

A REVIEW ON: THE DESIGN AND DEVELOPMENT OF EGFR TYROSINE KINASE INHIBITORS IN CANCER THERAPY

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ABSTRACT

Cancer chemotherapy has entered a new era of molecularly targeted therapeutics, in which structure based approaches are employed to create small molecule. This design has been successfully applied for the making of drugs for epidermal growth factor receptor tyrosine kinase (EGFR-TK). The rationale to inhibit the EGFR-TK family as an approach to cancer therapy has continued to grow stronger over the last 20 years. Recently significant progresses have been made in the area of EGFR tyrosine kinase inhibitors (EGFR-TKIs). Overexpression of EGFR has been shown to promote cell proliferation and growth, metastasis, angiogenesis, inhibition of apoptosis and resistance to standard cytotoxic therapies. The EGFR gene family is of eminent importance as prognostic marker in cancer patients. Four members have been identified in humans erbB1-erbB4 (HER1-HER4), of which HER1 (EGFR, erbB1) and HER2 (erbB2, C-neu) are best characterized. EGFR overexpression is seen in breast cancer, ovarian cancer, lung cancer and prostate cancer. Currently the most useful inhibitors of the EGFR family are derived from three chemical series which include 4-anilino-quinazolines, 4-[ar(alkyl)amino]pyridopyrimidines and 4-phenyl amino pyrrolo-pyrimidines. EGFR tyrosine kinase inhibitors in development include anti EGFR monoclonal antibodies such as cetuximab (Erbix) and small molecule inhibitors such as gefitinib (Iressa) and erlotinib (Tarceva). Many more EGFR-TKIs are still under evaluation in clinical trials for the treatment of cancer. This review discusses the current status of EGFR-TKIs and their design, development in cancer therapy.

KEY WORDS : EGFR, cancer, tyrosine kinase, monoclonal antibodies, small molecule inhibitors

INTRODUCTION

Cancer is one of the most widespread and feared diseases in the world today, feared because

it is known to be difficult to cure. The main reason for this difficulty is that cancer results from the uncontrolled multiplication of subtly modified normal human cells. Currently there are three major ways of treating cancer: radiation therapy, surgery and cytotoxic drugs. All of these have significant limitations, but drugs offer the only approach to treat cases where the cancer has spread through the body.

For decades, the hallmark of medical treatment for cancer has been intravenous cytotoxic chemotherapy. These drugs target rapidly dividing cells, including cancer cells and certain normal tissues. As a result, many patients experience the classic toxicities of alopecia, gastrointestinal symptoms, and myelosuppression. In the past decade, however a dramatic shift in cancer therapy has occurred. Although traditional cytotoxic chemotherapy remains the treatment of choice for many types of cancer, including breast, colorectal, lung and pancreatic cancers as well as lymphoma, leukemia and multiple myeloma.¹

A major challenge is to design new drugs that will be more selective for cancer cells, and thus have lesser side effects. Over the last fifty years about 5 lakhs natural and synthetic chemical compounds have been tested for anticancer activity, but only about 25 of these are in wide use today. This gives an indication of the difficulty of this problem.²

In the past decade tremendous progress has been made toward a new class of therapeutics termed 'targeted covalent drugs', in which structure-based approaches are employed to create small molecules that inactivate their protein target.³ Targeted cancer therapies are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules involved in tumor growth and progression. Targeted cancer therapies are sometimes called "molecularly targeted drugs" or "molecularly targeted therapies". By focusing on molecular and cellular changes that are specific to cancer, targeted cancer therapies may be more

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effective than other types of treatment.

Many targeted cancer therapies have been approved by the U.S. FDA for the treatment of specific types of cancer. Others are being studied in clinical trials and many more are in preclinical testing. The development of targeted therapies requires the identification of good targets that is targets that are known to play a key role in cancer cell growth and survival. This molecularly targeted therapy has been successfully applied to making drug candidates for epidermal growth factor receptor (EGFR).

