ISSN: 2319-6505

Available Online at http://journalijcar.org

International Journal of Current Advanced Research
Vol 5, Issue 11, pp 1478-1485, November 2016

REVIEW ARTICLE

International Journal of Current Advanced Research

ISSN: 2319 - 6475

CANCER THERAPY TARGET: THE EPIDERMAL GROWTH FACTOR RECEPTOR

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A R T I C L E I N F O

Article History:

Received 16th August, 2016
Received in revised form 12th September, 2016 Accepted 30th October, 2016
Published online 28th November, 2016

Key words:

EGFR1, Antibodies, TKIs, cancer, chemotherapy

A B S T R A C T

Epidermal growth factor receptor (EGFR) signaling plays an essential role in cell proliferation, survival, and migration. EGFR is a transmembrane protein whose extra cellular domain binds to its physiological ligand EGF and its intracellular domain possesses an intrinsic kinase activity which leads to transcription activation via downstream signalling pathways. Mutations in EGFR which lead to ligand independent and/or constitutive activation of EGFR have been implicated in several cancers such as non-small cell lung cancer (NSCLC), squamous cell carcinoma of the head & neck (SCHNN), colorectal cancer (CRC), and tumors of the ovary, cervix, bladder, esophagus, stomach, brain, endometrium, breast, and liver and provide a basis for targeted therapy. This review summarizes the structure of EGFR1 and structure-function correlation of EGFR mutations characterized in neoplastic tissue and the pharmaceutical drugs that have been developed to target different domains of EGFR1. The use of these drugs in India and their impact on therapy has also been discussed. The clinical data has been obtained from those reported in indexed journals. While the data may not be comprehensive, they nonetheless emphasize the importance of mutational analyses of EGFR1 prior to the use of EGFR1 targeted therapy in India for better management of the disease.

INTRODUCTION

Treatment of cancer usually consists of surgery, chemotherapy and radiation or a combination of these modalities. Despite improvements in diagnosis, earlier detection and targeted therapy, the prognosis of several types of tumors still remains poor.

Extensive analyses have defined tumor-associated or tumor-specific, surface antigens / receptors (coded by oncogenes) displayed on the malignant cell surface or in neoplastic tissue. For example, many growth factor receptors are often overexpressed on the surface of cancer cells and drive tumor growth. These surface oncogenes / surface receptors cause the tumor to become addicted to certain stimuli or signaling pathways and in many cases cessation of signalling through the oncogene can induce cell death in tumors and hence to tumor regression.

For example, switching ON (overexpressing) the *c-myc* gene exclusively in hematopoietic cells in mice models gives rise to T cell and myeloid leukemias. However, when *c-myc* was turned OFF, cells begin to differentiate and/or apoptose (Felsher and Bishop, 1999). Similarly, the diffuse large B-cell lymphomas signal through the B-cell receptor and is addicted to NF-KB signalling (Davis *et al*., 2010). Hepatocellular carcinomas are believed to be due to aberrant signalling of the Ras/Mapk pathway (Delire and Stärkel, 2015). Such receptors / oncogenes / signalling pathways therefore become targets for drug development, since inhibiting their activity would turn OFF the driving force behind neoplastic growth.

Targeted therapy in these cases can be accomplished by using monoclonal antibodies (Mabs) alone or Mabs armed with radionuclides, drugs, prodrugs, or toxins, that kill tumor cells while not destroying normal cells by targeting specifically the receptor / oncogene on the surface of the cell. In some cases, simply targeting the receptor / oncogene is insufficient and leads to partial regression of tumors and regrowth of an receptor / oncogene independent tumor, which has to be then screened for mutations in downstream targets of said receptor / oncogene. Thus targeting cells selectively through these receptors / oncogenes / siganlaing pathways is inherently different from surgery, radiation, and chemotherapy and could emerge as a favoured modality for cancer therapy due to target specificity. This review summarizes EGFR1 structure-function correlation in the light of the drugs that have been used and its implications in clinical use particularly in India.

