Review Article **Medicinal chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

TARGETING EPIDERMAL GROWTH FACTOR RECEPTOR: AN IMPORTANT STRATEGY IN ONCOLOGY

MAHESH G. KHARATMOL*[1] AND DEEPALI M. JAGDALE[1]

[1]Department of Pharmaceutical Chemistry, Bharati Vidyapeeth's College of Pharmacy, CBD Belapur, Navi Mumbai, Maharashtra, India.

ABSTRACT

Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase included in the Human Epidermal Receptor (HER) family plays an essential role in signal transduction pathways that regulate differentiation and cell division which causes normal organ development by mediating morphogenesis and differentiation through effects on apoptosis, cell proliferation, differentiation, invasion, and angiogenesis. The role of EGFR is to help maintain mucosal integrity, aid in mucosal repair in the gut, and maintain the protective barrier of the skin. Unlike normal cells that have tight regulatory mechanisms controlling EGFR pathways, tumor cells often have dysregulated EGFR signaling, allowing them to proliferate under adverse conditions, invade surrounding tissues, and increase angiogenesis. Epidermal growth factor can be found in saliva, macrophages, urine, plasma and milk. EGFR inhibitors are used to treat pancreatic cancer, non-small-cell lung cancer, colon cancer, breast cancer and some other cancers which show epidermal growth factor receptor up-regulation. Various heterocyclic scaffolds which have been reported as EGFR inhibitors in literature are pyrazoline, isoquinoline, quinoxalline, pyrimidine, indole and purine derivarives. Considering the importance of EGFR in targeted anticancer treatment, in the present article we are emphasizing on various targeted therapies, role of EGFR in cell growth, mechanism of action of EGFR inhibitors, structural requirements, resistance of the EGFR inhibitors based upon the literature survey.

KEYWORDS: Epidermal growth factor receptor (EGFR), HER-1/2, Tyrosine kinase inhibitors, Overexpression of EGFR, Resistance in EGFR, EGFR monoclonal Antibodies.

MAHESH G. KHARATMOL*

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth's College of Pharmacy, CBD Belapur, Navi Mumbai, Maharashtra, India.

Received on : 22-09-2016
Revised and Accepted on : 14-12-2016
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.p174-182

INTRODUCTION

EGFR is Epidermal Growth Factor Receptor. Growth factors are essential for the development, growth and homeostasis of multicellular organisms.[1] Acting through cell surface receptors, growth factors are required for cell-cell communications underlying embryonic tissue induction, fate determination, cell survival, apoptosis, tissue specialization and cell migration. Growth factor receptors transducer extracellular signals through the activation of intracellular messengers or directly through receptor translocation to the nucleus.[2-3] The ErbB receptor tyrosine kinases evolved as key regulatory entities enabling the extracellular environment to communicate with the intracellular machinery to bring forth the appropriate biological response in an ever-changing environment. [4]

EGFR FAMILY

The EGF receptor (ErbB) family consists of four closely related tyrosine kinase transmembrane receptors[5]:

- ErbB-1 (EGFR/HER-1),
- ErbB-2 (HER-2/neu),
- ErbB-3 (HER-3) and
- ErbB-4 (HER-4)

Two out of the four ErbB proteins, namely ErbB-1 and ErbB-4, are self-determining; when bound by a ligand growth factor they undergo dimerization and generate intracellular signals terminating in cell proliferation, migration or differentiation. The other two receptors are non- self-determining: ErbB-2 (HER-2 in human and Neu in rodents) does not depend on ligands for dimerization or activation, but acts as a preferred partner in heterodimeric complexes with other, ligand-bound ErbBs. On the other hand, ErbB-3 cannot generate signals in isolation, because the kinase function of this receptor is impaired. Nevertheless, in the context of a heterodimer, primarily with ErbB-2, ErbB-3 can compute potent intracellular signals.[4]

RECEPTOR STRUCTURE

The epidermal growth factor receptor (EGFR) is a potent tyrosine kinase, which through downstream signaling networks regulates such basic cell functions as proliferation, chemotactic migration, invasion, and avoidance of apoptosis.[6] A simple model by which to understand the complex system of growth factor signalling is based upon perceiving the network as three individual, sequential layers.

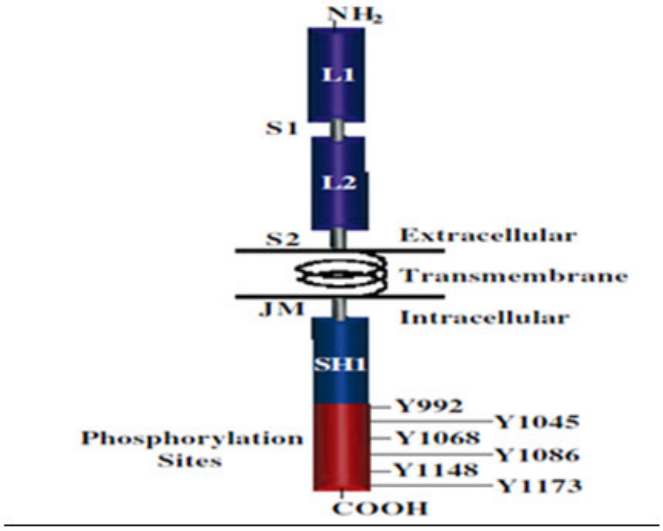

Figure 1
Structure of EGFR[5]

