EGFR: An important perspective in cancer therapy

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ABSTRACT

Cancer is the second leading cause of death in the western world. Despite advances in diagnosis and treatment, overall survival of patients remains poor. Scientific advances in recent years have enhanced our understanding of the biology of cancer. Human protein tyrosine kinases (PTKs) play a central role in human carcinogenesis and have emerged as the promising new targets. Several approaches to inhibit tyrosine kinase have been developed. These agents have shown impressive anticancer effects in preclinical studies and are emerging as promising agents in the clinic. The remarkable success of BCR-ABL tyrosine kinase inhibitor Imatinib (STI571) in the treatment of chronic myeloid leukaemia has particularly stimulated intense research in this field. In this review, we focus on the role of tyrosine kinases in cancer and the development of specific small molecule inhibitors for therapy. We also provide a critical analysis of the current data on epidermal growth factor receptor (EGFR) inhibitors and highlight areas for future research. Innovative approaches are needed to fully evaluate the potential of these agents, and a concerted international effort will hopefully help to integrate these inhibitors in cancer therapy in the near future.

Key words: Tyrosine kinase inhibitors, cancer, EGFR, EGF.

INTRODUCTION

Cancer is the second leading cause of death in the western world. Despite advances in diagnosis and treatment, overall survival of patients still remains poor. Until recently, surgery, chemotherapy, radiotherapy, and endocrine therapy have been the standard treatment options available for patients. This has improved survival in several types of solid tumours, but treatment-related toxicity and emergence of drug resistance have been the major cause of morbidity and mortality. Hence, there is an urgent need to develop newer more effective therapies to improve patient outcomes. Rapid scientific advances in recent years have enhanced our understanding of the biology of cancer. Consequently, several novel targets have been identified. Tyrosine kinases have emerged as a new promising target for cancer therapy. This review will focus on the role of EGFR in cancer and the development of specific EGFR blockers for cancer therapy with emphasis on small molecule inhibitors (Manning et al., 2002).

Human protein tyrosine kinases (PTKs)

Human genome sequence analysis has identified about 518 human protein kinases (constituting about 1.7% of all the human genes). Within this large protein kinase complement, at least 90 tyrosine kinase genes have been identified (Krupa et al., 2002). Among these, 58 are receptor tyrosine kinases (RTKs) and 32 are non receptor tyrosine kinases (NRTKs). Based on their extracellular and non catalytic domain sequences, the RTKs and NRTKs have been further grouped into 20 and 10 subfamilies, respectively (Robinson et al., 2000), (Table 1 and 2).

RTKs contain an amino-terminal extracellular Ligand-binding domain, a hydrophobic transmembrane helix, and a cytoplasmic domain, which contains a conserved protein tyrosine kinase core and additional regulatory sequences. Ligand binding to the extracellular domain results in receptor imerisation/oligomerisation, leading to activation...
Table 1: Receptor tyrosine kinases and cancer.

<table>
<thead>
<tr>
<th>Receptor TK subfamilies</th>
<th>Cancer associated</th>
<th>Receptor TK subfamilies</th>
<th>Cancer associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Breast, ovary, lung, glioblastoma, multiforme, stomach, colon, granulosa cell tumours and others</td>
<td>RYK</td>
<td>Ovarian</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Tumour angiogenesis, Kaposi sarcoma, haemangiosarcoma</td>
<td>DDR</td>
<td>Breast, ovarian cancer</td>
</tr>
<tr>
<td>PDGFR</td>
<td>Glioma, glioblastoma, ovary, Chronic myelomonocytic leukaemia (CML), malignant histiocytosis, glioma, endometrium GIST, myelodysplasia, Acute myeloid leukaemia (AML)</td>
<td>RET</td>
<td>Thyroid (papillary and medullary), multiple endocrine neoplasia</td>
</tr>
<tr>
<td>FGFR</td>
<td>AML, lymphoma, solid tumours, stomach, breast, prostate, Multiple myeloma</td>
<td>ROS</td>
<td>Glioblastoma, astrocytoma</td>
</tr>
<tr>
<td>NGFR</td>
<td>Papillary thyroid cancer, Neuroblastoma, myeloid leukaemia</td>
<td>LMR</td>
<td></td>
</tr>
<tr>
<td>HGFR</td>
<td>Papillary thyroid, rhabdomyosarcoma, liver, kidney, Colon, liver, Lymphoma, Stomach, oesophagus, colon, Breast</td>
<td>LTK</td>
<td>non-Hodgkin lymphoma, LTK</td>
</tr>
<tr>
<td>EPHR</td>
<td>Melanoma, Stomach, oesophagus, colon, Breasts, capillary haemangioblastoma</td>
<td>Insulin-R</td>
<td>Cervix, kidney (clear cell), sarcomas</td>
</tr>
<tr>
<td>AXL</td>
<td>AML</td>
<td>MUSK</td>
<td></td>
</tr>
<tr>
<td>KLG/CCK</td>
<td>--</td>
<td>ROR</td>
<td></td>
</tr>
<tr>
<td>TIE</td>
<td>Stomach, capillary haemangioblastoma, TEK Tumour angiogenesis</td>
<td>RTK 106</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Non-receptor tyrosine kinases and cancer.