EGFR

The type I tyrosine kinase epidermal growth factor receptors (EGFRs) are expressed in a broad spectrum of tumor types, which classifies them as one of the most frequently implicated oncogenes for human cancers. The *EGFR* (*c-erbB1*) proto-oncogene located in chromosome 7p11.2 contains 28 exons. The gene encodes a transmembrane glycoprotein of 464 amino acids which has an extracellular cellular ligand (EGF) binding domain and an intracellular domain possessing intrinsic tyrosine kinase activity of molecular mass of 170 kDa and multiple autophosphorylation sites clustered at the C-terminal tail **(Fig. 1,** Ferguson *et al*., 2003, Bocharov *et al*., 2008, Stamos *et al*., 2002).

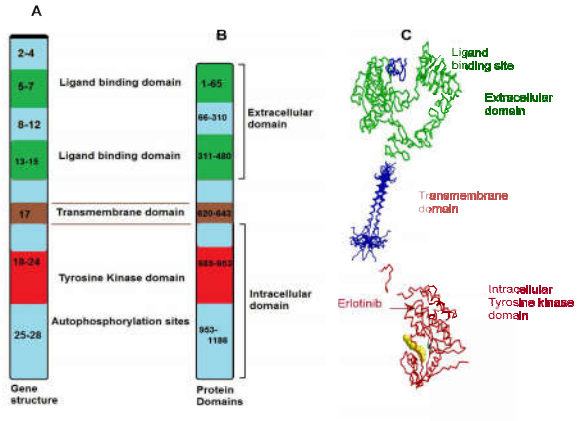

Fig. 1. A The Gene structure of EGFR. The numbers indicate exons**. B. Domains of the EGFR protein.** Numbers indicate amino acid numbers. **C. 3D-EGFR protein structure** compiled from structures of the extracellular (PDB ID: 1NQL), [4] transmembrane (PDB ID: 2JWA) [5] and an intracellular domain bound to Erlotinib (PDB ID: 1M17). [6] Only the protein backbone of a monomeric unit of EGFR is shown.

On EGF binding to its receptor, EGFR undergoes a transition from an inactive monomeric form to an active homodimer (Yarden and Schlessinger, 1987) and the intracellular domain of two molecules housing the tyrosine kinase domain phosphorylate each other on selected tyrosine residues. The phosphorylated tyrosine residues function like scaffolds to recruit and activate two downstream intermediate pathways: (1) PI3K/Akt1/mTOR pathway and (2) Ras/Raf1/Map2K1/MapK1 pathway, leading to transcription factor activation and controlled normal growth. Thus, during normal growth and development, EGFR stays predominantly in an inactivated state through autoinhibition and is activated on ligand binding or through increase in its concentration, which can happen due to a lack of control on its expression in the neoplastic state (Zhang *et al*., 2006).

Initially, (prior to mutational studies) monoclonal antibodies (mAbs) or inhibitors targeting the extracellular domain of EGFR were developed based on the rationale that EGFR driven growth was the driver of tumor growth in many cancers. However, sequencing of EGFR in gliomas led to the observation that the EGFR gene harboured an in frame deletion of exons 2-7 (in its intracellular domain) which led to its constitutive activation and in turn to neoplastic growth. This correlation provided the most conclusive evidence that mutations in EGFR were the driving force behind oncogenesis (Libermann *et al*., 1985). Since then mutations in the both extra and intracellular domains of EGFR have been characterizes and have provided the distinguishing features between tumors of different tissue lineage. While glioblastomas (GBMs) and SCCHNs harbor mutations in the extracellular domain of EGFR, NSCLCs harbor most mutations in or around the tyrosine kinase domain and this criteria drives targeted therapy.