The initial, extracellular layer (Fig 1) is composed of the ligands, and its nature is therefore determined by their concentration and bioavailability. These two parameters dictate whether the receptors that reside within the cell membrane (and comprise the second layer of the system) will dimerise to become active. If the information in the first layer is sufficient to induce receptor dimerisation and consequently increase catalytic activity, the third, intracellular layer of second messenger proteins can bind to specific sites on the receptors and initiate the signals required to induce the appropriate response.[7-8]

DOMAINS

Member of the ErbB family comprises a conserved protein tyrosine kinase domain that resides within the cytoplasm, a transmembrane domain that makes a single pass through the plasma membrane, and a glycosylated, extracellular ligand-binding domain[9] (Fig 1). In the EGF receptor family, this last domain exhibits four subdomains denominated L1, S1 (CR1), L2 and S2 (CR2) (or, more simply, I, II, III and IV respectively).[10] Of these domains, S1 and S2 are homologous, cysteine-rich regions (CR1 and CR2), while L1 and L2 form the ligand-binding site.[11]

DIMERISATION AND PHOSPHORYLATION

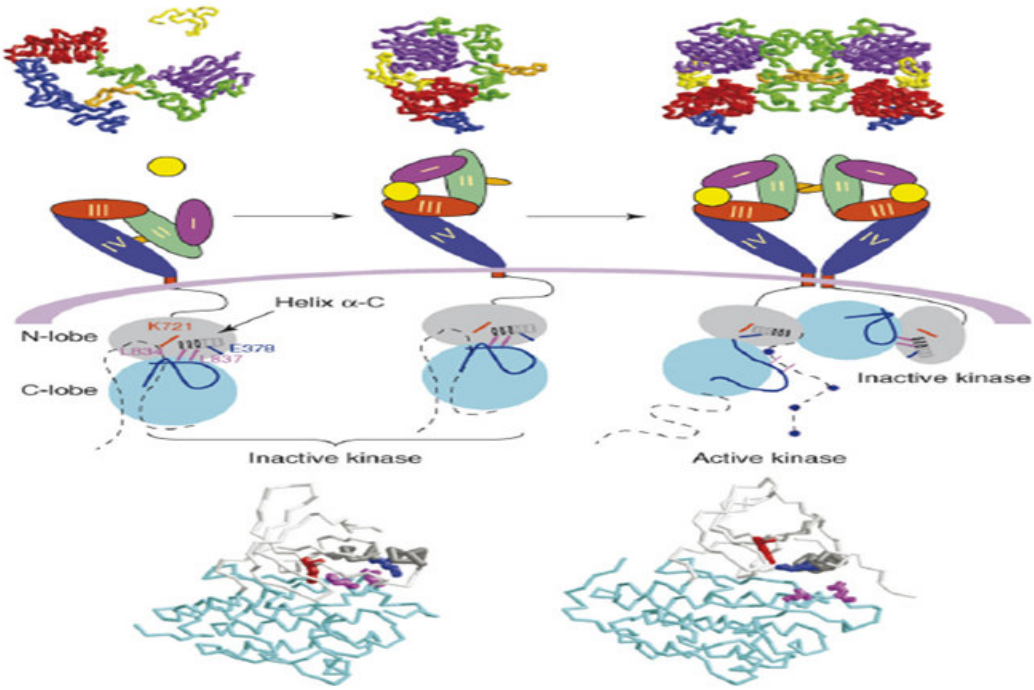

Figure 2
3D structure of ErbB receptor activation and a schematic representation of the extracellular and intracellular domains of ErbB receptors.[4]

Each S1 domain projects an extracellular 'dimerisation loop', and it is thought that the homophilic interactions between the two loops drive dimerisation. This scenario was elucidated by the crystal structure. The cytoplasmic region of the EGFR comprises three distinct domains[12]: The juxt amem brane domain, required for feedback by protein kinase C (PKC); The noncatalytic carboxy-terminal tail, possessing the six tyrosine transphosphorylation sites mandatory for recruitment of adaptor/effector proteins (e.g. Grb2) and phospholipase Cg (PLCg) containing SH2 domains (src homology domain 2), they have amino acid sequences conserved in regions of v-src and c-src[17] or PTB (phosphotyrosine binding) domains, plus the structures necessary for internalisation and degradation of the receptor; The central tyrosine kinase domain (src homology domain 1 (SH1) that is responsible for mediating transphosphorylation of the six carboxy terminal tyrosine residues[13] (Fig 2).Within seconds of ligand binding the kinase is activated and phosphorylates the receptor's carboxyl terminus on tyrosine residues.[14] Autophosphorylation is precise, specifying selected tyrosine residues for phosphorylation; these become docking sites for proteins bearing regions capable of recognizing and binding to phosphotyrosines localized within defined amino acid sequences.[15-16.] Second messenger- downstream signalling Consecutive to transphosphorylation of the receptor dimer, second messenger proteins possessing one of the two main classes of domains that recognise site-specific phosphorylation ('docking sites') can interact with the receptors. The larger of the two groups of second messenger proteins interact with the receptor via an SH2 structure, whereas the others exhibit what is known as a PTB domain. An example of a well-characterised second messenger/ receptor interaction is the recruitment of the enzyme phospholipase C gamma (PLCg).[9] This phosphorylation triggers a complex program of intracellular signals to the cytoplasm and then to the nucleus known as downstream signalling.[10]