<table>
<thead>
<tr>
<th>Non-receptor TK subfamilies</th>
<th>Cancer associated</th>
<th>Non-receptor TK subfamilies</th>
<th>Cancer associated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL</td>
<td>Chronic myeloid leukaemia (CML), AML, ALL, CMML</td>
<td>FAK</td>
<td>Adhesion, invasion and metastasis of tumours</td>
</tr>
<tr>
<td>FRK</td>
<td>Breast</td>
<td>ACK</td>
<td>-</td>
</tr>
<tr>
<td>JAK</td>
<td>AML, ALL, T-cell childhood ALL, atypical CML, Leukaemia, B-cell malignancies</td>
<td>CSK</td>
<td>-</td>
</tr>
<tr>
<td>SRC-A, B</td>
<td>AML, CLL, EBV-associated lymphoma, colon, breast, pancreas, neuroblastoma, melanoma</td>
<td>FES</td>
<td>-</td>
</tr>
<tr>
<td>SYK</td>
<td>Breast</td>
<td>TEC</td>
<td>-</td>
</tr>
</tbody>
</table>

of cytoplasmic tyrosine kinase activity and phosphorylation of tyrosine residues. Autophosphorylated tyrosine residues serve as a platform for the recognition and recruitment of a specific set of signal transducing proteins that modulate diverse cell signalling responses. Non receptor tyrosine kinases have a common conserved catalytic domain (similar to RTKs) with a modular N-terminal, which has different adapter protein motifs (Weiner et al., 2000). Tyrosine kinases play a critical role in the regulation of fundamental cellular processes including cell development, differentiation, proliferation, survival, growth, apoptosis, cell shape, adhesion, migration, cell cycle control, T-cell and B-cell activation, angiogenesis, responses to extracellular stimuli, neurotransmitter signalling, platelet activation,
transcription, and glucose uptake (Fabbro et al., 2002).

**Tyrosine kinases and human cancer**

Tyrosine kinases play a central role in oncogenic transformation of cells. This is achieved in several ways. Gene amplification and/or over expression of PTKs (e.g. EGFR and HER-2 over expression that is commonly seen in several cancers) cause enhanced tyrosine kinase activity with quantitatively and qualitatively altered downstream signalling (Blume-Jensen et al., 2001). Genomic rearrangements, such as chromosomal translocation, can result in fusion proteins with constitutively active kinase activity. Gain of function mutations or deletion in PTKs within the kinase domain or extracellular domain result in constitutively active tyrosine kinase (Nicholson et al., 2001).

EGFR family of tyrosine kinases is the most widely investigated. EGFR (HER-1) over expression is associated with a poor prognosis in ovarian, head and neck, oesophageal, cervical, bladder, breast, colorectal, gastric, and endometrial cancer (Srinivasan et al., 2004).

EGFR is activated by binding of its specific ligands, including EGF and TGF-α. Upon activation of EGFR by its growth factor ligands, it undergoes a transition from an inactive monomeric form to an active homodimers. EGFR may pair with another member of ErbB receptor family, such as HER2 (erbB-2/neu), to create an activated heterodimer. Its dimerization stimulates the intrinsic intracellular protein tyrosine kinase activity. As a result, autophosphorylation of several tyrosine (Y) residues in the C-terminal domain (Y992, Y1045, Y1068, Y1148 and Y1173) of EGFR occurs. This autophosphorylation elicits downstream activation and initiate several signal transduction cascades leading to DNA synthesis, cell proliferation, cell migration, adhesion, apoptosis and angiogenesis (Figure 1). Changes to EGFRs gene (over expression, dysregulation or mutation) can lead to continual or abnormal activation of receptor causing unregulated cell division, which can account for cancer (Neelam et al., 1998).

*Figure 1: Potential targets for cancer therapy.*

1 = Tyrosine kinase domain inhibitors, 2 = Tyrosine kinase receptor blockers (e.g., monoclonal antibodies), 3 = Ligand modulators (e.g., monoclonal antibodies), 4 = RNA interference and antisense technology, 5 = Gene therapy strategy, 6 = Inhibitors of Src tyrosine kinase, 7 = BCR-ABL inhibitors, 8 = Downstream signal transduction pathway inhibitor.
Architecture of the EGFR

EGFR is a transmembrane receptor/protein present on cell membranes. It is synthesized from a 1210-residue polypeptide precursor (Ullrich et al., 1984). It is a member of the ErbB family of receptor, a subfamily of four closely related receptor tyrosine kinases: EGFR (HER1/erb-1), HER2 (erbB-2/neu), HER3 (erbB3) and Her-4 (erbB-4) (Figure 2). These kinases have extracellular component/domain, a hydrophobic transmembrane component/domain and an intracellular tyrosine kinase component/domain (Slieker et al., 1986).

