rapidly through to late phase clinical trials and it seems very likely that one or more will be approved for clinical use in the near future. Because of their excellent oral bioavailability and the relatively mild side-effects reported to date they hold great promise as members of a new generation of signal transduction inhibitor anti-cancer drugs. Nevertheless, as we have attempted to highlight here, it may be some time before issues of predicting patient responsiveness and identifying the most effective combination therapies are understood better. The development of highly potent and specific inhibitors of ErbB receptors should also further advance our understanding of how these molecules contribute to the myriad of biological functions in which they appear to be involved.

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