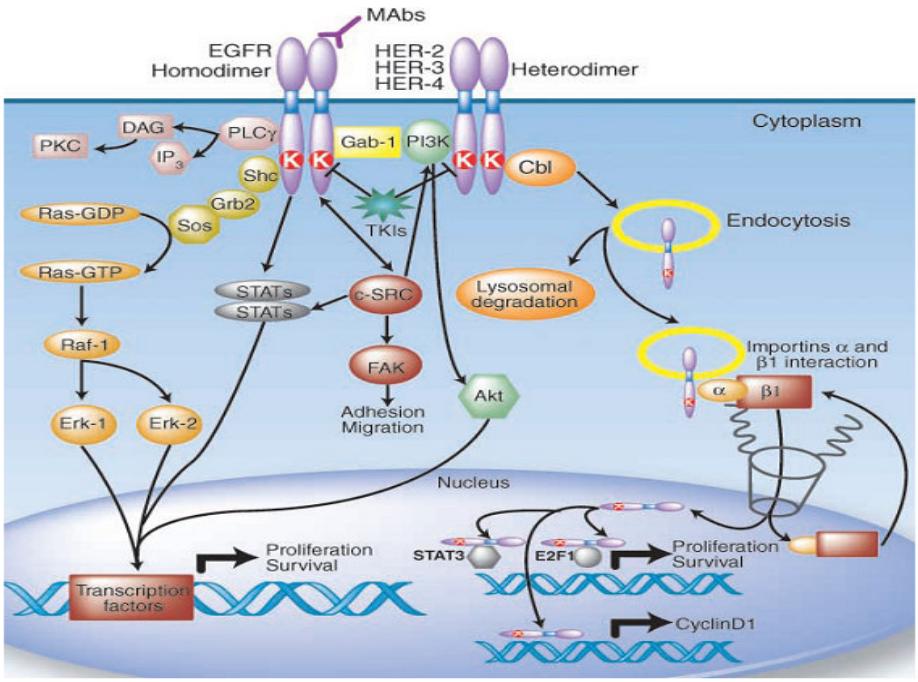

Figure 3

Signaling pathways of EGFR[3]

The two major intracellular pathways activated by EGFR are the RAS-RAF-MEK-MAPK pathway (Fig 3), which controls gene transcription, cell-cycle progression from the G1 phase to the S phase, and cell proliferation, and the PI3K-Akt pathway, which activates a cascade of anti-apoptotic and prosurvival signals. bFGF (basic fibroblast growth factor), HB-EGF (heparin-binding EGF), MAPK (mitogen-activated protein kinase), PI3K (phosphatidylinositol 3,4,5-kinase), TGFalpha (transforming growth factor alpha) and VEGF (vascular endothelial growth factor).[4]

OVEREXPRESSION OF EGF

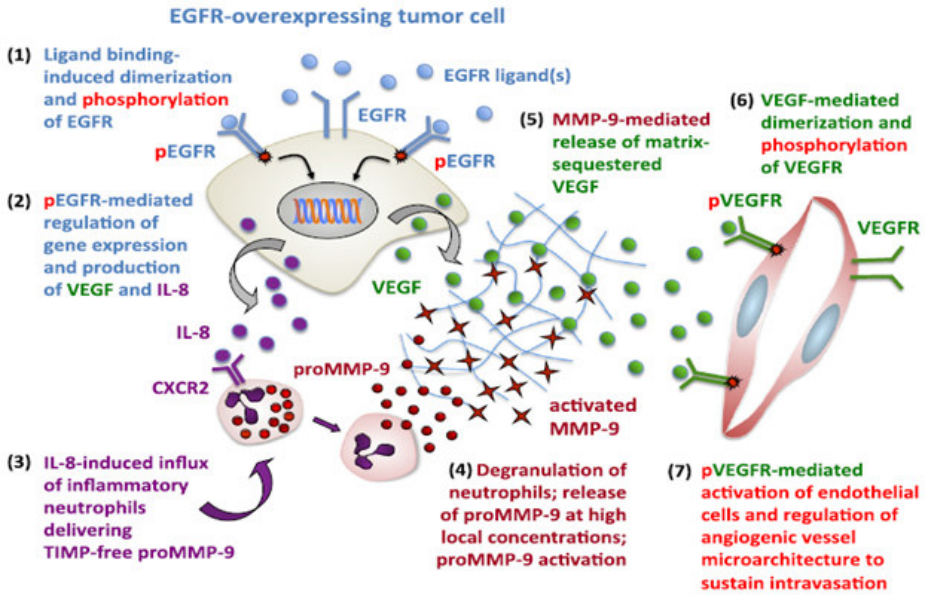

Figure 4

EGFR overexpressing tumor cell[6]