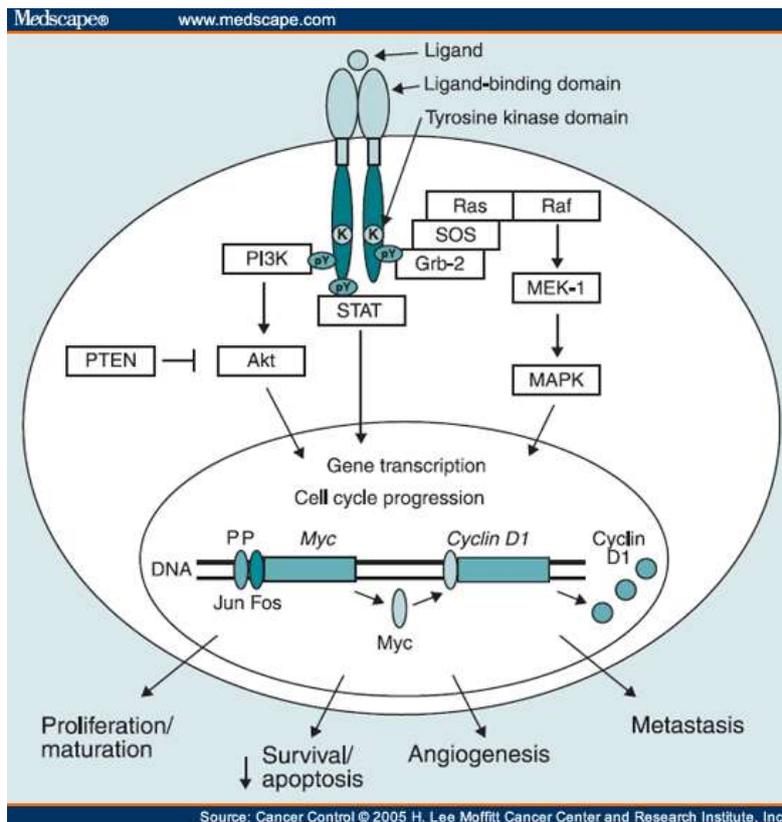
EGFR AS A THERAPEUTIC TARGET

The epidermal growth factor receptor (EGFR) gene family is of eminent importance as prognostic marker in cancer patients. EGFR and its ligands are involved in over 70% of all cancers. This receptor is a 170-kDa plasma membrane glycoprotein, having an extracellular ligand binding domain, a single hydrophobic transmembrane region, an intracellular domain possessing PTK activity, and a C-terminal tail that contains specific

tyrosine containing sequences, that upon phosphorylation, become binding sites for SH2-containing signaling proteins.⁴ The extracellular domain contains 621 amino acid residues, and its key feature is the ligand-binding domain, which is formed by two domains called L1 and L2.^{5,6} The role of the transmembrane domain, which contains 23 amino acid residues, in EGFR signaling remains uncertain, although recent studies suggest that it contributes to receptor stability.⁷ The intracellular domain of EGFR is composed of 542 amino acid residues and has tyrosine kinase activity, which functions as an activator of the cytoplasmic targets of the receptor.⁵

EGFR activation normally occurs by a 3-step mechanism. The first step is binding of specific ligands such as epidermal growth factor(EGF), amphiregulin or transforming growth factor- α (TGF- α) to the extracellular ligand binding domain of the receptor. The second step is dimerization of the ligand-bound receptor with one of the subclass I RTKs (HER1-HER4), leading to the third step, intracellular binding of ATP molecules and

Figure1. EGFR signaling pathways



After ligand activation, the EGFR phosphorylates and activates the Ras-Raf-MAPK, P13K/Akt and Stat/Jak pathways. This in turn results in activation of transcription factors and modulation of the cell cycle, growth, apoptosis and angiogenesis processes.

autophosphorylation of specific tyrosine residues within the tyrosine kinase domain of the receptor.⁸ Four members have been identified in humans erbB1-erbB4 (HER1-HER4), of which HER1 (EGFR, erbB1) and HER2 (erbB2, C-neu) are best characterized. HER1 and HER2 are frequently over expressed in tumors and are associated with short survival times of patients and therapy resistance of tumors.

