ejpmr, 2017,4(10), 174-183

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

EGFR INHIBITORS: ROLE IN CANCER THERAPY

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Article Received on 21/07/2017 **Article Revised on 12/08/2017** **Article Accepted on 04/09/2017**

ABSTRACT

Among various strategies for cancer therapy, molecular targeting has been reported to have better potential to provide tumor specificity. One particular molecular target of high promise in oncology is the Epidermal Growth Factor Receptor (EGFR). EGFR is overexpressed, dysregulated or mutated in many epithelial malignancies, responsible for tumor growth and progress. EGFR belongs to ErbB family of Receptor Tyrosine Kinases (RTK). These are trans-membrane proteins which are activated by binding with peptide growth factors of the Epidermal Growth Factor family of proteins. The growth and survival of carcinoma cells appear to be sustained by a network of receptors/ligands of ErbB family. It has been found that in cancer patients, EGFR gene amplification and EGFR tyrosine kinase domain mutation occur. This phenomenon is also important for therapeutic approaches, since the response to anti-EGFR agents might depend on the total level of expression of ErbB receptors and ligands in tumor cells. A number of agents targeting EGFR include specific antibodies directed against its ligand-binding domain and small molecules inhibiting tyrosine kinase activity are either in clinical trials or are already approved for clinical treatment. This article deals with the role of various approved EGFR inhibitors in the treatment of different type of cancer.

KEYWORDS: Epidermal growth factor receptors**,** Receptor Tyrosin kinases, Colorectal cancer, Head and neck squamous cell carcinoma.

INTRODUCTION

Cancer is the collection of related diseases in which uncontrolled cell growth and division occurs.[1] There are over 100 different types of cancer, and each is classified by the type of cell that is initially affected. In all types of cancer, certain body's cells begin to divide without stopping and spread into surrounding tissues where they grow, invade and destroy other healthy tissues. This process is called metastasis, is a serious condition often very difficult to treat.[2] They form lumps of tissue called tumours which grow, multiply and interfere with the body's systems (digestive, nervous and circulatory) where they release hormones which alter normal functions. Majority of the cancers solid except leukaemia.[1]

According to the American Cancer Society, cancer is the second most common cause of deaths in the US and accounts for nearly 1 of every 4 deaths.[3] In 2017, there will be an estimated 1,688,780 new cancer cases diagnosed and 600,920 cancer deaths in the US.[4,5] Between 30–50% of cancers can be prevented by avoiding risk factors and implementing existing evidence-based prevention strategies. Many cancers have a high chance of cure if diagnosed early and treated adequately.[6]

HISTORY OF EPIDERMAL GROWTH FACTOR RECEPTORS

Stanley Cohen, American biochemist and zoologist, and Rita Levi-Montalcini shared the 1986 Nobel Prize for medicine or physiology for their work on growth factors, discoveries of fundamental importance for understanding the mechanisms regulating cell and organ growth. EGF was discovered by Cohen. He purified, sequenced EGF and studied its mechanism of binding to receptors on the surfaces of cells. He described the hormonal regulation in growth and proliferation of cancer cells.[7]

STRUCTURE OF EGFR AND LIGANDS

EGFR (EGFR; ErbB-1; HER1 in humans) is the cell surface receptor for members of the EGF-family of extracellular protein ligands.[8] EGFR is a member of the ErbB family of receptors which is a subfamily of four closely related RTK: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). Mutations affecting EGFR expression or activity could result in cancer.[9] The ErbB family of receptors and their ligands ErbB1 (or EGFR), and ErbB4 are fully functional

receptors which possess an extracellular ligand-binding domain and a cytoplasmic protein tyrosine kinase domain and can function as homo or heterodimers. In contrast, ErbB2 (or neu), which lacks a ligand-binding domain, and ErbB3, which is defective in its intrinsic tyrosine kinase activity as shown in FIG.1, must heterodimerize with another member of the ErbB family for signal transduction.[10]

