EGFR Inhibitors in Treatment of Lung Adenocarcinoma

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The aim of this review is to provide an overview of current molecular targeted therapies for epidermal growth factor receptor (EGFR) mutation-positive lung adenocarcinoma. Current strategies aim to identify patients with driver mutations such as epidermal growth factor receptor gene mutations and anaplastic lymphoma kinase rearrangements. Previous treatment strategies have been less than satisfactory regarding response rates and significant systemic toxicities. EGFR has been shown to be deregulated by various mechanisms in lung adenocarcinoma (AC), including overexpression, amplification and mutation. Reversible EGFR tyrosine kinase inhibitors (TKIs) such as Erlotinib and Gefitinib offered a therapeutic alternative that has proven its superiority over standard platinum-based chemotherapy for patients with metastatic EGFR mutation-positive lung cancer. Irreversible EGFR tyrosine kinase inhibitors (TKIs) such as Afatinib and Dacomitinib came into practice to provide sustained disease control in adenocarcinoma of the lung with primary or acquired resistance to first generation EGFR TKIs.

Key words: Targeted therapy, NSCLC, Mutation, TKIs, Resistance.
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INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide. Approximately 80% of lung cancers are grouped as non-small cell lung carcinoma (NSCLC), which are clinically and pathologically different from small cell lung carcinoma (SCLC). Adenocarcinoma (AC) of lung constitutes about 40% to 50% of all lung cancers. NSCLC also includes squamous cell carcinoma (SQCC) and large cell carcinoma (LCLC)\(^1,2\).

The epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (RTKs), referred to as the HER or ErbB family, consists of four members namely: (HER1/ErbB1), (HER2/ErbB2), (HER3/ErbB3) and (HER4/ErbB4). They regulate many developmental, metabolic and physiological processes. The intracellular TK activity of EGFR is increased as a consequence of the binding of various cognate ligands, which include EGF, transforming growth factor-α, amphiregulin and others leading to the homodimerization of two EGFRs or the heterodimerization of EGFR with other family members, most commonly HER2.\(^3\) Heterodimerization with HER2, which is over-expressed in some tumors, is a more potent activator of EGFR TK than is EGFR homodimerization. The activation of receptor TK leads to the autophosphorylation of the intracellular domain of EGFR, and the phosphotyrosine residues that are formed act as a docking site for various adapter molecules, resulting in the activation of the Ras/mitogen-activated protein kinase pathway, the PI3K/Akt pathway and signal transducers and activators of transcription signaling pathways\(^4\).

EGFR mutations have been observed in NSCLC especially in adenocarcinoma which results in dysregulation of cellular growth and proliferation.\(^5\) Based on these findings EGFR tyrosine kinase inhibitors (TKIs) have been utilized as part of treatment strategies for selected patients with NSCLC. The presence of an EGFR mutation strongly predicts likelihood of response to TKI therapy, with an observed response rate of about 80% among individuals whose tumors express the mutation and only 10% among those whose tumors do not\(^6\).

Several types of activating mutations are known to occur in EGFR in NSCLC: Class I - exon 19 in-frame deletions (44% of all EGFR mutations), Class II - single amino acid changes (L858R 41%, G719 4%, other missense mutations 6%) and Class III - exon 20 in-frame duplication/insertions (5%). These mutations occur in the tyrosine kinase domain of EGFR. Eighty-five percent of all EGFR-activating mutations are exon 19 in-frame deletions or L858R, and they tend to be sensitive to currently available EGFR inhibitors. Class III mutations (exon 20) are generally insensitive to EGFR inhibitors.\(^7\)
Higher EGFR mutation frequency is observed in non-smokers, women, and in non-mucinous tumors. EGFR has been shown to be dysregulated by various mechanisms in adenocarcinoma, including overexpression, amplification, and mutation\(^6\).

In unselected patients, clinicopathological features like adenocarcinoma histology (40% vs 3% in other histologies), East Asian descent (30% vs 8% in non-Asians), female sex (42% vs 14% in male patients), and particularly never-smoking status (51% vs 10% in current or ever-smokers) are associated with an increased EGFR mutation frequency within the tumor\(^4\).