Receptor overexpression, mutations or autocrine stimulation, leads to excessive ErbB signaling, which is a indication of a wide variety of solid tumors.[6]High ErbB-1 expression, for instance, was found in the majority of cancers, and amplification of the ErbB-2 gene can be found in 20–30% of metastatic breast cancer.[4]Besides direct regulation of tumor cell proliferation, migration and invasion, cancer cell–expressed EGFR can also regulate the production of definite molecules involved in the establishment and modification of the tumor micro-environment. Among those molecules are VEGF, the major carcinogenic factor, and interleukin 8 (IL-8), the major attractant for tumor-infiltrating neutrophils (Fig 4), the inflammatory cell type playing a critical early role in tumor angiogenesis and finally gaining the appreciation it deserves in cancer research.[6]

EGFR INHIBITORS

The earliest molecules to reach the clinic were cetuximab, gefitinib, and erlotinib.[18] Although the anti-

EGFR antibody cetuximab did not show significant clinical activity (as a single agent or in combination with chemotherapy), in large clinical trials both gefitinib and erlotinib had single-agent activity, with response rates <10%.[19]Two predominant classes of EGFR inhibitors have been developed including monoclonal antibodies (mAbs) that target the extracellular domain of EGFR, such as cetuximab (Erbitux), and small molecule tyrosine kinase inhibitors (TKIs) that target the intracellular domain of EGFR, such as gefitinib (Iressa) and erlotinib (Tarceva).[20]The mAbs function at the extracellular ligand-binding site of the receptor, whereas the small molecule TKIs functions at the intracellular tyrosine kinase domain of the EGFR. Mechanisms of action of EGFR inhibitors mAbs directed against EGFR are as follows[20-23](Fig 5):

(i) extracellular binding;
(ii) internalization of receptor–antibody complexes;
(iii) inhibition of EGFR signaling pathways; and
(iv) potential stimulation of an immunological response.

TKIs directed against EGFR have the following mechanisms of action:
(i) intracellular binding;
(ii) prevention of tyrosine kinase activation; and
(iii) inhibition of EGFR signaling pathways.

Monoclonal Antibodies (mAb)

Two anti-EGFR monoclonal antibodies, panitumumab and cetuximab, are currently in widespread use in cancer treatment.[24] There are mAb in clinical trials such as EMD 72000 which is Humanised IgG1, MDX-447 which is Humanised bispecific: EGFR/FcRy1, h-R3 which is Humanised and Mab 806 which is Anti-EGFR VIII[20]. Cetuximab (ErbituxTM) is an immunoglobulin (Ig) G1 human–murine chimeric analogue of the murine monoclonal antibody M225. It binds to the EGFR with a 2-log higher affinity compared with the natural ligands TGF-α and EGF.[25] Binding of cetuximab to the EGFR promotes receptor internalization and consecutive degradation without receptor phosphorylation and activation.[26] This results in receptor down-regulation, reducing the availability of EGFR on the cell surface and preventing activation of EGFR-associated, downstream signalling pathways.It binds to the mutant receptor EGFRvIII, inducing internalization of 50% of antibody-receptor complexes after 3 hours, and an 80% reduction in phosphorylated EGFRvIII.Binding of cetuximab to EGFR inhibits the progression of the cell cycle at the G0/G1 boundary, increases expression of the cell cycle regulator p27KIP1 and induces apoptosis by increasing expression of pro-apoptotic proteins (e.g. Bax and caspase-3, caspase-8 and caspase-9)[27] or by inactivation of anti-apoptotic proteins (e.g. Bcl-2) inducing decreased expression or phosphorylation.[28] It has also been reported to inhibit the production of pro-angiogenic factors such as VEGF, IL-8 and the bFGF; inhibition of these factors is associated with a decrease in new blood vessel formation and the development of distant metastases in orthotopic cancer models.[29]Cetuximab is administered i.v. at doses of 200-400 mg/ml and has a mean half-life of 114 hr (range 75-188 hr), thus allowing weekly administration. The kinetics of cetuximab is nonlinear, with complete saturation of systemic clearance, and are unaffected by co-administration with cisplatin.[21, 30]5.1.2. Panitumumab (Vectibix) is a fully human IgG2 targeting the extracellular domains of EGFR monoclonal antibody. It is developed by Abgenix's XenoMouse Technology, which creates antibodies that do not contain murine proteins, it offers effective high affinity therapy (Kd = 5 × 10^{-11} M) with a minimum rate of allergic reactions or anaphylaxis.[24, 31]Well tolerated – its main toxic effect is dermatological – it has never reached grade 4 in clinical trials. Because of its structure (fully human antibody), infusion-related reactions are minimal. Panitumumab could be administered weekly, fortnightly or every 3 weeks.[32]