ErbB FAMILY AND ITS RECEPTORS

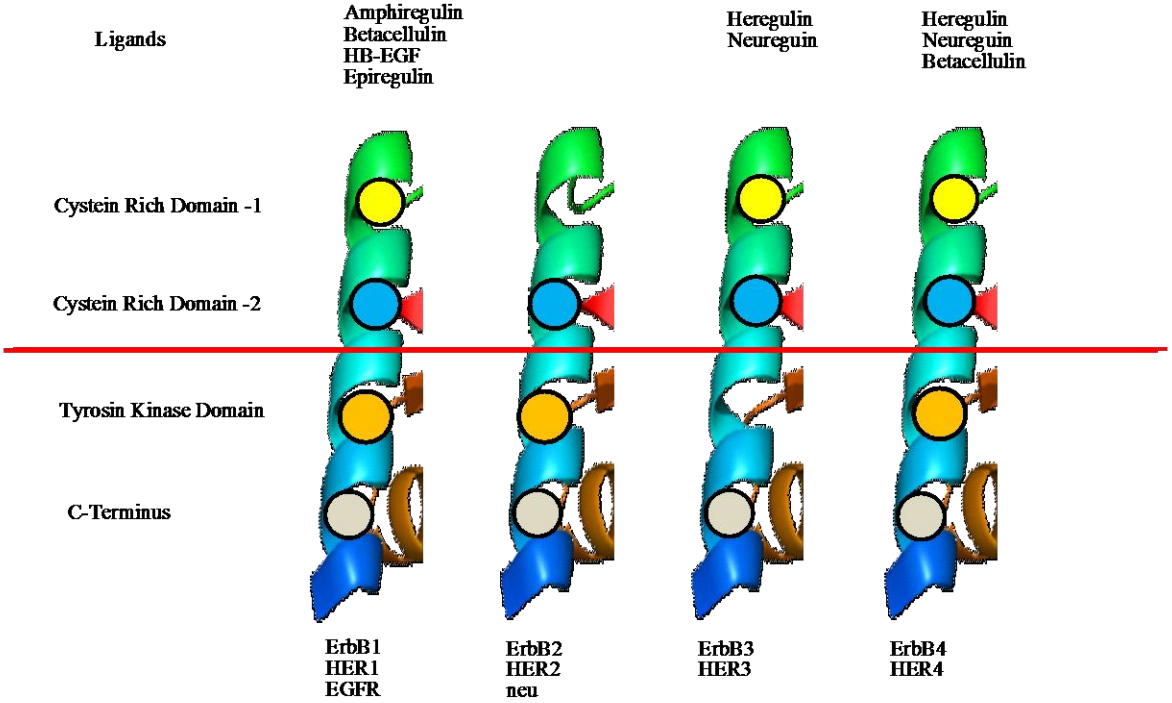


FIG.1: Schematic diagram of the four ErbB family members and their ligands.

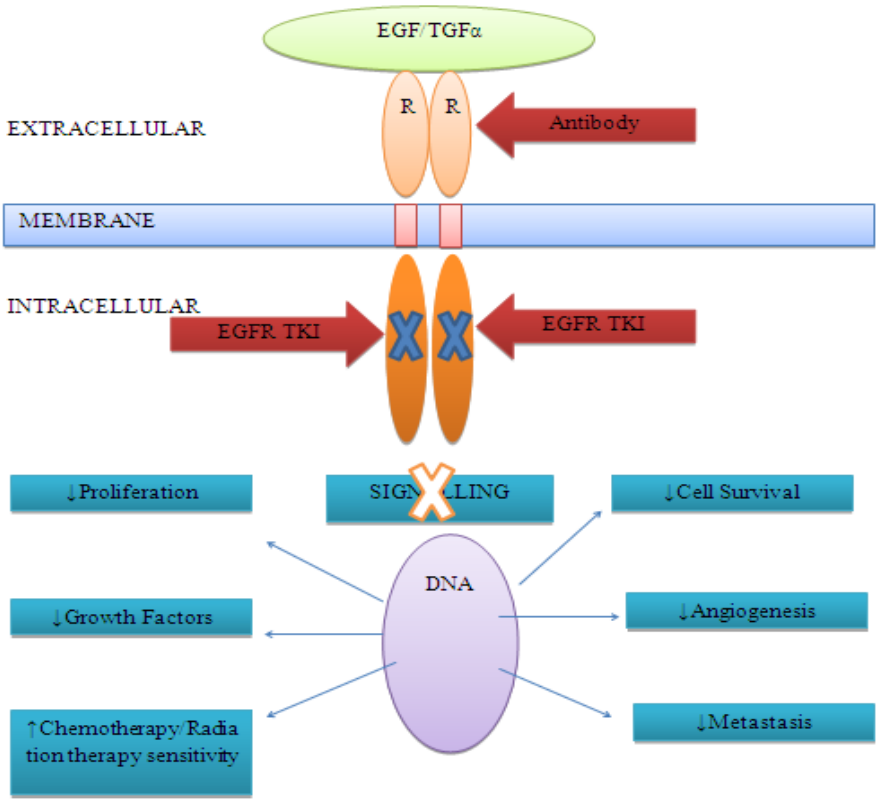


Fig. 2: Mechanism of action of EGFR inhibitors.