EGFR Mutations and implications on targeted therapy

In the late 90's seven genomic variants of EGFR which were detected in biopsies were classified as class I mutants which lacked the extracellular domain of the encoded protein; class II: mutants which contained an in-frame deletion of 83 aa in the extracellular domain outside the ligand site; class III: mutants which contain an in-frame deletion of exons 2 to 7 with a novel glycine at the junction of 1/8 exon in the extracellular domain; class IV and V: mutants which carried deletions in the intracellular (or cytoplasmic) domain and class VI and VII mutants (class IV and V respectively), coexisting with one of the defined extracellular domain deletions (**Table 1**, Wikstrand *et al*., 1998). Type vIV mutants of EGFR including deletion mutations in exon 25 - 27 and 25 - 28 deletion which result in the truncation of the C-terminal domain of EGFR have also been identified in GBM patients [Ekstrand *et al*., 1992, Frederick *et al*., 2000) and only a few studies characterizing their oncogenic potential have been reported thus far.

Table 1 Mutations of the EGFR detected in tumour cells; residues that occur at the splice sites are not shown

Type	Alteration in sequence
EGFR vI	Translation starts at aa 543
EGFR vII	Deletion of aa 521–603
EGFR vIII	Deletion of aa 6–273
EGFR vIII/12–13	Deletions of aa 6–273 and 409–520
EGFR vIV	Deletion of exon 19 deletion (aa747-749), aa 959 – 1030, Exon 18-21 point mutations.
EGFR vV	Truncation at residue 958
EGFR.TDM/–7	Tandem duplication of 6–273
EGFR.TDM/18–25	Tandem duplication of 664–1030
EGFR.TDM/18–26	Tandem duplication of 664–1014

aa: amino acid(s)

Mutations in Extracellular domain of EGFR: Implications in glioma, GBM, metastatic CRC and SCCHN.

The most common EGFR variant is the type III EGFR deletion mutant EGFRvIII (also called Δ801EGFR or del2-7 EGFR), has an in-frame deletion of exons 2 to 7 with a novel glycine at the junction of 1/8 exon **(Fig. 2,** Ferguson *et al*., 2003, Pederson *et al*., 2001**).** EGFRvIII was shown to occur commonly in gliomas (Libermann wt al., 1985). The occurrence of EGFRvIII was observe to be 57% of high-grade gliomas, 24% - 67% of GBMs, 42% of SCCHNs (Sok *et al*., 2006) and only about 16% of NSCLCs (Kris *et al*., 2011). EGFRvIII expression is lost in vitro; consequently SCCHN cells must be stably transfected with an EGFRvIII construct to establish a model for preclinical investigations. Data for mutant EGFRvIII expression in tumors from an Indian population is limited. Gaitonde *et al*., 2005 reported that in grade IV GBMs 5 out 13 samples harbored the EGFRvIII mutation.

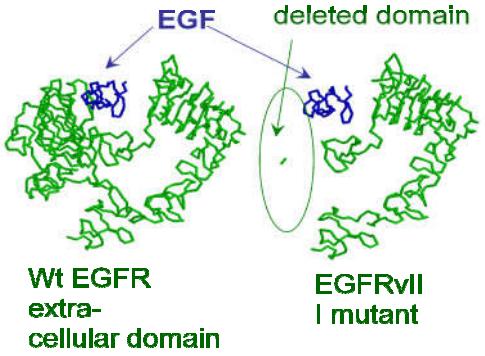

Fig. 2. 3D structures of the extracellular domain of EGFR (LHS) and EGFRvIII mutant (RHS) when complexed with EGF. The protein backbones are shown in green and EGF in blue have been generated using the co-ordinates deposited in RSCB PDB (PDB ID: 1NQL). [4] The structure of EGFRvIII was generated by deleting corresponding residues in 1NQL. Therefore this is only a model and the mutant may have a completely different conformation / architecture.

Pharmacodyamics of EGFRvIII

Since exons 2-7 code for the extracellular domains of EGFR (See **Fig. 1, 2 and 3**), EGFRvIII is unable to bind its ligand EGF. However, the truncated extracellular domain in a novel extracellular domain architecture that mimics the EGF bound activated EGFR, but also confers resistance to antibody mediated therapies (see below).