EGFR Tyrosine Kinase Inhibitors

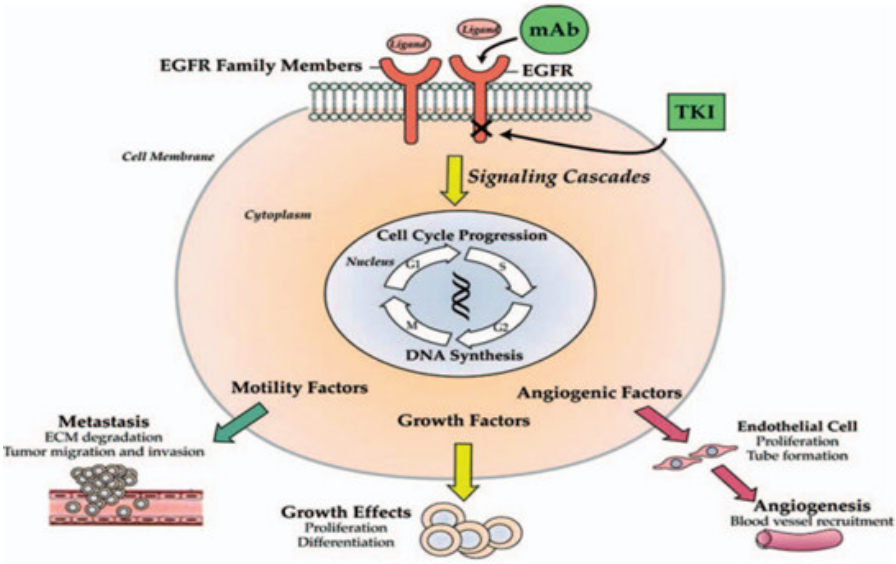

Figure 5
Schematic figure of the EGFR pathway highlighting potential downstream

cellular and tissue effects of EGFR inhibition.[20]

Tyrosine Kinase Inhibitors are classified as follows[18]:

1) First generation reversible EGFR-TKIs: Gefitinib and Erlotinib
2) Second generation irreversible EGFR-TKIs: Afatinib
3) Third generation mutant-specific and irreversible EGFR-TKIs: Rociletinib

There are TKIs which are under clinical trials such as ZD1839, OSI-774 which is type of ErbB-1, EKB-569, EKB-569, PKI-166 which is type of ErbB-1/2 and CI-1033 which is type of Pan ErbB.[20]

First generation reversible EGFR-TKIs: Gefitinib & Erlotinib

First-generation EGFR-TKIs reversibly compete with the ATP binding sites at the tyrosine kinase domain of EGFR. These results in EGFR deactivation, and subsequently deactivation of the downstream signaling networks.[33]The first available EGFR-TKIs were gefitinib and erlotinib, two orally active inhibitors. [34-36]5.2.1.1. Gefitinib have been clinically validated as the most effective first-line treatment in EGFR mutation-positive NSCLC (Non-Small Cell Lung Cancer).[10]Gefitinib, the recommended dose is 250 mg daily. Dosage can be increased to 500 mg daily with belonging CYP3A4 inducer use, in the absence of severe adverse drug reaction.[34]In terms of toxicity profile, most of the reported toxicities related to first-generation EGFR-TKIs were mild (Grade 1 or 2). Diarrhea and rash were more frequently seen with the gefitinib, whereas hematologic abnormalities (cytopenias) were mainly seen in the chemotherapy group. On the other hand, liver dysfunction and nail changes were more frequent with gefitinib.[38]5.2.1.2. Erlotinib is also first-line treatment in locally advanced or metastatic NSCLC.[35] However, these agents may be deleterious in patients with EGFR wild-type tumors.[37] The recommended dose for erlotinib is 150 mg daily, which should be continued unless there is disease progression or unacceptable toxicity. The dose should be increased by 50 mg increments until 300 mg daily for patients who are actively smoking. Since first-generation EGFR-TKIs are metabolized mostly in the liver, via cytochrome (CYP) P450, CYP3A4, special dosage consideration must be paid to CYP3A4 inducers and inhibitors. Hence US FDA recommends increasing the erlotinib dose by 50 mg with the concomitant use of CYP3A4 inducers. Oppositely, the use of CYP3A4 inhibitors requires a decrease in the dose by 50 mg as tolerated to a minimum of 25 mg daily.[10] On one hand, treatment with erlotinib was mainly complicated by rash, fatigue, stomatitis, and anorexia. Most adverse effects associated with first generation EGFR-TKIs are treated with supportive care. However, erlotinib, at its maximum tolerated and approved dose of 150 mg daily, results in a plasma trough concentration that is almost 3.5 times that for gefitinib, administered at its approved dose of 250 mg once daily.[39]In brief, erlotinib is an effective and well-tolerated first-line treatment option for advanced disease in mutation selected elderly patients. It is also a reasonable option for the second or consecutive line of treatment in this patients.[40]