Owing to prominent importance of EGFR signaling in cancer development, both anti-EGFR monoclonal antibodies and small-molecule EGFR tyrosine kinase inhibitors have been developed. Their mechanism of action is shown in FIG 2. Anti-EGFR antibodies, e.g. Cetuximab and Panitumumab, bind to the extracellular domain of EGFR monomer and compete for receptor binding by the endogenous ligands; resulting in blocking of ligand-induced receptor activation. The small molecule EGFR inhibitors, such as Erlotinib, Gefitinib and Lapatinib compete with ATP to bind to the catalytic domain of kinase which in turn inhibits EGFR auto phosphorylation and downstream signaling. However, these inhibitors are known to be effective in only a small subset of patients. Mutations in the EGFR gene and possible down-stream effectors have been shown to be associated with various clinical outcomes associated with EGFR inhibitor treatments.[11]

EGFR INHIBITORS AS ANTICANCER THERAPY

EGFR inhibitors are used in the treatment of metastatic non-small-cell lung cancer, colorectal cancer, squamous-cell carcinoma of the head and neck, and pancreatic cancer in which family of RTK has been found to be deregulated which leads to overexpression and amplification of EGFR which results in appropriate cellular stimulation.[12]

Head and neck squamous cell carcinoma

Head and neck squamous cell carcinoma involving squamous cell carcinomas of the lip, oral cavity, pharynx and larynx, is the sixth most prevalent cancer worldwide.[13] In 2015, about 45,000 new cases were diagnosed and 9000 patients died due to HNSCC in the United States.[14] The 5-year relative survival rate of HNSCC is about 66%.[14] Currently, six agents have received Food and Drug Administration (FDA) approval for the treatment of HNSCC. These include Cisplatin, 5-Fluorouracil, Docetaxel, Methotrexate, Bleomycin and Cetuximab.[15] However, an important disadvantage of cytotoxic agents is their lack of selectivity in targeting cells. Till now, Cetuximab remains the only targeted agent for the treatment of HNSCC. In patients suffering from HNSCC, GFs/RTKs are highly expressed which contributes to EGFR inhibition resistance in syngeneic mouse model of human EGFR-expressing TUBO cancer cells on a BALB/c background model system, and may be a common mechanism of constitutive or acquired resistance to EGFR inhibition in HNSCC.[16] EGFR can modulate autophagy by regulating Beclin-1, a key component involved in the initiation of autophagy[17,18] J. Cai et al., compared various EGFR tyrosine kinase inhibitors which differentially affect autophagy in HNSSC and compared the mechanism of action of first and second generation TKIs on lysosomal homeostasis. According to his study, Sunitinib (first generation EGFR TKI) is a multi-targeted RTK inhibitor is weak base (pKa 8.95), which accumulates in lysosomes and neutralizes the acidic pH of lysosome and affects autophagy by disturbing lysosome function.[19] However, Dacomitinib or Afatinib (second generation EGFR TKIs) did not disturb the acidic environment of lysosome rather than they affect lysosomes homeostasis. He also studied that the combination of Flavopiridol (a pan-cyclin-dependent kinase (CDK) inhibitor) and the second-generation EGFR TKI Dacomitinib has synergistic effect to suppress the growth of HNSCC cells.[20] This suggests a novel strategy to improve the efficiency of anti-EGFR therapies.