Therapy using Anti-EGFR monoclonal antibodies

Anti-EGFR monoclonal antibodies, such as Cetuximab (Erbitux), Panitumumab (Vectibix), and Nimotuzumab, bind to the extracellular domain of the EGFR monomer and block ligand-induced EGFR activation by competing with endogenous ligands. Cetuximab, binds to the L2 domain of EGFR (Li *et al*., 2005, Yun *et al*, 2007), and is a chimeric protein antibody composed of variable and constant regions from mouse and human sources, respectively, while panitumumab and nimotuzumab are fully humanized EGFR antibodies. Both Cetuximab (Erbitux) and Panitumumab have been shown to be equally effective atleast in 1 study for treatment of CRC (Price *et al*, 2014).

Most of these antibodies bind domain III (region away from the exon 2-7 deletion as shown in **Fig. 3**) of the extracellular region but recognize distinct but overlapping epitopes and work through prevention of ligand binding. (Ferguson *et al*., 2003, Li *et al*., 2005). However, since EGFRvIII is active, independent of ligand binding; it represents a mechanism of cetuximab resistance **(see Fig. 3,** Patel *et al*, 2007).

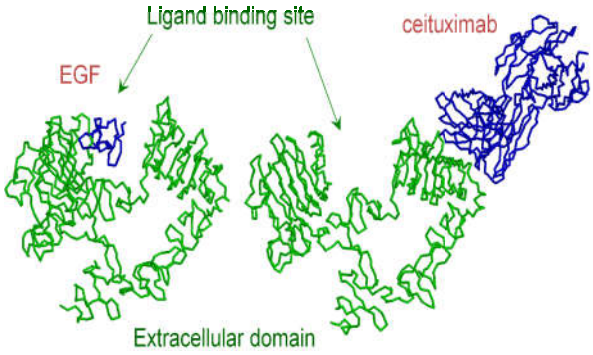

Fig. 3 Structure of the extracellular domain of EGFR bound to EGF (LHS) and when bound to ceituximab (RHS). The protein backbones are shown in green and EGF in blue and have been generated using the co-ordinates deposited in RSCB PDB database (PDB ID: 1NQL; [4] PDB ID: 1YY9 [17])

Cetuximab has been approved in many countries world-wide for treating patients with metastatic CRC (mCRC) or with SCCHN. In India, it is indicated for the treatment of patients with *EGFR*-expressing, *K-ras* wild-type mCRC in combination with chemotherapy or as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy and who are intolerant to irinotecan. For SCCHN, it has been approved in combination with radiation therapy for locally advanced disease or in combination with platinum-based chemotherapy for recurrent and/or metastatic disease. **Table 2** summarizes the anitibodies developed against EGFR for chemotherapy. **Table 3** summarizes clinical data reported only from Indian centres (Dattatreya and Goswami, 2011, Agarwal *et al*., 2011, Rangaraju *et al*, 2012, Basavaraj *et al*., 2010).

Table 2 Anti-EGFR antibodies available for treatment

Antibody	Status *
Rindopepimut	Phase 3 trial for glioblastoma (Cancer Research UK)
Panitumumab	Approved USA 2006, EU 2007 for *K-ras* wild-type, *EGFR*-expressing metastatic colorectal cancer in combination with FOLFOX, Phase 3 for CRC in combination with chemotherapy before / after surgery (Cancer Research UK).
Cetuximab	Approved UK 2004, USA 2004, EU 2006 for *K-ras* wild-type, *EGFR*-expressing metastatic colorectal cancer, for recurrent or metastatic head and neck cancer, for head and neck cancer, for colorectal cancer (National Institute of Health)
Nimotuzumab	Discontinued after phase III, Apr 2014 (Japan)
Zalutumumab	Discontinued, Apr 2011 (from Genmab)
Matuzumab	Terminated in 2008

*Information from various sources including Cancer research UK, National Institute of health, Astra Zeneca etc.