Nearly 50%–60% of patients with NSCLC have at least one identifiable driver mutation, with the most common mutations being in the Kirsten ras (KRAS) gene (24%) and the epidermal growth factor receptor (EGFR) gene (13%–22%), with translocations involving anaplastic lymphoma kinase (ALK) in another 5%–6\(^{11,109}\%\).

**EGFR INHIBITORS**

Patients with adenocarcinoma and large cell histology NSCLC should be tested at diagnosis for EGFR mutations as those who harbor such mutations benefit from upfront EGFR inhibitors. Many clinical trials have shown that patients with EGFR mutations have improved progression-free survival (PFS) and overall survival (OS) when treated with EGFR inhibitors compared to patients with the same mutations who received the standard chemotherapy regimen\(^5\)\(^{12,14}\).

In a pivotal trial of Erlotinib versus chemotherapy, PFS was improved from 4.6 months to 13.1 months with Erlotinib compared to those who received standard doublet chemotherapy\(^9\).

Early phase II trials of Erlotinib and Gefitinib in the first line setting demonstrated modest efficacy in unselected patients with rather low and disappointing response rates (4–23%), PFS (1.6–3.5 months) and OS (5–13 months)\(^{16–22}\). This concurs with results of the large second- and third-line trials BR.21 and ISEL which showed again minor clinical benefit of EGFR TKI therapy compared to placebo in unselected patient population\(^{23,24}\). Subgroup analyses and the discovery of activating EGFR gene mutations as predictors for TKI effectiveness since then yielded a conceptual change in selecting patients for EGFR-targeted therapies\(^{16,18,19,25,26}\). The following trials have used clinical criteria (such as smoking status, sex, histology, and ethnicity) and some have used the EGFR mutational status, whereas others have selected patients with potential intolerance to chemotherapy based on poor performance status and higher age\(^{20,22,27–29}\). In contrast, several phase II trials, and in particular the phase III study IPASS, selected patients for clinical surrogate markers (i.e. female patients, Asians, never- or former light-smokers). All of these trials reported an increase in ORR (13–43%) and PFS (4–5.9 months). Additional EGFR-mutational analyses revealed that the benefit was consistently highest in patients with tumors harboring the activating EGFR gene mutations\(^{14,19,30–32}\). These findings eventually stimulated 4 randomized phase III trials comparing first-line Erlotinib or Gefitinib with cytotoxic chemotherapy in patients with proven EGFR-mutant NSCLC. In such molecularly selected populations, the OPTIMAL, WJTOG3405, and NEJSG002 trials in Asian patients and the EURTAC trial in Caucasian patients clearly demonstrated the superiority of Erlotinib or Gefitinib over platinum doublet chemotherapy – the standard of care – in terms of PFS (OPTIMAL: 13.1 vs. 4.6 months, hazard ratio (HR) 0.16; WJTOG3405: 9.2 vs. 6.3 months, HR 0.48; NEJSG002: 10.8 vs. 5.4 months, HR 0.30; EURTAC: 9.7 vs. 5.2 months, HR 0.37). Also the objective response rate (ORR) was more than doubled in the EGFR TKI arms (OPTIMAL: 83% vs. 36%; WJTOG3405: 62% vs. 32%; NEJSG002: 74% vs. 31%; EURTAC: 58% vs. 15%)\(^{33–36}\). Recently published meta-analyses consistently confirmed that EGFR mutations predict response to EGFR TKI with much higher sensitivity (0.78; 95% confidence interval (CI) (0.74–0.82) than do EGFR gene copy numbers or EGFR expression levels\(^37\). First-line treatment with Erlotinib or Gefitinib in selected patients with EGFR mutations increases the chance of obtaining an ORR more than 2-fold (70% vs. 33%) when compared to chemotherapy and the same time the hazard of progression is reduced by 65\(^{38}\). In EGFR mutation- positive patients treated with Erlotinib or Gefitinib, those with exon 19-deleted tumors appear to have a longer PFS (14.6 vs. 9.7 months) and OS (30.8 vs. 14.8 months) as compared to patients with EGFR L858R-mutant tumors, although this was not consistently reproducible throughout all trials\(^{35,36,41,42}\). The European Medicines Agency (EMA) approved Gefitinib in 2009 due to results from the IPASS and INTEREST trials, and Erlotinib in 2011 due to EURTAC trial results for the first-line treatment of patients with EGFR-mutant NSCLC, but both drugs have yet to be licensed for this indication by the U.S. Food and Drug Administration (FDA). This is in part due to the fact that none of these trials nor the meta-analysis (HR 0.96, 2p = 0.71) has actually demonstrated a significant improvement in OS for recipients of first-line EGFR TKI therapy\(^38\). There is evidence, however, that the response rates are higher when TKIs are given upfront to chemotherapy- naive patients compared to chemotherapy treated patients\(^31\). It is highly unlikely that such an overall survival advantage will ever be demonstrated, as nearly all patients with known EGFR mutations who receive first-line chemotherapy cross over to TKI treatment.
Furthermore, EGFR TKIs are also active in the second-line and maintenance treatment (SATURN, INFORM trial) – especially in EGFR-mutant tumors. In many trials the HR for OS was slightly in favor of first-line EGFR TKI and that the quality of life was maintained for much longer in TKI-treated patients. Patients who are older or have a poor performance status will have a greater benefit from first-line EGFR TKIs. These trials also clearly show that proper selection of patients is absolutely crucial for treatment with EGFR-targeted agents. First-line EGFR TKIs in patients with unknown or wild-type EGFR status were detrimental in terms of PFS and OS as compared to chemotherapy. In line with this, early trials combining EGFR TKIs with first-line chemotherapy in unselected patients (INTACT-1 and -2 trials) did not lead to an increased treatment efficacy, but recent trials in selected patients suggest that a combination may work (CALGB 30406 trial).