Colorectal cancer

It is currently the third most common cancer in both men and women. Improvement in screening and prevention decreases the Incidence and mortality rates of CRC. In the United States in 2017, there are projected to be 135,430 individuals newly diagnosed with CRC and 50,260 deaths from the disease.[21]

Approval of new drugs, including Irinotecan, Oxaliplatin, Capecitabine, several humanized monoclonal antibodies that target either vascular endothelial growth factor like Bevacizumab, Aflibercept, and Ramucirumab or the epidermal growth factor receptor like Cetuximab and Panitumumab and most recently, Regorafenib and Trifluridine/ Tipiracil (TAS-102),[22-27] leads to the increased survival of patients with metastatic colorectal cancer in recent decades. Clinical benefit from these drugs is now well established for patients with mCRC, with median overall survival of over 30 months.[28-30] The only established biomarker for the treatment of mCRC patients is tumor RAS mutational status, which is a negative predictive marker for anti-EGFR therapy.[31] Currently, BRAF mutational testing is also recommended by the National Comprehensive Cancer Network (NCCN)[32] and the European Society for Medical Oncology (ESMO).[33] Cetuximab and Panitumumab are both monoclonal antibodies used to treat this. Cetuximab being IgGI antibody exerts additional antitumor effects by mediating antibody-dependent cellular cytotoxicity.[34] More recent studies have found that resistance to anti-EGFR therapy can also be mediated by lower-frequency mutations in KRAS exon 3 or 4, or in NRAS exon 2, 3 or 4.[25,35] If patients with any RAS mutation were excluded then most of the patient with mCRC are more likely to benefit from anti-EGFR therapies.[36] Outcomes for metastatic colorectal cancer (mCRC) patients have been improved by treatment with anti-EGFR antibodies, particularly when combined with predictive biomarkers to select patients lacking RAS mutations. Potential mechanisms of resistance to anti-EGFR therapies act through acquired mutations of KRAS and the EGFR ectodomain is revealed by new technologies such as liquid biopsy and next-generation sequencing. Mutations in molecular effectors that participate in downstream EGFR signaling are also negative predictors for anti-EGFR therapy. Cetuximab has clinically significant activity when given alone or in combination with Irinotecan in patients with Irinotecan-refractory colorectal cancer.[37]

Non-small-cell lung cancer

Lung cancer is the leading cause of deaths in both men and women not only in the United States but also throughout the world. In 2016, this disease caused approximately 158,000 deaths in the United States, more than colorectal, breast, and prostate cancers combined.[38] Lung cancers are of two type: small cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC).

NSCLC is a type of epithelial lung cancer which accounts for about 85% of all lung cancers.[39] It usually grows and spreads more slowly than SCLC. NSCLCs are relatively insensitive to chemotherapy and are primarily treated by surgical resection with curative intent, although chemotherapy is increasingly being used both pre-operatively (neoadjuvant chemotherapy) and post-operatively (adjuvant chemotherapy).

The mutation of EGFR TKs receptor can lead to over expression of the tyrosine kinase domain in the cell membrane which results in unregulated cell growth and proliferation.[40,41] TKIs such as Erlotinib can achieve an average tumor control in 11 months;[42] in EGFR mutated non-small-cell lung cancers[43,44] however, resistance mechanisms such as the T790M mutation within exon 20 can develop with loss of tumor control.[44,45] Osimertinib is a third generation EGFR TKI that exhibits activity towards the T790M mutation.[46]

The most common EGFR oncogenic mutations are either an amino acid substitution at position 858 from leucine (L) to arginine (R; L858R) or in-frame deletion within exon 19 (Ex19del).[47] Together, these account for about 90% of all EGFR mutations. It is well established that lung cancers bearing these common mutations are highly responsive to first-generation EGFR inhibitors Gefitinib and Erlotinib with objective response rates of approximately 70%. Erlotinib was found to be more effective in NSCLC patients who had undergone four rounds of Platinum chemotherapies. Hence, trials with 150 mg/Kg Erlotinib in lung cancer patients, already exposed to Platinum-based chemotherapy, revealed increased progression-free survival and overall survivals at a rate much higher than Erlotinib alone.[39] Generally, as a second or third-line treatment, Erlotinib had minimum side effects, limited to dysentery and minor skin irritations. Erlotinib is also remarkably effective as a second as well as third-line treatment in combination with Docetaxel and Pemetrexed chemotherapeutics. In fact, Erlotinib emerged as the most efficient third-line therapeutic choice for patients with deteriorated performance status where chances of survival and quality of lives had prominently worsened.[48] As a third-line treatment, it not only improved the quality of lives but also the palliative symptoms of lung cancer,[49] and the drug was well tolerated as well.[50] The main advantage for Erlotinib co-treatment was its easier affordability compared to chemotherapeutics.[51]