The EGFR extracellular portion (or ectodomain) consists of four domains that we refer to as the L1, CR1, L2, and CR2 domains (Figure 3). The structure determinations of ectodomain fragments of the EGFR and ErbB3 show the L1 and L2 domains that consist of the so-called β-solenoid or β-helix folds, which resemble the corresponding domains of the IGF-1 receptor (Garrett et al., 2002). Ligand binds between the L1 and L2 domains of the EGFR. The CR1 and CR2 domains consist of a number of small modules, each appearing to be held together by one or two disulfide bonds (Ogiso et al., 2002). The first module of the CR1 and CR2 domains contain conserved tryptophan residues (Trp 176 and Trp 492) that intercalate between the fourth and fifth helical turns of the β-helical L domain and sit in a hydrophobic environment that includes other conserved tryptophan residues (Trp 140 and Trp 453). Cytoplasmic domain indicates that residues 626–647 are β-helical (Rigby et al., 1998) suggesting that the transmembrane β-helix continues into the juxta membrane domain. The juxta membrane region appears to have a number of regulatory functions, that is, down regulation and Ligand dependent internalization events (Kil et al., 2000). The carboxy-terminal domain of the EGFR contains tyrosine residues where phosphorylation modulates EGFR mediated signal transduction. There are also several serine threonine residues (and another tyrosine residue) where
Table 3: Signaling proteins and their function.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRB-2</td>
<td>Adaptor</td>
</tr>
<tr>
<td>Nck</td>
<td>Adaptor</td>
</tr>
<tr>
<td>Crk</td>
<td>Adaptor</td>
</tr>
<tr>
<td>Shc</td>
<td>Adaptor</td>
</tr>
<tr>
<td>Dok-R</td>
<td>Adaptor</td>
</tr>
<tr>
<td>PLC-y</td>
<td>Phospholipase</td>
</tr>
<tr>
<td>P120 Ras GAP</td>
<td>Ras attenuator</td>
</tr>
<tr>
<td>PTB-1B</td>
<td>Phosphatase</td>
</tr>
<tr>
<td>SHP-1</td>
<td>Phosphatase</td>
</tr>
<tr>
<td>Src</td>
<td>Tyrosine kinase</td>
</tr>
<tr>
<td>Ab1</td>
<td>Tyrosine kinase</td>
</tr>
</tbody>
</table>

phosphorylation has been inferred to be important for the receptor down regulation processes and sequences thought to be necessary for endocytosis. Residues 984–996 in the C-terminus have been identified as a binding site for actin and may well be involved in the formation of higher order receptor oligomers and/or receptor clustering after ligand activation of the kinase domain (Hartigh et al., 1992).

The abbreviations used: L and CR, for the Ligand binding and the cysteine-rich domains [also known as I(L1), II(CR1), III(L2), and IV(CR2)]; JM and CT, juxtamembrane domain and carboxy-terminal terminus. The transmembrane domain (residues 622–644) is between the CR2 and the juxta membrane domains (Kim et al., 2002).

Signaling pathways activated by the EGFR

In the absence of ligand binding, the EGFR exists on cells as both monomers and dimmers (Moriki et al., 2001). Yet Ligand binding to the EGFR kinase is required to elevate the receptor’s tyrosine kinase activity (Yu et al., 2002).

Given the functional diversity of proteins that complex with, or are phosphorylated by, the EGFR, it is hardly surprising that EGF stimulation of a cell results in the simultaneous activation of multiple pathways (Table 3) (Jorissen et al., 2003):

1. Shc, Grb2, and the Ras/MAPK pathway
2. The JAKs and STATs pathways
3. Phospholipid metabolism: PLD, PLCγ, and PI3-K
4. The Src family of kinases

**Ligand binding to the EGFR**

Ligand interacts with the L1 and L2 domains of a given EGFR molecule. The conserved EGF residue Arg 41 makes bidentate hydrogen bonds with Asp 355. Tyr 13 interacts with Phe 357 of the receptor. The side chain of Gln 384 of the EGFR makes two hydrogen bonds to the EGFR main chain atoms Gln 43 O and Arg 45 N. The side chain of Leu 47 projects into a hydrophobic pocket consisting of Leu 382, Phe 412, and Ile 438 with the side chain of Ala 415 at its base. The EGFR L1 residues Gln 16 and Gly 18 contribute three main chain-to-main chain hydrogen bonds to Cys 31 and Cys 33 of EGFR, thus extending the larger of the two ligand β-sheets into the receptor (colored green in Figure 4), (Groenen et al., 1994; Nice et al., 2002).