Afatinib is an irreversible ErbB family blocker that covalently binds to the cysteine residue of EGFR (as well as HER2), providing longer inhibition of EGFR. In a decisive large randomized trial, Afatinib in a preselected EGFR-mutant population was compared to the state-of-the-art chemotherapy with pemetrexed and cisplatin, and was shown to be superior. Afatinib is undergoing clinical testing as single therapy or in combination treatments in many clinical trials. In July 2013 Afatinib was approved as the first-line treatment for advanced stage NSCLC with class I and class II EGFR mutations.

Icotinib is another EGFR inhibitor clinically developed in China and reported to be active against mutant and wild-type forms of the receptor. A phase 3 randomized trial of Icotinib versus Gefitinib showed the non-inferiority of Icotinib and a much better profile in terms of drug-related side effects.

Dacomitinib, a pan-EGFR-family irreversible inhibitor is currently in several trials in NSCLC. In a recent trial, treatment with Dacomitinib was associated with a median PFS of 12.4 weeks compared with 8.3 weeks for Erlotinib in patients with adenocarcinoma. However, a superior PFS with Dacomitinib compared to Erlotinib was not observed in other histological subtypes of NSCLC.

There are a number of other trials evaluating the efficacy of combining first- or second-generation EGFR inhibitors with other molecularly targeted agents.

Many clinical trials for recurrent or advanced NSCLC involve Erlotinib in combination with other therapies. Results of a trial using combination of Erlotinib and Tivantinib/ARQ 197, a non-ATP-competitive inhibitor of MET, showed clinical activity in patients with NSCLC, with 6 of 8 patients achieving stable disease.

A phase 3 randomized trial explored addition of Erlotinib to the doublet chemotherapy with cisplatin/gemcitabine as a first-line treatment in patients with advanced NSCLC. Addition of Erlotinib significantly prolonged PFS in patients with mutant EGFR.

The use of RTK inhibitors in wild-type EGFR NSCLC patients remains a subject of controversy, with some investigators advocating it, while others provide data analysis indicating no benefit in this large heterogeneous population of patients.

The First-Line Erbitux in Lung Cancer (FLEX) phase 3 worldwide study demonstrated that Cetuximab, a monoclonal antibody directed against EGFR when to a platinum-based doublet (cisplatin and vinorelbine) can extend median OS in patients with advanced EGFR-expressing NSCLC (stage wet IIIB or stage IV). As a result, the use of Cetuximab combined with cisplatin-vinorelbine has been endorsed by National Comprehensive Cancer Network (NCCN) guidelines as a first-line option for the treatment of advanced NSCLC.