Second generation irreversible EGFR-TKIs: Afatinib

Comparatively, 10–30% of NSCLC develop resistance to first generation EGFR-TKIs, and these patients experience disease progression after 10–13 months of therapy[41]. Acquired resistance to EGFR-TKIs occurs because of either a secondary EGFR mutation (EGFR T790M missense mutation in exon 20 being the most common), which allows continued EGFR signaling even with the effect of EGFR-TKIs, or amplification of the oncogene mesenchymal-epithelial transition factor.[42-44] This resistance to first generation EGFR-TKIs led to the development of the second generation EGFR-TKIs, including afatinib and dacomitinib. These novel medications are irreversible pan HER inhibitors. The combined targeting of HER-1 (EGFR), HER-2 and HER-4 is essential in conquering drug resistance to first-generation EGFR-TKIs.[45] To date; dacomitinib remains an investigational compound and has not received regulatory approval in any country. Currently, afatinib is approved as a first-line option for the treatment of metastatic NSCLC with EGFR exon 19 deletions or exon 21 (L858R) substitution mutations. It is also recommended for subsequent therapy in patients who progressed after first-line chemotherapy.[35] The FDA-approved daily dose of afatinib is 40 mg, continued until disease progression or drug intolerability, at which time afatinib can be resumed at a lower dose. Being an irreversible inhibitor of all ErbB family receptors, afatinib exhibits more serious toxicities compared to its reversible counterparts. In fact, the role of EGFR is to help maintain mucosal integrity, aid in mucosal repair in the gut, and maintain the protective barrier of the skin. Therefore, afatinib related toxicities are mainly gastrointestinal and dermatological.[46] Diarrhea is the most common adverse effect.[47]

Third generation mutant-specific and irreversible EGFR-TKI: Rociletinib

Rociletinib, a third generation EGFR-TKIs, showed promising results in phase I and II trial, in treatment of advanced EGFR mutated NSCLC with acquired resistance to first and second generation EGFR-TKIs.[48] This new drug selectively inhibits tumors that harbor the acquired T790 mutation. The trial showed a mild treatment related adverse effect, the most being hyperglycemia in 22% of patients. Therefore, the role of this novel generation of EGFR-TKIs in the elderly is currently difficult to predict.

RESISTANCE

Primary resistant mutation T790M

The EGFR gene, located in the 7p12-14 region in the short arm of chromosome7, consists of 28 exons. The tyrosine kinase function is encoded by exons 18-24. The rate of mutation in exon 19 is the highest, accounting for more than 60% of overall mutations.[44]A primary mutation of the EGFR gene consulted acquired resistance to EGFR-TKIs.[49] This mutation (located in exon 20) results in the substitution of methionine for threonine at position 790 (T790M) in the kinase domain. Threonine 790 has been nominated as a “gatekeeper” residue, important for regulating inhibitor specificity in the ATP binding pocket. The T790M mutation enhances affinity of the ATP binding pocket for ATP, thus successfully competing with the TKIs, thereby consulting resistance.[50] Currently, two theories can explain the production of these mutations: subcloning and induced

mutation/acquisition.[51]The T790M mutation can coexist with other mutations, like L858R and D761Y. The T790M mutation also possesses enhanced phosphorylation activity, especially in combination with the L858R mutation. The combination leads to lung cancer cell survival, indicating that the T790M mutant is actually an oncogene.[52]

Secondary resistance mutations: L747S, D761Y and T854A

The secondary mutations mainly include D761Y, L747S and T854A.[53-55] They reduce the sensitivity of mutant EGFR to EGFR-TKIs, but the resistance mechanism remains unknown. A possible explanation may be that these secondary resistance mutations modify the conformation.of EGFR and the combination between EGFR and TKIs.[56]

Table 1
EGFR mutation pathways[56]

Mutation	Activation of alternate pathways	Other pathways
EGFR T790M	HER2 amplification	Activation of PD-1
EGFR D761Y	MET amplification	Epithelial-mesenchymal Transition
EGFR T854A	mTORC1	Small cell transformation
BRAF V600E	AXL	
NRAS mutation	IGFIR	
	FGFR activation	