**DEVELOPMENT OF TYROSINE KINASE (EGFR) INHIBITORS**

Several approaches to target PTKs have been developed (Figure 1). Classification of such inhibitors is based on their mode of action. Receptor tyrosine kinases are multidomain proteins. The catalytic domain (Mg-ATP complex binding site) has emerged as the most promising target for drug design in recent years. Random screening of compound libraries initially identified small molecule chemical inhibitors of the catalytic domain. Combinatorial chemistry, in-silico cloning, structure-based drug design, and computational chemistry have now become indispensable tools in lead compound identification and optimisation of these inhibitors. Highly sensitive, accurate, and reliable high throughput assays for screening inhibitors have been developed (scintillation proximity assay, fluorescence polarisation assay, homogenous time resolved fluorescence assay, and the heterogeneous time resolved dissociation- enhanced fluorescence technology). Knowledge about tertiary structure of protein kinases has expanded, and the X-ray crystallographic structure for over 50 protein kinases has been resolved (Levitzki, 2002). Understanding of the molecular interactions of the various parts of the ATP
Table 4: Approaches for targeting EGFR.

**Approaches for targeting EGFR**

### 1) Small molecule inhibitors:
- ZD1839 (Iressa, Gefitinib)
- OSI-774 (Tarceva, Erlotinib, CP-358774)
- MV833
- Soluble Flt-1 and Flk-1
- CI-1033 (PD183805) GFB 116
- PKI-166 VEGF Trap
- CGP-59326A NM3
- EKB-569 VEGF 121-diphtheria toxin
- GW 572016 conjugate

### 2) Monoclonal antibodies against receptors:
- IMC-C225 (Cetuximab)
- ABX-EGF
- Y10
- MDX-447 (EMD 82633)
- h-R3
- EMD 72000

### 3) Gene therapy approaches

binding site’ (adenine region, sugar region, hydrophobic pocket, hydrophobic channel, and the phosphate-binding region) has accelerated drug development. Although ATP-binding site is highly conserved among tyrosine kinases, minor differences in kinase domain architecture have allowed development of highly selective inhibitors. Data on EGFR co-crystallised with its inhibitor OSI-774 (Erlotinib) were published and provide valuable insight into the mechanism of action of compound (Stamos et al., 2002). Most small molecules bind in the vicinity of the ATP-binding site of their target kinases, using a part of their scaffold to mimic the binding of the adenine moiety of ATP. Such ATP mimics are competitive inhibitors of the substrate-binding sites within the catalytic domain and compete with endogenous ATP (often present in mill molar levels in cells) for binding. Early potent lead compounds had poor solubility and so to increase solubility, new compounds were generated, but they had reduced affinity toward the kinase domain. To overcome these problems, irreversible inhibitors were developed in the hope that covalent attachment of a selective inhibitor to the kinase domain would completely abolish catalytic activity and would translate into potent drugs (Laird et al., 2003). By considering this, CI-1033 (Pfizer) and EKB-569 (Wyeth) inhibitors were developed which bind irreversibly to EGFR and HER-2, respectively (Denny et al., 2002).

Small molecules that target more than one tyrosine kinase have also been developed, and they have the potential to block multiple pathways and produce enhanced anticancer effect. PKI-166 inhibits EGFR and HER-2 (Slichenmyer et al., 2001). In the 1980s, first natural tyrosine kinase inhibitors quercetin and genistein were reported (Mellinghoff et al., 2002; Glossmann et al., 1981). Since then, an overwhelming number of natural and synthetic small molecules inhibitors have been described. Tyrosine kinase inhibitors can be broadly categorised into natural products and related derivatives (quercetin, genistein, staurosporine, erbastatins, davilactones); quinazolines, pyridopyrimidines, and related heterocyles (e.g., ZD1839); phenylamino-pyrimidines (e.g., STI 571); tryphostins and analogues (e.g., SU1498, SU0020); indoles and oxindoles (e.g.,SU5416, SU5402) (Akiyama et al., 1987).

Development of monoclonal antibodies against tyrosine kinase receptors particularly against EGFR has been reviewed (Al-Obeidi et al., 2000). Herceptin (Trastuzumab: monoclonal antibody against HER-2) is licensed for use in HER-2 over expressing breast cancer. Monoclonal antibodies block ligand–receptor interaction and hence ligand-induced signalling. They also increase receptor down regulation and internalisation. Immune-mediated responses including complement-mediated lysis and antibody-dependent cellular toxicity induced by monoclonal antibodies have been described. Some evidence suggests that, by cross-linking or binding to receptors, they can modulate signalling activity such that they may trigger apoptosis or growth inhibition in cells (Farah et al., 1998). Antisense oligonucleotides are designed to interact with mRNA and block transcription. Such an approach has been developed to target EGFR, VEGFR 1GF-1R, and TGF-a (Zwick et al., 2002).