RESISTANCE TO EGFR INHIBITORS

Resistance to EGFR inhibitors arises because of cellular heterogeneity within an oncogene-addicted tumor. Tumor shrinkage indicates a response to a molecularly targeted therapy, but residual tumor may be a source of slow growing drug-tolerant "persistor" cells that promote tumor regrowth, regeneration, and heterogeneity.

Nearly all patients with EGFR-mutant- AC develop resistance to the first-generation inhibitors Erlotinib and Gefitinib, with a median PFS of 14 months.

Although EGFR kinase mutations are associated with an enhanced sensitivity to Gefitinib and Erlotinib, not all tumors that have activating mutations are associated with an enhanced response. Tumors that fail to respond to EGFR TKIs despite the presence of an activating mutation might have an additional genetic lesion that relieves the tumor of its dependence on the EGFR signaling pathway. One mechanism that has been linked to insensitivity of NSCLC to EGFR TKIs is the occurrence of insertion point mutations in exon 20 of the EGFR gene.

The most common and first identified secondary mutation is the threonine-790 to methionine (T790M) point mutation in exon 20 which represents...
approximately 50% of all acquired resistance in NSCLC. The development of such genetic alteration restores the EGFR TK affinity to ATP, rendering first-generation TKIs inactive. T790M mutation has been identified in pre-treatment tumors and is responsible for both a lower sensitivity and duration of response to the first generation TKIs.

Other critical resistance mechanisms have been identified to be linked to the inhibition of EGFR and include MET amplification, PTEN loss, HER2 kinase domain or PIK3CA and transformation to small cell lung cancer.

Concerns have been raised about resistance to TKIs due to mutations in the KRAS gene (which encodes for a protein downstream to the EGFR in the pathway initiated by the activation of the tyrosine kinase) or due to mutations that occur in other proteins of the same cascade such as BRAF.

Other resistance point mutations, such as aspartic acid-761 to tyrosine (D761Y), have been reported, some of which may weaken the interaction of EGFR TKI with its target.

In common with resistance mechanisms identified for EGFR, ALK-rearranged cancers have been reported to develop secondary mutations.

OVERCOMING RESISTANCE TO EGFR INHIBITORS

Management of EGFR tumor resistance has become the next challenge in order to extend these patients' overall survival; identification of the molecular resistance mechanisms will allow for the treatment of TKI-resistant tumors.

A new class of drugs, the so-called second-generation TKIs, may be able to overcome the T790M mutation resistant cell. Compared to first-generation TKIs, these molecules show higher affinity for the ATP-binding domain, form an irreversible covalent bond to the ATP-binding site and are able to stimulate other receptors (e.g. HER2).

The second-generation TKIs Afatinib irreversibly inhibit RTKs of EGFR family, as well as the T790M variant of EGFR.

The second-generation TKIs Neratinib hasn't shown good RR when tested on patients with known T790M mutation.

Dacomitinib is another irreversible TKI able to target the activity of all HER TKs and has shown activity in NSCLC cell lines harboring the T790M mutation. This molecule has been evaluated in two phase II trials: The first one was after failure of one or two chemotherapy regimens and failure on Erlotinib; the second one compared it with Erlotinib in the second and third-line in patients with advanced NSCLC with promising results.

Another approach for overcoming resistance to reversible EGFR TKIs involves targeting parallel- or convergent signaling pathways. The mammalian target of the rapamycin (mTOR) signaling pathway integrates nutrient and mitogen signals to regulate cell proliferation, survival and angiogenic pathways, and has been implicated in resistance to EGFR inhibitors. In both sensitive and resistant tumor cell lines, the mTOR inhibitor, Everolimus, reduces the expression of EGFR signaling effectors and cooperates with Gefitinib to overcome resistance.

Various drugs or antibodies capable of inhibiting MET (e.g., Crizotinib, Foretinib, ARQ 197, MetMAb) could, in principle, be combined with the first (Erlotinib) or second (Dacomitinib, Afatinib) generation EGFR-TKIs. Concurrent inhibition of both may improve patient outcomes. Small-1399 molecule inhibitors of MET and MetMAb/Onartuzumab are currently being tested in NSCLC. However, the phase III trial of Onartuzumab combined with Erlotinib in MET positive EGFR mutant NSCLC failed to improve PFS or OS in spite of the positive results from a phase II trial.