CONCLUSION AND FUTURE PERSPECTIVES

Epidermal Growth Factor Receptors are considered promising targets for cancer therapy due to their strong involvement in development, growth and homeostasis of multicellular organisms, cell survival, apoptosis and cell migration. Preclinical studies analyzing EGFR inhibition in tumor brought positive results rising the idea that the development of strategies to inhibit EGFR may be powerful therapy to help defy cancer.In a large number of tumours, EGFR are mutated or overexpressed. The EGFR gene is often amplified and deletion mutations found in cancer cells have been shown to have constitutive active tyrosine kinase. This suggests that the EGFR plays an important role in the development of the malignant phenotype of many cancers.The uses of anti-EGFR monoclonal antibodies are diffuse in cancer therapy; however, clinical responses have been observed in only 15% of patients treated.On the basis of reported trials and current debate, complex efforts are needed that take into consideration not only the mechanistic effect of blocking EGFR, but also the further exploiting of mAb effects on the immune system.Molecular inhibitors of EGFR signaling represent a highly promising class of molecular targeted anticancer agents.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Wieduwilt M, Moasser M. The epidermal growth factor receptor family: Biology driving targeted therapeutics. Cell Molecular Life Science. 2008: 65(10): 1566 – 1584.
2. Linggi B, Carpenter G. ErbB receptors: New insights on mechanisms and biology. Trends Cell Biol. 2006:16: 649 – 656.
3. Scaltriti M, Baselga J. The epidermal growth factor receptor pathway: A model for targeted therapy. Clinical Cancer Research. 2006:12: 5268 – 5272.
4. Erez M, Bublil, Yosef Yarden. The EGF receptor family: spearheading a merger of signaling and therapeutics. Current Opinion in Cell Biology. 2007:19: 124 – 134.
5. Henson E, Johnston J, Los M, Gibson S. Clinical activities of the epidermal growth factor receptor family inhibitors in breast cancer. Biologics: Targets & Therapy. 2007:1(3): 229 – 239.
6. Minder P, Zajac E, Quigley J, Deryugina. EGFR regulates the development and microarchitecture of intratumoral angiongenic vasculature capable of sustaining cancer cell intravasation. Neoplasia. 2015:18(8): 634 – 649.
7. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008:358: 1160 – 1174.
8. Wheeler DL, Dunn EF, Harari PM. Understanding resistance to EGFR inhibitors-impact on future treatment strategies. Nat Rev Clin Oncol. 2010:7: 493 – 507.
9. Bazley LA, Gullick WJ. The epidermal growth factor receptor family. Endocrine-Related Cancer. 2005:12: S17 – S27.
10. Pawson T. Protein modules and signaling networks. Nature. 1995:373: 573 – 580.
11. Stein RA, Staros JV. Evolutionary analysis of the ErbB receptor and ligand families. Journal of Molecular Evolution. 2000:50: 397 – 412.
12. Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Zhu H-J, Walker F, Frenkel MJ, Hoyne PA, Jorissen RN, Nice EC, Burgess AW, Ward CW. Crystal structure of a truncated epidermal growth factor receptor

extracellular domain bound to transforming growth factor alpha. Cell. 2002:110: 763–773.
13. Shelton HE, Dawson T, Xiong Li, Hong Yu. Heterodimerization and functional interaction between EGF receptor family members: A new signaling paradigm with implications for breast cancer research. Breast Cancer Research and Treatment. *1995:*35: 115–132.
14. Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S. Oncogenes and signal transduction. Cell 1991:64: 281 – 302.
15. Crews CM, Erikson RL. Extracellular signals and reversible protein phosphorylation: What to make of it all. Cell. 1993:74: 215 – 217.
16. Birge RB, Hanafusa J. Closing in on SH2 specificity, Science. 1993:262: 1522–1524.
17. Fry MJ, Panayotou G, Booker GW, Waterfield MD. New insights into protein-tyrosine kinase receptor signaling complexes. Protein Science. 1993:2: 1785 – 1797.
18. Sai-Hong Ignatius Ou. Second-generation irreversible epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs): A better mousetrap?. Critical Reviews in Oncology/Hematology. 2012:83: 407 – 421.
19. Thatcher N, Chang A, Parikh P. Gefitinib plus best supportive care in previously treated patients with refractory advanced nonsmall- cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet. 2005:166; 1527 – 1537.
20. Harari P. Epidermal growth factor receptor inhibition strategies in oncology. Endocrine-Related Cancer. 2004:11: 689 – 708.
21. Harari PM, Huang S-M, Herbst R, Quon H. Molecular targeting of the epidermal growth factor receptor in head and neck cancer. In Head and Neck Cancer: a Multidisciplinary Approach. 2003: 1001 – 1016.
22. Earp HS III, Calvo BF, Sartor CI. The EGF receptor family – multiple roles in proliferation, differentiation, and neoplasia with an emphasis on HER4. Transactions of the American Clinical and Climatological Association. 2003:114: 315 – 333.
23. Arteaga CL, Baselga J. Clinical trial design and end points for epidermal growth factor receptor-targeted therapies: implications for drug development and practice. Clinical Cancer Research. 2003:9: 1579 – 1589.
24. Martinelli E, De Palma R, Orditura M, De Vita F, Ciardiello F. Anti-epidermal growth factor receptor monoclonal antibodies in cancer therapy. Clinical and Experimental Immunology The journal of Translational immunology. 2009:158: 1 – 9.
25. Kim ES, Khuri FR, Herbst RS. Epidermal growth factor receptor biology. Curr Opin Oncol. 2001:13: 506–13.
26. Hadari YR, Doody JF, Wang Y. The IgG1 monoclonal antibody cetuximab induces degradation of the epidermal growth factor receptor. J Clin Oncol. 2004:22: 234.
27. Mayo LD, Donner DB. A phosphatidylinositol 3-kinase/Akt pathway promotes translocation of Mdm2 from the cytoplasm to the nucleus. Proc Natl Acad Sci USA. 2001:98: 1598 – 603.
28. Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N. A repressor of mRNA translation is phosphorylated and inactivated by the Akt (PKB) signaling pathway. Genes Dev. 1998:12: 502 – 13.
29. Perrotte P, Matsumoto T, Inoue K. Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. Clin Cancer Res. 1999:5: 257 – 65.
30. Baselga J. The EGFR as a target for anticancer therapy- focus on cetuximab, European Journal of Cancer. 2001:37(Suppl 4): S16 – S22.
31. Mendez MJ, Green LL, Corvalan JR. Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice. Nat Genet. 1997:15: 146 – 56.
32. Mitchell EP, Rose L, Ramirez M, Jacobs M. Dermatological toxicities of panitumumab in the treatment of patients with metastatic colorectal cancer from two clinical studies. Annual Meeting Proceedings. Gastrointest Cancers Symp. 2007: 449.
33. Zaarour M, Weerasinghe C, Nazha B, Hassan S, Atallah J. Epidermal growth factor receptor tyrosine kinase inhibitors in elderly patients with non-small cell lung cancer. Expert Review of Anticancer Therapy, 2015: 1 – 9.
34. Gao G, Ren S, Li A. Epidermal growth factor receptor-tyrosine kinase inhibitor therapy is effective as first-line treatment of advanced non-small-cell lung cancer with mutated EGFR: A meta-analysis from six phase III randomized controlled trials. Int J Cancer. 2012: 31(5): 822 – 9.
35. Ettinger DS, Wood DE, Akerley W. Non-small cell lung cancer. J Natl Compr Canc Netw. 2015:13(5): 515 – 24.
36. Remon J, Mora´n T, Reguart N. Beyond EGFR TKI in EGFR-mutant nonsmall cell lung cancer patients: main challenges still to be overcome. Cancer Treat Rev. 2014:13(5): 723 – 9.
37. Mok TS, Wu YL, Thongprasert S. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009:361(10): 947 – 57.
38. Yoshida T, Yamada K, Azuma K. Comparison of adverse events and efficacy between gefitinib and erlotinib in patients with non-small-cell lung cancer: a retrospective analysis. Med Oncol. 2013: 30(1): 349.
39. Li J, Karlsson MO, Brahmer J. CYP3A phenotyping approach to predict systemic exposure to EGFR tyrosine kinase inhibitors. J Natl Cancer Inst. 2006:98: 1714 – 23
40. Yap TA, Popat S. Toward precision medicine with next-generation EGFR inhibitors in non-small-cell lung cancer. Pharmgenomics Pers Med. 2014:7: 285 – 95.
41. Oxnard GR, Arcila ME, Chmielecki J. New strategies in overcoming acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer. Clin Cancer Res. 2011:17(17): 5530 – 7.
42. Kobayashi S, Boggon TJ, Dayaram T. EGFR mutation and resistance of non-small-cell lung