Gefitinib treatment is used in NSCLC patients who had already got exposure to two regimens of chemotherapy. This drug showed improved response rates and prevented metastasis even where Platinum-based and Docetaxel chemotherapies had failed. However, patients with higher expression of EGFR gene and with a history of smoking responded more positively to Gefitinib.[52] Patients, who had acquired a secondary missense mutation at exon 10 of EGFR-tyrosine kinase, termed as the T790M "gate-keeper mutation", showed greater resistance to Erlotinib and Gefitinib.[53] In fact, around 50-60% of patients resistant to Erlotinib and Gefitinib had T790M mutation within their EGFR gene.[53,54] Second-generation EGFR inhibitor, Afatinib appeared effective in lung cancer patients who were resistant to Erlotinib and Gefitinib, and in lung cancer cell lines possessing HER2 as well as T790M mutations within EGFR gene.[39] It has also been approved as first-line treatment of advanced NSCLC harboring activating EGFR mutations.[55] Third generation TKIs like Osimertinib (Tagrisso) can inhibit EGFR resistance mutation[45] by forming an irreversible covalent bond with EGFR and specifically targeting the T790M mutation.[46] Osimertinib has been licensed for use in the European union since February 2016 for patients with EGFR T790M mutation positive NSCLCs. It showed objective response rate (ORR) of 67% in phase II studies.[45] Progression free survival has been recorded at 9.6 months. Treatment is generally well tolerated with third generation TKIs.

Table 1: New FDA approved EGFR and their indications.

Drugs	Molecular Properties	Route of administration	Approval Date	Approved Uses
Erlotinib[56]	Reversible EGFR tyrosine kinase inhibitor (quinazoline-derivative molecule)	Oral	14th May 2013	As monotherapy for the treatment of NSCLC that is refractory to platinum-based chemotherapy. Approved by the FDA and the European medicines evaluation agency (EMEA) for use in combination with Gemcitabine as first-line treatment for advanced pancreatic cancer.
Gefitinib[56]	Reversible EGFR tyrosine kinase inhibitor (quinazoline-derivative)	Oral	5th May 2003	Used as third-line treatment of NSCLC that is refractory to platinum-based and Docetaxel based chemotherapy regimens.
Cetuximab[56]	Cetuximab Human–mouse chimeric monoclonal antibody (IgG1 subtype)	Intravenous	2004 6th July 2012	It has been approved for the treatment of advanced CRC that is refractory to Irinotecan based chemotherapy. Cetuximab in combination with radiotherapy is also approved for the treatment of locally advanced squamous-cell carcinoma of the head and neck.
Panitumumab[56]	Fully human monoclonal antibody (IgG2κ subtype)	Intravenous	27th September 2006 December 2007 approved by EMEA	Approved as monotherapy for third-line treatment of CRC that is refractory to fluoropyrimidines, oxaliplatin, or irinotecan. Approved for use in patients with CRC who carry a normal, wild-type *K-RAS* gene.
Afatinib[57]	Tyrosin kinase inhibitor	Oral	15th April 2016	First-line treatment of patient with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 (L858R) substitution mutations as detected by an FDA-approved test.
Osimertinib[58]	Tyrosin kinase inhibitors		November 2015. EMA approval in February 2016	For the treatment of metastatic non-small cell lung cancer (NSCLC) in cases where tumor EGFR expression is positive for the T790M mutation as detected by FDA-approved testing

ONGOING RESEARCH FOR DEVELOPMENT OF EGFR INHIBITORS

Zhang *et al*. synthesized a series of 4-anilinoquinazoline derivatives containing substituted diaryl urea or glycine methyl ester moiety which were identified as EGFR and Vascular Endothelial Growth Factor Receptors VEGFR-2 dual inhibitors. Compounds **1, 2, 3,** exhibited the most potent inhibitory activities against EGFR (IC_{50} = 1 nM, 78 nM and 51 nM, respectively) and VEGFR-2 (IC_{50} = 79 nM, 14 nM and 14 nM, respectively). They showed good anti proliferative activities as well.[59]

EGFR T790M mutant is found in about 50% of clinically acquired resistance to gefitinib among patients with non-small cell lung cancer (NSCLC). New derivatives of 4(3*H*)-quinazolinones were synthesized by Patel *et al.* and evaluated for their inhibitory activity against NSCLC. The results of the study demonstrated that compound **4,** 7-Chloro-3-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)-2-phenylquinazolin-4(3*H*)-one was found to be the most potent compounds of the series with IC_{50} value of 0.031 lM against mutant T790M/L858R EGFR.[60]