Not much reported clinical data is available, probably due to the fact that a Cetuximab (Erbitux) from Merck can be ill-afforded by many patients in India and opt for alternate therapies (for example see Patil *et al*., 2015). However "an Observational Study to Evaluate the Safety and Efficacy of FOLFIRI / FOLFOX Plus Cetuximab as First-line Therapy in Patients With KRAS Wild-type Metastatic Colorectal Cancer" is presently underway at many hospitals in India. (http://clinicaltrials.gov/show/NCT01134666).

Mutations in the intracellular domain of EGFR: Implications for NSCLC

EGFR mutations commonly found in biopsied lung tissue of patients diagnosed with non-small cell lung cancer (NSCLC) exist in exons 18 – 21 (see **Figs. 1 and 4**) and are associated with the tyrosine kinase activity in the intracellular domain of EGFR. The mutations characterized are predominantly small, in-frame deletions in exon 19 or a L858R point mutation in exon 21 clustered around the ATP-binding pocket of the tyrosine kinase domain such as those depicted in (Fig. 4, Yun *et al*., 2007). *EGFR* exon 19 deletions are in-frame deletions occurring within exon 19, which encodes part of the kinase domain and leading to a deletion of three amino acids 747-749. This mutation occurs with a frequency ~48% in *EGFR* mutant lung tumors and the L858R mutation occur in ~20% cases (Mitsudomi and Yatabe, 2010).

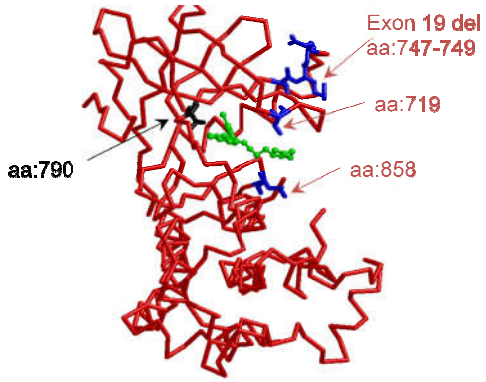

Fig. 4 Structure of the intracellular domain of the Wt EGFR when bound to Gefitinib (PDB ID: 1ITY [19]) showing the sites most commonly mutated in NSCLC. Protein backbone is shown in red. Gefitinib is shown in green. Sites with mutations sensitizing to TKI are shown in blue. The mutation at 790 is resistant to TKI and is shown in black. Aa: amino acid.

A screen of 220 NSCLC tissue samples from India analyzed for EGFR mutations detected mutations in 51.8% of the study population.

Table 3 EGFR mAb treatment in India

Inclusion criteria	No. of patients	Drug used use	Response comparisons*	Overall conclusions	Reference
Advanced unresectable (Stage IV) SCHNN	18	Cetuximab + RT	22% CR, 50% PR, 22%S, 11% PD	---	[21]
Stage III 7 IV SCHNN Ineligible for platinum based CT	37	Cetuximab + RT		At 16 months the 2-year loco-regional control was 35.5%, disease-free survival was 29.5%, and overall survival was 44.4%	[22]
Recurrent/metastatic SCHNN	35	Cetuximab + RT	3.1% CR, 53.1% PR 18.8% SD		[23]
SCHNN (Phase II study)	92	Nimotuzumab (N) +/- (RT) +/- cisplatin (C)		At 5 yr OS was 57% with CRT + N, 39% with N + RT, 26% with CRT (P = 0.03), and 26% with RT (P>0.05)	[24]

* All values indicated are percentages reported in the respective studies. CR: complete remission, PR: Partial remission; S: stable disease PD: progressive disease, RT; Radiation therapy, C; chemotherapy.

Among the mutant positive cases, the deletions in exon 19 (52%) and the L858R mutation in exon 21 (26%) were most predominant (Sahoo *et al*., 2011). Similarly, out of 1018 Indian patients approximately 53% mutations were in-frame deletions in exon 19, whereas 38% are L858R in exon 21, 6% of the mutations were found in exon 18 and 3% in exon 20, which is similar to that described in other populations (Mitsudomi and Yatabe, 2010, Choughule *et al*, 2013). These mutations render the EGFR1 constitutively active. Inhibitors which suppress / inhibit the activity are called tyrosine kinase inhibitors (TKIs). Mutations leading to TKI resistance such as an exon 19 insertion and a T790 mutation have also been characterized (Cho *et al*., 2011).