Recombinant antibody technology has allowed the design, selection, and production of humanised/human antibodies, human–mouse chimeric, or bispecific antibodies (Zeng et al., 2002). Various approaches and certain developed drugs for inhibition of EGFR are shown in Table 4 (Husdon, 1999).
In this review, we focus on the current status of small molecule inhibitors of EGFR in cancer therapy.

SMALL MOLECULE INHIBITORS

In the past few years, a large number of preclinical and clinical studies of tyrosine kinase inhibitors have been reported. The most promising and extensively investigated and used drugs are shown in Table 3.

Targeting EGFR family

Selective small-molecule inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR) commonly referred to as EGFR tyrosine kinase inhibitors (EGFR-TKIs) are in the vanguard of the new attack on cancer directed at specific biochemical signal-transduction pathways (Table 5). Some remarkable success of the EGFR family inhibitors are described hereafter (Grunwald et al., 2003).

ZD1839 (Gefitinib, AstraZeneca Pharmaceuticals)

Gefitinib is an anilinoquinazoline small molecule inhibitor of EGFR tyrosine kinase (Table 5). Specifically, this agent competes with the binding of ATP to the tyrosine kinase domain of EGFR, thereby inhibiting receptor autophosphorylation and resulting in inhibition of signal transduction. Gefitinib may also induce cell cycle arrest and inhibit angiogenesis. It is a selective tyrosine kinase receptor inhibitor used in the therapy of non-small cell lung cancer (Hirata et al., 2002).

OSI-774 (Erlotinib, Genetech)

Erlotinib is a quinazoline derivative and highly selective inhibitor of EGFR tyrosine kinase. It is competing with adenosine triphosphate (Townesley et al., 2006). It reversibly binds to the intracellular catalytic domain of epidermal growth factor receptor (EGFR) tyrosine kinase, thereby reversibly inhibiting EGFR phosphorylation and blocking the signal transduction events and tumorigenic effects associated with EGFR activation. It is used in the treatment of non-small cell lung cancer (Grunwald et al., 2003).

CI-1033 (Canertinib, Pfizer Oncology)

Canertinib is a 4-anilinoquinazoline and an irreversible pan-erbB inhibitor. It is highly selective for the EGFR family of tyrosine kinases erbB-1, erbB-2 and erbB-4. Although erbB-3 does not have an active tyrosine kinase domain, signalling through this pathway is also blocked by CI-1033 as it inactivates the catalytically active hetero dimerisation partners by covalently modifying a specific cysteine residue (Cys-773) in the ATP binding site of these receptors. It inhibits the growth of a panel of cell lines (colon cancer, breast carcinoma, and epidermoid carcinoma cell lines), (Allen et al., 2002).

GW2016 (Lapatinib, GlaxoSmithKline)

Lapatinib is a synthetic, orally-active quinazoline with potential antineoplastic properties. Lapatinib reversibly blocks phosphorylation of the epidermal growth factor receptor (EGFR), ErbB2, and the Erk-1 and-2 and AKT kinases; it also inhibits cyclin D protein levels in human tumor cell lines and xenografts. It is used in the therapy of advanced breast cancer and other solid tumors (Rusnak et al., 2001).

PKI166 (Novartis)

It is a reversible pyrrolo-pyrimidine inhibitor of the EGFR tyrosine kinase. PKI-166 treatment inhibits EGFR autophosphorylation, c-fos mRNA expression and cell proliferation in cancer cells (Mellinghoff et al., 2002).

AG1478 (Tyrphostin)

It is a quinazoline derivative and competitive inhibitor of the ATP binding site in the kinase domain, is a highly potent and specific reversible tyrosine kinase inhibitor of the EGFR. It has shown significant anti-proliferative effects in glioblastoma, leiomyoma, colorectal carcinoma and nasopharyngeal carcinoma cells (Zhang et al., 2008).

BIBW 2992 (Afatinib)

Afatinib is an orally bioavailable anilino-quinazoline derivative and inhibitor of the receptor tyrosine kinase (RTK) epidermal growth factor receptor (ErbB; EGFR) family. Additionally, afatinib inhibits the EGFR T790M gatekeeper mutation which is resistant to treatment with first-generation EGFR inhibitors (Bordoni et al., 2011). EGFR, HER2 and HER4 are RTKs that belong to the EGFR superfamily; they play major roles in both tumor cell proliferation and tumor vascularization and are overexpressed in many cancer cell types. It is used in the therapy of selected forms of metastatic non-small cell lung cancer (Solca et al., 2007).