cancer to gefitinib. N Engl J Med. 2005:352(8): 786 – 92.
43. Bean J, Brennan C, Shih JY. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Ac ad Sci USA. 2007:104(52): 209 – 327.
44. Yu HA, Arcila ME, Rekhtman N. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. Clin Cancer Res. 2013:104(52): 2240 – 7.
45. Kosaka T, Yamaki E, Mogi A, Kuwano H. Mechanisms of resistance to EGFR TKIs and development of a new generation of drugs in non-small-cell lung cancer. J Biomed Biotechnol. 2011: 165 – 214
46. Salomon DS, Brandt R, Ciardiello F, Normanno N. Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol. 1995:19(3): 183 – 232.
47. Miller VA, Hirsh V, Cadranel J. Afatinib versus placebo for patients with advanced, metastatic non-small-cell lung cancer after failure of erlotinib, gefitinib, or both, and one or two lines of chemotherapy (LUX-Lung 1): a phase 2b/3 randomised trial. Lancet Oncol. 2012:13(5): 528 – 38.
48. Sequist LV, Soria JC, Goldman JW, Rociletinib in EGFR-mutated non-small-cell lung cancer, N Engl J Med, 2015;372(18):1700 – 9.
49. Kobayashi S, Boggon TJ, Dayaram T, Jänne PA, Kocher O, Meyerson M. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005:352: 786 – 92.
50. Yun CH, Mengwasser KE, Toms AV, Woo MS, Greulich H, Wong KK. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A. 2008:105: 2070 – 5.
51. Ma C, Wei S, Song Y. T790M and acquired resistance of EGFR TKI: a literature review of clinical reports. J Thorac Dis. 2011:3: 10 – 8.
52. Suda K, Onozato R, Yatabe Y, Mitsudomi T. EGFR T790M mutation: a double role in lung cancer cell survival?. J Thorac Oncol. 2009:4: 1 – 4.
53. Costa DB, Halmos B, Kumar A, Schumer ST, Huberman MS, Boggon TJ. BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. PLoS Med. 2007:4: 1669 – 79.
54. Toyooka S, Date H, Uchida A, Kiura K, Takata M. The epidermal growth factor receptor D761Y mutation and effect of tyrosine kinase inhibitor. Clin Cancer Res. 2007:13: 3431.
55. Bean J, Riely GJ, Balak M, Marks JL, Ladanyi M, Miller VA. Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A mutation in a patient with EGFR-mutant lung adenocarcinoma. Clin Cancer Res. 2008:14: 7519 – 25.
56. Woo HS, Ahn HK, Lee HY, Park I, Kim YS, Hong J. Epidermal growth factor receptor (EGFR) exon 20 mutations in non-small-cell lung cancer and resistance to EGFR-tyrosine kinase inhibitors. Invest New Drugs. 2014:32: 1311 – 5.