Therapy using Tyrosine kinase inhibitors (TKIs) for NSCLC

EGFR1-specific small molecule inhibitors such as gefitinib (Iressa) and erlotinib (Tarceva) are taken orally, translocate across the plasma membrane and interact with the cytoplasmic domain of EGFR. In the cell, TKIs compete with ATP to bind the catalytic domain of the kinase, which in turn inhibits EGFR autophosphorylation and downstream signaling, including cell proliferation and survival. Several EGFR tyrosine kinase inhibitors (TKIs) have been developed or are in development (1) First-generation: Gefitinib (Iressa) and erlotinib (Tarceva). Icotinib is another reversible inhibitor like gefitinib and erlotinib that was recently developed and is available only in China. (2) Second-generation: Afatinib (Gilotrif), dacomitinib, neratinib. These are irreversible inhibitors with activity against both EGFR and family members. (3) Third-generation: CO-1686, AZD9291 (Asami and Atagi, 2014, Stasi and Cappuzzo, 2014, Liao *et al*., 2015). These are mutant-selective as they were designed to target mutant EGFR better than wildtype EGFR. Apart from these there are general tyrosine kinase inhibitors which targ*et al*l tyrosine kinases including EGFR1. **Table 4** summarizes the present status of TKIs in clinical use for the treatment of NSCLC.

Table 4 Tyrosine kinase inhibitors

Inhibitors	Status (FDA approval)*
Gefitinib (EGFR Inhibitor)	US approved 2003, withdrawn 2005, EU approved 2009
Erlotinib (EGFR Inhibitor)	US approved 2013 for tumors having EGFR exon 19 deletions or exon 21 (L858R) substitution mutations. UK 2008: Erlotinib is recommended as an alternative to docetaxel for patients with non-small-cell lung cancer (NSCLC) who have already tried one chemotherapy regimen but it has not worked.
Icotinib (EGFR Inhibitor)	China approved 2011, Available only in China, Phase II ongoing 2012-2014
Afatinib (General TKI)	US approved 2013, EU approved 2013 for EGFR mutation positive lung cancer
AEE-788 (General TKI)	Phase I studies in 2012-2013
BMS-690514 (EFGR, VEGFR inhibitor)	Phase I studies in 2012-2013
XL647 (EGFR, VEGFR2, HER2, and EphB4 inhibitor)	Phase II study in 2012-2013 in NSCLC patients known or suspected of having tumors harboring T790M)

*Information from various websites sources including Cancer research UK, National Institute of health, Astra Zeneca, Medscape etc.

Table 5 Effect of different mutations in the intracellular domain of EGFR on physiological activity and TKI binding*

Mutant	Physiological Activity	TKI	Binding to TKI	Reference
Wt enzyme				[4]
L858R	Kinase Activity = 2.5 fold Wt enzyme			[4]
Y485R	Kinase Activity = 2.5 fold Wt enzyme			[4]
Wt enzyme		Gefitinib	Dissociation constant Kd ~ 50 μM	[19]
L858R	Tyrosine kinase activity = 50 times Wt enzyme	Gefitinib	20 times more tightly than Wt (Kd ~ 2.5 nM)	[19]
G719S	Tyrosine kinase activity = 10 times Wt enzyme	Gefitinib	3 times more weakly than Wt	[19]
Wt enzyme		Erlotinib	Inhiibition Ki = 17.5 nM	[33]
L861Q	~ 5 times lower the Km ATP of Wt enzyme	Erlotinib	3 times more inhibited than Wt	[33]
Exon 19 deletion	~ 25 times the Km ATP of Wt enzyme	Erlotinib	5 times more inhibited than Wt (Ki = 6 nM)	[33]
L858R	~ 2 times the Km ATP of Wt enzyme	Erlotinib	2.5 times more inhibited than Wt	[33]