PF299 (Dacomitinib)

Dacomitinib is a highly selective, orally bioavailable small-molecule inhibitor of the HER family of tyrosine kinases.
Table 5: Chemical structures of small molecules that inhibit growth factor signalling pathways.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ZD1839 (Gefitinib)</strong></td>
<td>(N-(3\text{-}\text{chloro}\text{-}4\text{-}\text{fluorophenyl})\text{-}7\text{-}methoxy\text{-}6\text{-}(3\text{-}\text{morpholin}\text{-}4\text{-}yloxypropoxy)quinazolin-4\text{-}amine)</td>
</tr>
<tr>
<td><strong>OSI-774 (Erlotinib)</strong></td>
<td>(N-(3\text{-}\text{ethynylphenyl})\text{-}6,7\text{-}\text{bis(2\text{-}methoxyethoxy)quinazolin-4\text{-}amine})</td>
</tr>
<tr>
<td><strong>CI1033 (Canertinib)</strong></td>
<td>(N\text{-}[4\text{-}(3\text{-}\text{chloro}-4\text{-}\text{fluorophenyl})\text{-}amino]\text{-}7\text{-}(3\text{-}\text{morpholin}\text{-}4\text{-}yloxypropoxy)quinazolin-6\text{-}yl]prop-2\text{-}enamide)</td>
</tr>
<tr>
<td><strong>GW2016 (Lapatinib)</strong></td>
<td>(N\text{-}[3\text{-}\text{chloro}-4\text{-}(3\text{-}\text{fluorophenyl})\text{-}methoxy]\text{-}phenyl]\text{-}6\text{-}[5\text{-}(2\text{-}methylsulfonyl)ethylamino]methyl[2\text{-}furyl]quinazolin-4\text{-}amine)</td>
</tr>
<tr>
<td><strong>PKI166</strong></td>
<td>(4\text{-}[4\text{-}[(1R)\text{-}1\text{-}\text{phenylethyl})\text{-}amino]-7\text{H}\text{-}\text{pyrrolo[2,3-d]pyrimidin-6\text{-}yl]}\text{phenol})</td>
</tr>
<tr>
<td><strong>AG1478</strong></td>
<td>(N\text{-}(3\text{-}\text{chlorophenyl})\text{-}6,7\text{-}\text{dimethoxyquinazolin-4\text{-}amine})</td>
</tr>
</tbody>
</table>
Table 5: Conts. Chemical structures of small molecules that inhibit growth factor signalling pathways.

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BIBW 2992</strong> (Afatinib)</td>
<td>(E)-N-[4-((3-chloro-4-fluoroanilino)-7-([3S]-oxolan-3-yl)oxyquinazolin-6-yl)-4-(dimethylamino)but-2-enamide</td>
</tr>
<tr>
<td><strong>PF299</strong> (Dacomitinib)</td>
<td>(E)-N-[4-((3-chloro-4-fluoroanilino)-7-methoxyquinazolin-6-yl)-4-piperidin-1-ylbut-2-enamide</td>
</tr>
<tr>
<td><strong>HKI-272</strong> (Neratinib)</td>
<td>(E)-N-[4-([3-chloro-4-(pyridin-2-ylmethoxy)anilino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide</td>
</tr>
<tr>
<td><strong>AG 490</strong></td>
<td>(E)-N-benzyl-2-cyano-3-(3,4-dihydroxyphenyl)prop-2-enamide</td>
</tr>
<tr>
<td><strong>ZD6474</strong> (Vandetanib)</td>
<td>N-[4-(bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine</td>
</tr>
<tr>
<td><strong>Rociletinib</strong></td>
<td>N-[3-[[4-acetyl(piperazin-1-yl)-2-methoxyanilino]-5-(trifluoromethyl)pyrimidin-4-yl]amino]phenyl]prop-2-enamide</td>
</tr>
<tr>
<td><strong>AZD8931</strong> (Sapitinib)</td>
<td>2-[[4-((3-chloro-4-fluoroanilino)-7-methoxyquinazolin-6-yl]oxypiperidin-1-yl]-N-methylacetamide</td>
</tr>
<tr>
<td><strong>AST1306</strong> (Allitinib)</td>
<td>N-[4-[[3-fluorophenyl]methoxy]anilino]quinazolin-6-yl]prop-2-enamide</td>
</tr>
</tbody>
</table>

with potential anti-neoplastic activity. Dacomitinib specifically and irreversibly binds to and inhibits human Her-1, Her-2, and Her-4, resulting in the proliferation inhibition and apoptosis of tumor cells that overexpress these receptors. It has been investigated for the treatment of Lung Cancer (Tony et al., 2010).