Many cancers have increased levels of active Hsp90, which is involved in protein folding. HSP90 is a molecular chaperone that is critical for tumor growth and proliferation. Client proteins of HSP90 include many signaling kinases such as RTKs and intracellular kinases essential for cancer cell survival, since lack of HSP90 triggers protein degradation. Hsp90 inhibitors may thus block multiple signaling pathways that are functioning aberrantly in cancer cells. Hsp90 inhibitors such as AUY922 and STA9090 are in many clinical trials for lung cancer. Both inhibitors showed good efficacy in preclinical models of NSCLC.

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toxicities, severe myelosuppression and neutropenic sepsis, with treatment-related fatal events in up to 8% of patients, especially in patients with poor performance status. Fatal events during EGFR TKI treatment in the form of lung or liver toxicity have been rarely reported. The most common adverse events leading to TKI dose reductions are cutaneous reactions (acneiform ‘rash’), paronychia and diarrhea, because of the abundance of EGFR in skin and mucosa. Meta-analysis confirmed that the appearance of skin rash was an independent predictive factor for survival (HR 0.30; p< 0.00001) and tumor progression (HR 0.50; p< 0.00001) during Erlotinib and Gefitinib treatment.

In first-line NSCLC trials of Erlotinib and Gefitinib, most patients developed rash of grades 1 or 2. Only few patients experienced grade 3 rash (Erlotinib: 67%, 2–13% grade ≥ 3; Gefitinib: 66%, 3% grade ≥ 3). Skin reactions normally appear after 1 week and reach maximum severity following 2–3 weeks of TKI treatment, after which they gradually and spontaneously disappear.

Diarrhea also affects a substantial part of EGFR TKI-treated patients. It is thought to result from excess chloride secretion (‘secretory diarrhea’), which can lead to dehydration, electrolyte imbalances, renal insufficiency, and malnutrition. In first-line NSCLC trials, the diarrhea incidence was reported as high as 52% (5% grade ≥ 3) for Erlotinib and 47% (4% grade ≥ 3) for Gefitinib.

A much less frequent but potentially lethal (30–50% lethality) side effect of EGFR TKIs therapy is interstitial lung disease (ILD), which in general occurs during the first 3 months of treatment (median 24–42 days) in < 1% of all patients, with higher risk in Japanese populations (1.6–3.5%) and Chinese patients. Pre-existing pulmonary fibrosis, prior thoracic irradiation and smoking history have been identified as further risk factors for developing ILD and should be taken into account when considering a patient for first-line EGFR TKI treatment.

Other side effects may occur and mostly reversible include fatigue, nausea/vomiting, and increased liver enzyme levels. Advanced or metastatic NSCLC, palliative chemotherapy is associated with only modest survival prolongation and indeterminate impact on quality of life. Personalized therapy for NSCLC patients should include a genetic testing of the EGFR mutational status for individual patients. Genomic testing for personalized treatment of lung cancer is now associated with improved survival, likely due to getting targeted kinase inhibitors to the indicated patients. All patients with EGFR mutation-positive lung adenocarcinoma must be offered an EGFR TKI therapy. The main approach to block the EGFR pathway is by competing with ATP for binding to the tyrosine kinase domain. The EGFR TKIs Erlotinib and Gefitinib are reversible inhibitors of the EGFR kinase and are also called “first-generation” small molecular inhibitors. The second-generation TKIs Afatinib may overcome resistance to the treatment of Erlotinib or Gefitinib through the T790M gatekeeper mutation. Several irreversible EGFR inhibitors blocked multiple EGFR family members, interrupting the cooperative signal pathway among EGFR members and resulted in a more complete blockade. EGFR inhibitors are better tolerated than chemotherapy. Side effects like rash and diarrhea are manageable in the outpatient setting under close surveillance, in particular during the first weeks of treatment. The problem with targeted therapies is that most of the time clinical responses to them are short-lived. The mechanisms of resistance are multifaceted, and might involve new resistance-conferring mutations in the target protein itself (such as T790M in EGFR) or activate lateral signal-transduction pathways via new mutations or changes in the expression level of the key proteins. Close cooperation between clinicians, surgeons, molecular biologists and pathologists is crucial for a continuous improvement in the field of NSCLC target therapy.

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