The role of EGFR has been most thoroughly studied in breast cancer, where it is over expressed in 25-30% of cases and is correlated with a poor prognosis. EGFR over expression is also seen in ovarian cancer,⁹ lung cancer¹⁰ and in hormone-refractory prostate cancer.¹¹

Activation of the EGFR is involved in malignant transformation and tumor growth through the inhibition of apoptosis, cellular proliferation, promotion of angiogenesis and metastasis. At the cellular level, three major signaling pathways mediate the downstream effects of EGFR activation (Fig.). The first pathway involved the Ras-Raf-MAPK pathway.¹² The second pathway involved phosphatidylinositol 3-kinase (PI3K) and Akt.¹³ The third pathway involves the stress activated protein kinase pathway, involving Jak/Stat and protein kinase C (Figure 1).¹⁴

Among the multiple signal transduction pathways activated by the EGF family, the MAPK pathway is one of the most relevant because it regulates cellular processes, such as gene transcription and proliferation, by activating a variety of substrates located in the cytosol, nucleus, and plasma membrane.^{15, 16} Another important signal transduction pathway activated by the EGF family of receptors is the P13K/Akt signaling pathway, which mediates cell survival.^{17, 18} The recruitment of Akt to the plasma membrane triggers a cascade of pro-survival signaling, which is mediated by increased expression of anti-apoptotic signals, decreased expression of pro-apoptotic signals, and the activation of mRNA translation.^{19, 20} In addition to activating the receptor, the binding of ligands initiates receptor internalization for signal termination, usually within seconds of receptor activation. After the ligand-receptor complex is internalized, it is either degraded, leading to signal termination, or recycled to the cell surface for another round of signaling.

EGFR have been researched since the early 1980s. It was discovered that some carcinogenic viruses, namely erythroblastosis tumour viruses, alter the operation of human epidermal growth factor receptor. The presence of those receptors in

large quantities in several tumors suggests that they are a significant factor of carcinogenesis. Since the 1980s, researchers have made attempts to selectively inhibit their signaling pathways. Human tumors of epithelial origin express high levels of EGFR. For this reason, this receptor tyrosine kinase was first proposed as a target for cancer therapy more than 20 years ago.

EGFR TARGETING AGENTS

Theoretically a variety of approaches and strategies can be used to target EGFR, such as (1) using monoclonal antibodies (mAbs) that compete with the binding of activating ligands to the extracellular domain of the receptor. (2) Using small molecule inhibitors of the intracellular tyrosine kinase domain of the receptor. (3) Using immunotoxin conjugates to deliver toxins that target EGFR of tumor cells. (4) Reducing the level of EGFR through the use of antisense oligonucleotides and (5) inhibiting downstream effectors of the EGFR signaling network.²¹

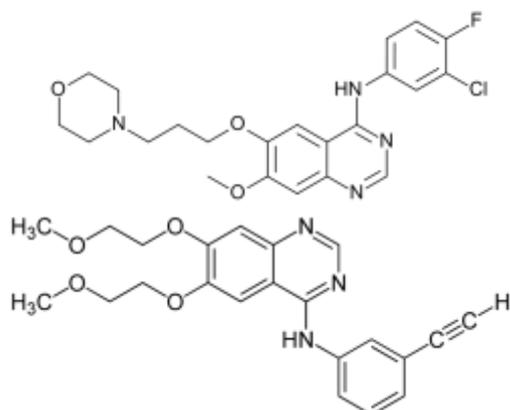
Most targeted therapies are either small molecule drugs (Figure 2) or monoclonal antibodies because they have been extensively explored in clinical trials. Small molecule drugs are typically able to diffuse into cells and can act on targets that are found inside the cell. Most monoclonal antibodies usually cannot penetrate the cell's plasma membrane and are directed against targets that are outside cells or on the cell surface.