Song *et al*. reported compound **5** for treatment of gefitinib-resistant NSCLC treatment. It has structurally modified diphenylpyrimidine derivatives bearing a morpholine functionality (Mor-DPPYs) which displayed high activity against EGFRT790M/L858R kinase (IC_{50} = 0.71 nM) and repressed H1975 cell replication harboring EGFRT790M mutations at a concentration of 0.037 mM. Inhibitor **5** demonstrated high selectivity (SI = 631.9) for T790M-containing EGFR mutants over wild type EGFR, suggesting that it will cause less side effects. Moreover, this compound also showed promising antitumor efficacy

in a murine EGFRT790M/L858R-driven H1975 xenograft model without affecting body weight.[61]

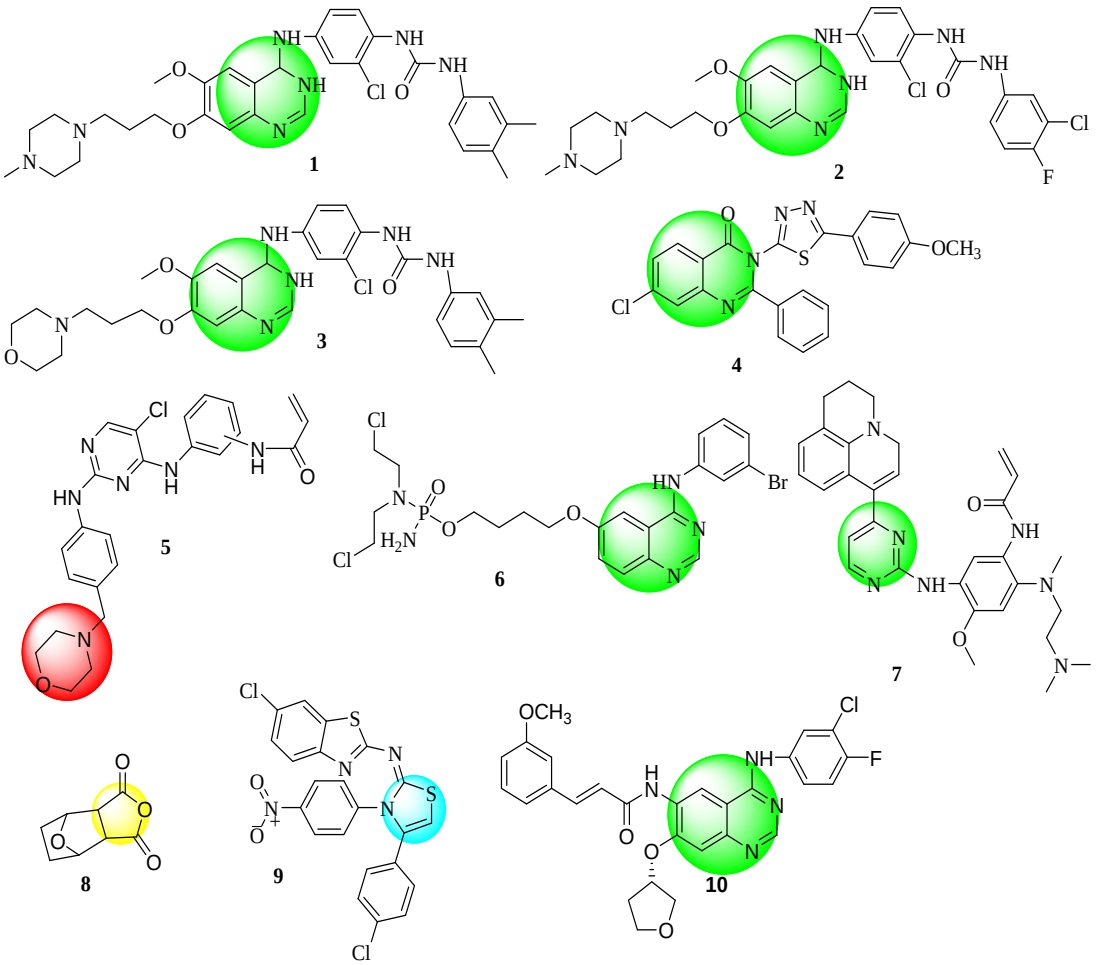
Lin *et al*. synthesized a series of novel compounds with phosphoramide mustard functionality incorporated into the quinazoline scaffold of EGFR/HER2 inhibitors as multi-target-directed ligands against tumor cells. Among all compounds, Compound **6** was one of the most potent inhibitors with IC_{50} of 7.4 nM and 82 nM against EGFR and HER2, respectively.[62]