Pharmacodyamics of mutations in the intracellular regions of EGFR (exon 19, L858R and other mutants)

In vitro, EGFR mutants have excess tyrosine kinase activity in response to ligand binding suggesting a specific gain of function. Structurally, it has been shown that L858R mutation locks the EGFR in a constitutively active conformation [Zhang *et al*., 2006, Yun *et al*., 2007, Carey *et al*., 2006) thus increase EGFR signaling in cells. **Table 5** summarizes mutations in EGFR which have been pharmacologically characterized (Ferguson *et al*., 2003, Libermann *et al*, 1985, Carey *et al*., 2006).

Gefitinib was originally approved in the US in 2003 and withdrawn in 2005 when it failed to comply with initial end point expectations. However gefitinib therapy has been more successful in Asian population compared to Western populations. The reported pharmacological data along with the frequency of mutations which occurs in Asian over Caucasian populations in addition to lifestyle provide an explanation to this observation. The exon19 deletion mutation and L858R mutations have been observed to about 10% in Caucasian populations (Lynch *et al*., 2004) and thus gefitinib benefits about 10% of the population. The frequency of these mutations goes upto 40-50% in Asian women patients who in general fall in the category of never-smokers as studies from India and other East Asian studies suggest. Since gefitinib has a superior affinity for the L858R mutated EGFR **(Table 5)** and presumably for exon 19 mutated EGFR (extrapolating results from Carey *et al*, 2006), [33] a larger population of Asian women stand to benefit from this therapy.

Data from phase I dose-escalation trials show that the expected mean trough steady state serum concentration of gefitinib at 250 mg/day is about 0.5 μM (Baselga *et al*., 2002) whereas erlotinib at 150 mg/day has mean trough steady state concentrations that exceed 2.5 μM (Hidalgo *et al*., 2001). These clinical doses are atleast 10 times more than the respective Kd or Ki values for gefitinib and erlotinib (**Table 5)** respectively and can be expected to completely inhibit EGFR signaling. A meta analysis of all reported responses to the inhibitors erlotinib gefitinib afatinib and icotinib using overall response rate, progress free survival, 1 year and 2 year overall survival as criteria reveal that therapy with afatinib and erlotinib were more efficacious but had more severe rash and diarrhea when compared to therapy with gefitinib and icotinib (Liang *et al*., 2014).

Table 6 summarizes the data reported from gefitinib use in India. [Parikh *et al*., 2008, Nag *et al*., 2010, Louis *et al*., 2012, Shahid *et al*., 2012, Mehta *et al*., 2013, Mok *et al*., 2009) Erlotinib use in India is limited due to cost (~ 1,00,000 / vial) and limited availability. In general, not all the references in **Table 6** reported correlation of success/failure of TKI therapy with mutations in EGFR, however they reported that the side-effect profile of gefitinib therapy was manageable when compared with the obvious adverse effects associated with chemotherapy. Contrary to chemotherapy, patients on gefitinib were managed in the outpatient department and rarely required discontinuation of therapy or admissions for supportive care. This is one major benefit in prescribing gefitinib as a first line therapy for advanced NSCLC. The IPASS study observed that among 261 patients positive for the EGFR mutation, gefitinib improved PFS compared to carboplatin-paclitaxel. In contrast, in 176 patients who were negative for the mutation, PFS was improved with standard chemotherapy than gefitinib (Mok *et al*., 2009). This was re-inforced by a recent study by Noronha *et al*, 2013 where response to TKI therapy was 74% in patients with EGFR mutations compared to 5% in patients with Wt EGFR. Non-smoking Asian women seem to harbour mutations in EGFR more sensitive to gefitinib, in contrast to caucasians highlighting that differences in basic genetics could lead to differences in differences in cause and subsequent therapy. Thus, the presence of a mutation of the EGFR gene is a strong predictor of a better outcome with gefitinib and underscores the need for diagnostic EGFR mutational analyses.