Monoclonal antibodies, which are usually water soluble and large (typically molecular weight of approximately 150,000 Da), target extracellular components of these pathways, such as ligands and receptor-binding domains. In contrast, small molecule inhibitors (typical molecular weight of approximately 500 Da) can enter the cells, thereby blocking receptor signaling and interfering with downstream intracellular molecules.

A first generation of erbB family targeted drugs including the reversible inhibitors like gefitinib, erlotinib and lapatinib and monoclonal antibodies like cetuximab and trastuzumab has been shown to produce therapeutic benefit against a variety of cancers. These drugs addresses the overexpression of EGFR and HER2 as well activating somatic mutations that drive increased signaling by erbB family proteins in many cancers. However their effectiveness has been blunted by issues such as a significant number of non-responding patients and a decreasing response-rate during drug treatment.^{22, 23}

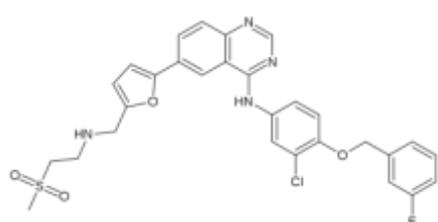
Monoclonal Antibodies

Figure 2. Small molecule EGFR inhibitors

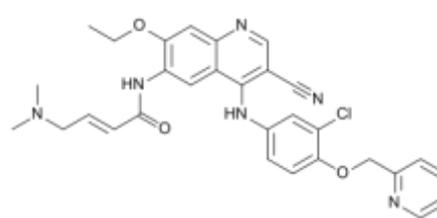


Gefitinib

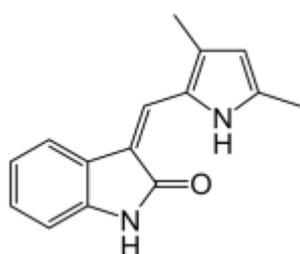
Erlotinib



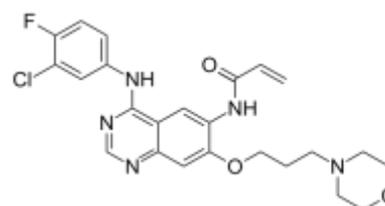
Lapatinib



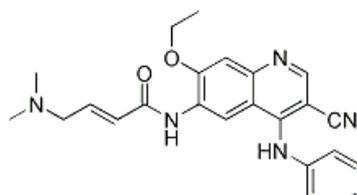
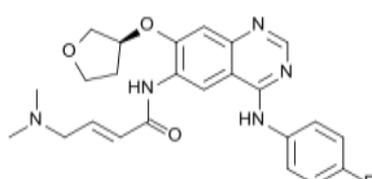
Neratinib



Semaxanib



Canertinib



The design of monoclonal antibodies has changed over the past 20 years as biotechnology has improved. Early drugs in this class were created by immunizing mice with the target antigen. The resulting monoclonal antibodies were composed entirely of mouse proteins, which were potentially highly antigenic to humans, carrying a risk of hypersensitivity reaction during infusion. Patients treated with these early drugs often

formed anti-mouse protein antibodies, which could neutralize the effect of the therapeutic antibody. To limit these undesirable effects, recently developed monoclonal antibodies contain an increased proportion of human components and a decreased proportion of murine components; chimeric antibodies are 65 percent human, humanized antibodies are 95 percent human, and human antibodies are 100 percent

human.²⁴ The type of antibody can often be identified by the suffix of the drug name: -momab (murine), -ximab (chimeric), -zumab (humanized), or -mumab (human).