**HKI-272 (Neratinib)**

Neratinib is an orally available, 6,7-disubstituted-4-anilinoquinoline-3-carbonitrile irreversible inhibitor of the HER-2 receptor tyrosine kinase with potential anti-neoplastic activity. Neratinib binds to the HER-2 receptor irreversibly, thereby reducing autophosphorylation in cells, apparently by targeting a cysteine residue in the ATP-binding pocket of the receptor. Treatment of cells with this agent results in inhibition of downstream signal transduction events and cell cycle regulatory pathways, arrest at the G1-S (Gap 1/DNA synthesis)-phase transition of the cell division cycle, and ultimately decreased cellular
proliferation. Neratinib also inhibits the epidermal growth factor receptor (EGFR) kinase and the proliferation of EGFR-dependent cells (Yoshinori et al., 2012).

**AG 490 (Tyrphostin B42)**

It is an inhibitor of EGFR with IC50 of 0.1 μM in cell-free assays, 135-fold more selective for EGFR versus ErbB2, and also inhibits JAK2 with no activity to Lck, Lyn, Btk, Syk and Src (Gyurkovska et al., 2012).

**ZD6474 (Vandetanib)**

Vandetanib is an orally bioavailable 4-anilinoquinazoline. Vandetanib selectively inhibits the tyrosine kinase activity of vascular endothelial growth factor receptor 2 (VEGFR2), thereby blocking VEGF-stimulated endothelial cell proliferation and migration, and reducing tumor vessel permeability (Morabito et al., 2009). This agent also blocks the tyrosine kinase activity of epidermal growth factor receptor (EGFR), a receptor tyrosine kinase that mediates tumor cell proliferation and migration and angiogenesis. Vandetanib is a multi-kinase inhibitor that is used in the therapy of advanced or metastatic medullary thyroid cancer (Herbst at al., 2007).

**CO1686 (Rociletinib)**

Rociletinib is Pyrimidine based and an orally available small molecule, irreversible inhibitor of epidermal growth factor receptor (EGFR) with potential anti-neoplastic activity. It binds to and inhibits mutant forms of EGFR, including T790M, thereby leading to cell death of resistant tumor cells (Tran et al., 2016).

**AZD8931 (Sapitinib)**

Sapitinib is a quinazoline derivative with erbB receptor tyrosine kinase inhibition activity (Scheipl et al., 2016). It inhibits erbB tyrosine receptor kinases and result in inhibition of cellular proliferation and angiogenesis in tumors expressing erbB. The erbB protein family is also called the epidermal growth factor receptor (EGFR) family (Zhaomei et al., 2014).

**AST-1306 (Allitinib)**

It is quinazoline derivative, a novel irreversible inhibitor of EGFR and ErbB2, highly selective for ErbB family than other kinases (Zhang et al., 2014).

**Other EGFR inhibitors**

**CP-724714**

It is a potent, selective inhibitor of HER2/ErbB2, 640-fold selectivity against EGFR, InsR, IRG-1R, PDGFR, and VEGFR2. It has been proposed that CP-724714 induced inhibition of hepatic efflux transporters that contributed to an accumulation of drug and bile levels in the liver, leading to hepatobiliary cholestasis. CP-724714 has since been discontinued in clinical development (Richard et al., 2014).

**EKB-569 (Pelitinib)**

Pelitinib is a 3-cyanoquinoline pan-ErbB tyrosine kinase inhibitor with potential antineoplastic activity (Table 6). It irreversibly binds covalently to EGFR ErbB-1, -2 and -4, thereby inhibiting receptor phosphorylation and signal transduction and resulting in apoptosis and suppression of proliferation in tumor cells that overexpress this receptors (Erlichman et al., 2006).

**TAK-285**

TAK-285 is an orally active irreversible potent dual EGFR/HER2 inhibitor (Table 6) (Ishikawa et al., 2011).

**AC-480 (BMS-599626)**

AC-480 is an orally bioavailable reversible EGFR, HER2, and HER4 inhibitor (Table 6) (Mylin et al., 2011).

**Side effects caused by small molecule inhibitors**

Small molecule inhibitors commonly cause gastrointestinal side effects including diarrhoea, nausea, and vomiting. Whether this represents the effect of EGFR inhibition on the intestinal epithelial cells or reflects the enterohepatic circulation of the drug or its metabolite or the effect of the drug on other kinase targets in the epithelium is unclear (Dancey et al., 2003). Wide spread use of ZD1839 has shown that it induces pulmonary fibrosis in about 2–4% of patients (0.5% deaths). VEGF/VEGFR target in agents could cause disruption of tumour endothelium and trigger a clotting cascade along the denuded vessels. Thromboembolic events (deep vein thrombosis, strokes, and transient ischaemic attacks) have been reported in a trial of SU5416 in combination with cisplatin and gemcitabine (Kuenen et al., 2002).

**Development of resistance to small molecule inhibitors**

Predclinical and clinical studies of Imatinib (STI571) in
Table 6: Chemical structures of Dual acting small molecules that inhibit growth factor signalling pathways.