Resistance to EGFR1 mediated therapy and future of targeted therapy

Although several cases of complete remission after TKI therapy of NSCLC have been reported, re-emergence of mAB and TKI insensitive tumors have also emerged. These tumors acquire secondary mutations in EGFR1 itself (Kobayashi *et al*., 2005) or in downstream targets. Activation of EGFR1 activates atleast two signaling pathways. The first pathway involves Ras-Raf-Mapk pathway, where phosphorylated EGFR1 recruits the guanine-nucleotide exchange factor via the Grb2 and Shc adapter proteins, activating Ras and subsequently stimulating Ras and the Map kinase pathway to affect cell proliferation, tumor invasion, and metastasis. The second pathway involves PI3K/Akt pathway, which activates the major cellular survival and anti-apoptosis signals via activating nuclear transcription factors such as NF-KB.

Table 6 Tyrosine kinase inhibitor (Gefitinib/Iressa) therapy for NSCLC in India

Inclusion criteria	Sample size	Sex	Histology	EGFR mutational Analysis	Gefitinib use	Response comparisons / Overall conclusions	Reference
Advanced NSCLC	77		Adeno-carcinoma	None	1st line	OS 14% compared to 0% in placebo	[38]
Advanced NSCLC	37	M&F	Adeno-carcinoma	12 out of 37 samples	1st line = 21 2nd line = 16	4.8 CR, 42.9 PR, 38 S, 14.3 PD 0 CR, 42.9 PR, 19.1S, 14.3 PD 0 CR, 23 PR, 42.5 S, 34 PD	**[39]**
Stage IIIB & IV advanced NSCLC	109	M&F	Adeno-carcinoma	None	1st line = 47 2nd line = 17	Significant PFS benefit for the female patients, non-smokers with significant OS benefit only for female NSCLC patients.	[40]
Metastatic Non-small Cell Lung Cancer	50	F	Adeno-carcinoma	22 +ve for EGFR (~50%)	1st line	Progression free survival (PFS) was seen in 19 (38%) patients	[41]
Advanced NSCLC	63	M&F	71% Adeno-carcinoma	None	1st line	1.6 CR, 7.9 PR, 38 S, 42. PD No survival benefit noted	[42]

* All values indicated are percentages reported in the respective studies. CR: complete remission, PR: Partial remission; S: stable disease PD: progressive disease. Clinical data has been obtained from those reported in Pubmed indexed journals.

Mutations in almost all signalling molecules downstream of EGFR1 such as Ras, (Pao *et al*., 2005), Raf (Paik *et al*., 2011) and MEK1 (Marks *et al*., 2008) have been detected in NSCLCs. They may occur independently in tumors or may occur in recurrent tumors as resistance strategy to EGFR1 therapy. So far data suggests that these mutations are by and large mutually exclusive from each other. Mutations in the second pathway such as those in the PI-3K have also been identified (Kang *et al*., 2005).

Nonetheless, these mutations activate signalling pathways downstream of EGFR1 independent of EGFR1 activation status and drive neoplastic growth. EGFR1 activation may also lead to phosphorylation of PLCγ and subsequent hydrolysis of phosphatidylinositol 4,5 biphosphate (PIP2) into inositol 1,4,5-triphosphate (IP3) and diacylglycerol, resulting in activation of protein kinase C and CAMK or through the JAK/STAT pathway which is also implicated in activating transcription of genes associated with cell survival. Detailed studies identifying mutations in these pathways have not yet been identified. Thus EGFR1 mutational analysis could provide to be a better prognostic indicator and management of EGFR1 targeted therapy.

Acknowledgement

All structures have been generated from structure files deposited by the respective authors at the RSCB PDB (RSCB protein database, http://www.rcsb.org) using the software RASMOL. The author expresses her sincere gratitude to Mr. Sahil Kulkarni, who gave a thorough reading of the manuscript.

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