Cetuximab (Erbix) is a chimeric (mouse/human) monoclonal antibody that is approved for treating some patients with squamous cell carcinoma of the head and neck or colorectal cancer. It binds to the external portion

myeloid leukemia, imatinib inhibits a continuously active tyrosine kinase that results from the translocation of chromosomes 9 and 22. Because this molecular abnormality occurs in essentially all patients with chronic myeloid leukemia, imatinib therapy results in a complete hematologic response in 98 percent of patients.^{31, 32} More recently, small molecule inhibitors targeting the EGFR pathway have been used in the treatment of

Table 1. Monoclonal Antibodies

Drug	Target	Antibody Type	FDA-approval
Cetuximab (Erbix)	EGFR	Chimeric, unconjugated head and neck cancers	Colorectal cancer
Trastuzumab (Herceptin)	HER2/neu	Humanized, unconjugated	Breast cancer with HER/2 neu over expression
Panitumab (Vectibix)	EGFR	Human, conjugated	Colorectal cancer

of EGFR, thereby preventing the receptor from being activated by growth signals, which may inhibit signal transduction and lead to antiproliferative effects.

Trastuzumab (Herceptin) is approved for the treatment of certain types of breast cancer. It is a monoclonal antibody that binds to the human epidermal growth factor receptor2 (HER2)/neu. Trastuzumab is also being studied for use with other cancers.²⁵ It has been used with some success in women with uterine papillary serous carcinomas that overexpress HER2/neu.²⁶ The combination of trastuzumab with chemotherapy has been shown to increase both survival and response rate, in comparison to trastuzumab alone.²⁷ Panitumumab (Vectibix) is a fully human monoclonal antibody. It was approved by the FDA in 2006 for the treatment of EGFR expressing metastatic colorectal cancer (Table1).²⁸ Nimotuzumab, another humanized antibody, is used clinically for treatment of head and neck squamous cell carcinoma²⁹ and glioblastoma.³⁰

Small Molecule Inhibitors

Small molecule inhibitors typically interrupt cellular processes by interfering with the intracellular signaling of tyrosine kinases. Tyrosine kinase signaling initiates a molecular cascade that can lead to cell growth, proliferation, migration and angiogenesis in normal and malignant tissues.¹

Imatinib, one of the first small molecule inhibitors, is also one of the most effective. Approved in 2002 for the treatment of chronic

solid tumors, such as non-small cell lung cancer.

The most promising small molecule selective EGFR tyrosine kinase inhibitors are currently three series of compounds, which include 4-anilinoquinazolines, 4-[ar(alk)ylamino]pyridopyrimidines and 4-phenylaminopyrrolopyrimidines.

Gefitinib (Iressa) is approved to treat patients with advanced non-small cell lung cancer. This small molecule drug inhibits the tyrosine kinase activity of the EGFR, which is overproduced by many types of cancer cells. Erlotinib (Tarceva) is approved to treat metastatic non-small cell lung cancer and pancreatic cancer that cannot be removed by surgery or has metastasized. This small molecule drug inhibits the tyrosine kinase activity of EGFR. Gefitinib and erlotinib are type I ATP competitive reversible inhibitors and are both based on a 4-anilinoquinazoline backbone structure.³³

Lapatinib (Tykerb) is a dual inhibitor of EGFR and HER2 that has shown activity in patients with HER2-over expressing advanced breast cancer whose disease has progressed after treatment with trastuzumab. Lapatinib is a relatively new drug which was approved by the FDA on 2007. It is an oral anticancer drug which is used to treat solid tumors for lung and breast cancer (Table 2).