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>Molecular Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP-724714 (Sesquisuccinate)</td>
<td>C38H38F2Cl2N6O4</td>
</tr>
<tr>
<td>EKB-569 (Pelitinib)</td>
<td>C37H28Cl2F2N6O4</td>
</tr>
<tr>
<td>TAK-285</td>
<td>C36H36ClF4N6O2</td>
</tr>
<tr>
<td>AC-480</td>
<td>C37H40ClF4N6O2</td>
</tr>
</tbody>
</table>

chronic myeloid leukaemia provide insights into the development of therapeutic resistance to tyrosine kinase inhibitors. Several lines of evidence indicate that BCR-ABL gene amplification is an important mechanism for resistance to STI571 in CML. Mutations in the ATP-binding pocket such that STI571 no longer has the affinity to bind to this site may also account for resistance in some patients (Paterson et al., 2003).

Increased levels of EGFR mRNA and protein but not those of HER2, HER3, and HER4 were observed in PC-9/ ZD cells that are resistant to Gefitinib (ZD1839) in a recently presented study. Increased EGFR/HER2 hetero dimer formation was demonstrated in PC-9/ ZD cells. Exposure to ZD1839 increased the formation of these dimers but resulted in only partial inhibition of the auto phosphorylation of EGFR in PC-9/ ZD cells. This difference in the inhibition by ZD1839 was most apparent at Tyr1068 of EGFR. Acquired resistance to ZD1839 in breast cancer cells may also result from increased IGF1-R signalling. Molecular mechanisms for acquired resistance to the HER1/EGFR tyrosine kinase inhibitor Erlotinib (OSI774) were addressed in another preclinical study of resistant cancer cell lines. Interestingly, resistance was associated with significant down regulation of HER1/ EGFR (protein) and phosphorylated (P) HER1/EGFR by Western blot analysis, but no changes in HER1/EGFR mRNA levels (Koizumi et al., 2003).

CRITICAL ISSUES IN THE DEVELOPMENT OF TYROSINE KINASE INHIBITORS

The clinical trials of tyrosine kinase inhibitors in solid tumours have been largely unsuccessful. Comparative analysis of trials of STI571 and EGFR inhibitors in solid tumours provides valuable insights. STI571 produced dramatic responses in chronic myeloid leukaemia (CML), gastrointestinal stromal tumours (GIST), chronic myelomonocytic leukaemia (CMML), hyper eosinophilic syndrome, and dermat fibro sarcoma protuberans. A constitutively activated tyrosine kinase plays a central role in driving the malignant phenotype in these disorders. The ‘kinase dependency’ correlates well with response (Sawyers et al., 2003). There are multiple mechanisms by which solid tumours can achieve ‘kinase dependency’ such as translocations which lead to fusion proteins with constitutively activated kinase, mutations within kinase genes, leading to constitutive activation, mutations within phosphatases that negatively regulate kinases and constitutively secreted ligand that leads to kinase activation (Shimizu et al., 1999).

 Comprehensive kinome sequencing analysis can be used to identify genetic alterations in a spectrum of tumours.

 Microarray-based technology to assess the expression of kinases in tumours is another approach. Phosphorylation status of kinases in tumours can also be evaluated using
phospho-specific antibodies, and mass spectroscopy can be used for protein identification.

Solid tumour studies are largely limited by the unavailability of serial tumour samples for analysis of target inhibition. Surrogate markers (e.g., in peripheral blood and skin) poorly predict what is happening in the tumour tissue (Bardelli et al., 2003). One approach to address the problem may be to test these inhibitors in patients where serial tumour sampling may be feasible (e.g., cervical cancer).

CONCLUSIONS

Elucidation of the various steps initiated in response to EGFR activation has clearly demonstrated that EGFR plays a crucial role in regulating cell functions, and its aberrant expression in cancer cells may influence prognosis and survival. This knowledge has provided a foundation for rational anticancer drug development. The two most widely investigated pharmacologic strategies for inhibiting EGFR function to date are the use of monoclonal antibodies to block ligand binding to the extracellular portion of EGFR and the use of low-molecular-weight inhibitory molecules to interfere with intracellular phosphorylation of intracellular EGFR tyrosine kinase. Both strategies have yielded agents that interrupt cellular proliferation and also facilitate apoptosis, indicating that these agents possess cytotoxic as well as cytostatic properties. Because these EGFR inhibitors have a different site and mechanism of action than traditional cytotoxic treatments, combinations of the EGFR with chemotherapy and radiation produce additive or synergistic anticancer activity with toxicity profiles that often do not overlap with traditional cytotoxic chemotherapy.

A concerted international effort by the pharmaceutical industry and academia is urgently needed to achieve these goals and fully realise the potential of tyrosine kinase inhibitors in cancer therapy. The need remains for a therapeutic agent that can have a long term impact on the treatment of cancer and to ensure disease free survival.

REFERENCES