Based upon the modeling binding mode of marketed AZD9291 with T790M, a series of 5,6-dihydro-4*H* pyrrolo[3,2,1-ij] quinoline derivatives was designed and synthesized by Zhang *et al*. with the purpose to overcome the drug resistance resulting from T790M/L858R double mutations. The most potent compound **7**, showed excellent enzyme inhibitory activities and selectivity with sub nanomolar IC_{50} values for both the single L858R and double T790M/L858R mutant EGFRs, and was more than 8-fold selective for wild type EGFR. Compound **7** exhibited good microsomal stability, pharmacokinetic properties and lower binding affinity to hERG ion channel than AZD9291. It also displayed strong antiproliferative activity against the H1975 NSCLC cells bearing T790M/L858R and *in vivo* anticancer efficacy in a human NSCLC (H1975) xenograft mouse model.[63]

Norcantharidin (NCTD), compound **8** is a Chinese FDA approved, chemically synthesized drug for cancer treatment. In a study conducted by Qiu *et al*., it was found that NCTD suppressed not only the expression of the total EGFR and the phosphorylated EGFR but also the expression of the total c-Met and the phosphorylated c-Met in colon cancer cells. In addition, NCTD-induced cell death was comparable to that of the anti-cancer drug gefitinib, a tyrosine kinase inhibitor for EGFR, based on the immunoblot analysis of the expressed proteins after the drug treatment.[64]

A new series of 19 compounds containing benzothiazole and thiazole was designed by Zhu *et al*. Molecular docking studies were performed on the designed series of molecules. Compounds showing good binding affinity towards the EGFR receptor were selected for synthetic studies. Among these, compound **9** showed potent activity against all the colon cell lines with the Growth inhibitory concentration (GIC_{50}) values in molecular range.[65]

Tu *et al*. synthesized a series of Afatinib derivatives bearing cinnamamide moiety which were evaluated for their IC_{50} values against four cancer cell lines (A549, PC-3, MCF-7 and Hela).The selected compound **10** was further evaluated for the inhibitory activity against EGFR and VEGFR2/KDRkinases.[66]



COMBINED VGEF AND EGFR INHIBITORS IN CLINICAL TRIALS FOR TREATMENT OF VARIOUS TYPES OF CANCER

It has been shown in preclinical studies that anti-VEGF and anti-EGFR treatments are additive in preclinical tumor xenografts models. Clinical trial studies also showed that by completely blocking VGEF and EGFR, an increased antitumor activity has been observed.

In metastatic colorectal cancer for which both Bevacizumab and Cetuximab are approved for use, concurrent administration of Bevacizumab plus Cetuximab, with and without Irinotecan is used in Irinotecan-refractory disease in a randomized phase II trial (BOND II) to determine the efficacy and safety of the drugs.[67]

CONCLUSION

Molecular inhibitors of EGFR signaling represent a highly promising class of molecular targeted anticancer agents. Despite the recent success of EGFR inhibitory agents in gaining FDA approval, there remain several major gaps in our knowledge regarding the function, activity and preferred clinical applications for EGFR inhibitors. The rational selection of cancer patients for EGFR inhibitor therapies remains a major challenge as the association between EGFR overexpression and response to EGFR inhibitor therapy does not appear straight forward.

VEGF signaling is up-regulated by EGFR expression and, conversely, VEGF up-regulation independent of EGFR signaling seems to contribute to resistance to EGFR inhibition. Preclinical studies have shown that VEGF and EGFR inhibitors can have additive effects and that combined inhibition is effective in EGFR inhibitor–resistant cell lines. Clinical trials have also produced promising data: combining the anti-VEGF monoclonal antibody Bevacizumab with the anti-EGFR antibody Cetuximab or the EGFR tyrosine kinase inhibitor Erlotinib increases benefit compared with either of these anti-EGFR agents alone or combined with chemotherapy.

ACKNOWLEDGEMENTS

Authors are thankful to Jamia Hamdard, New Delhi.

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