APPROACHES TO THE DEVELOPMENT OF EGFR INHIBITORS

The rationale to inhibit the epidermal growth factor receptor (EGFR) tyrosine kinase family as an

approach to cancer chemotherapy has continued to grow stronger over the last 10 years. Both preclinical and clinical data strongly supports the

specific pathways, such as drugs that inhibit angiogenesis by targeting vascular endothelial growth factor receptor (VEGFR). Another new

Table 2. Small Molecule Inhibitors

Drug	Target	FDA-approval
Gefitinib (Iressa)	EGFR	NSCLC
Erlotinib (Tarceva)	EGFR	NSCLC, Pancreatic Cancer
Lapatinib (Tykerb)	HER2/new, EGFR	Breast cancer with HER2/neu over expression

involvement of these receptors in the formation and progression of human cancers, as well as establish a high correlation in cancer patients between receptor/ligand expression and poor prognosis. New structural classes have emerged that exhibit enormous improvements with regard to potency, specificity, and *in vitro* and *in vivo* activity. Very recently further advancements in this field have been made whereby very specific, irreversible inhibitors of the EGFR family have been synthesized that provide unique pharmacological properties and exceptional efficacy.³⁴

Two irreversible inhibitors, BIBW2992 and PF-00299804 which are dual inhibitors of EGFR and HER2 are in pivotal phase 3 trials for non-small cell lung cancer (NSCLC).^{35, 36} A major challenges for the treatment of NSCLC patients some of whom achieve dramatic responses to the reversible EGFR inhibitors erlotinib/gefitinib, is the development of drug resistance. In approximately 50% of patients who relapse there is evidence of resistance of resistance due to mutation at T790M in the kinase binding site.²² Initially, it had been assumed that T790M resistance was due to loss of affinity of the drug to the kinase binding site, however x-ray crystallography studies shows that the drugs can adopt similar binding modes and have relatively modest differences in affinity to the T790M form of the protein and to WT.³⁷ It appears that the basis for resistance in cells is due to an increased affinity for ATP to the T790M mutant form of the enzyme making it more difficult for a reversible drug to compete with the mM levels of intracellular ATP.³⁸

To enhance EGFR targeted therapies, new directions were suggested and undertaken. One new direction was therapy with multiple drugs working in synergy, one drug inhibiting EGFR activation and other drugs targeting other cancer

direction was the development of drugs targeting EGFR along with other PTKs having a role in cancer cell proliferation, such as lapatinib (Tykerb), which also targets erbB2, canertinib (CI-1033), a pan-erbB inhibitor and semaxanib (SU5416) which also targets the VEGFR and KIT. Neratinib (HKI-272) is an irreversible, dual EGFR/HER2 inhibitor, under investigation for the treatment of breast cancer and other solid tumors.³⁹ It is in development for the treatment of early and late-stage HER2-positive breast cancer.⁴⁰ Pelitinib is an irreversible inhibitor, binds covalently to EGFR, erbB2 and erbB4.⁴¹ Afatinib is a candidate drug against non-small cell lung carcinoma, it also undergoing clinical trials for breast, prostate and head and neck cancers, as well as glioma.³⁵ A third new direction was the development of a reliable and accurate *in vivo* quantitative method to determine EGFR levels of expression and their in specific tumors and metastases so as to guide and monitor customized EGFR targeted cancer therapy.⁸

CONCLUSION

In conclusion, EGFR is valid target for therapeutic intervention in patients with cancer. Work conducted over the past two decades has culminated in the clinical development of a substantial number of specific inhibitors of this target. The results from the clinical studies completed thus far are promising and some of these drugs have already been approved for cancer treatment. With all of the advances in this new category of drugs, one can expect many promising results to emerge from the current clinical trials and future developments to come. Future research on molecularly targeted therapies will focus on the identification of new drugs and drug targets, improved selection of tumours sensitive to these drugs, and the rational design and optimization of combination therapies.

With the various combinations of specific-target Tyrosine Kinase inhibitor drugs, several types of cancer can be reduced, retarded, or eliminated through concentrated chemotherapy regimens. With Tyrosine Kinase inhibitors, patients with cancer are given more options and better choices for managing the disease without disrupting their quality of life to the degree that previous treatments have. The new wave of discoveries involving translational research will help transform oncology from its current state of empirically based patient management to one in which treatment decisions are based on mechanistic approaches that successfully integrate molecular biology, pathology, imaging, and clinical medicine.

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