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Treatment of Non Small Cell Lung Cancer: Overcoming the resistance to EGFR inhibitors

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Abstract

PURPOSE OF REVIEW—Testing for epidermal growth factor receptor (EGFR) mutations has become standard practice in treating patients with advanced nonsmall cell lung cancer (NSCLC). EGFR tyrosine kinase inhibitors (TKIs) are being offered as first line therapy in patients with EGFR activating mutations. These drugs offer an increased progression free survival and response rate compared to standard chemotherapy in this setting, however resistance invariably occurs. This review discusses the development of resistance to EGFR TKIs and the progress that is being made to better understand how to overcome this resistance.

RECENT FINDINGS—Results from recently published papers dealing with resistance to EGFR TKIs are allowing for a better understanding of this mechanism. No one treatment allows for overcoming this resistance. Understanding this resistance will likely become an individualized patient/tumor approach. Selecting which drug or drugs that may be suitable can only be determined based on the molecular mechanism of resistance.

SUMMARY—Progress is being made in our understanding of the multiple pathways of resistance. Using a tumors molecular signature at the time of progression can determine the best treatment option.

Keywords

Epidermal Growth Factor Receptor; Tyrosine Kinase Inhibitors; Resistance; Lung Cancer

Introduction

The epidermal growth factor receptor (EGFR) is part of the family of tyrosine kinase receptors comprising four receptors in the Human Epidermal Receptor (HER -1,-2,-3,-4) family. The EGFR/(HER-1) signals and activates downstream pathways that result in tumor cell proliferation, survival, and metastasis in nonsmall cell lung cancer (NSCLC).(1) Mutations in the EGFR receptor occur in up to 13% of North American and European populations and up to 50% in East Asian populations.(2, 3) The majority of these mutations are activating mutations or gain-of-function mutations occurring in exons 19 and 21.(4) Exon 19 mutations are often in-frame deletions and exon 21 mutations are usually point

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mutations (e.g., L858R); they result in increased activation of the downstream pathways.(5) These mutations cluster around the adenosine-5'-triphosphate (ATP)-binding domain (tyrosine kinase domain (TKD)) and confer sensitivity to the first generation tyrosine kinase inhibitors (TKIs) erlotinib (Genentech; San Francisco, CA, US) and gefitinib (AstraZeneca; Wilmington, DE, US). These activating mutations also confer oncogene-addiction. Blockage of this pathway in these selected advanced NSCLC patients by first generation TKIs have demonstrated high initial response rates and increased progression-free survival (PFS). Gefitinib was compared to the chemotherapy carboplatin-paclitaxel in the front line setting demonstrating 12-month PFS rates of 24.9% compared to only 6.7% with chemotherapy.(6) Erlotinib has also demonstrated increased PFS in a similar population of 13.1 months vs. 4.6 months in patients receiving the chemotherapy carboplatin-gemcitabine.(7) Meta-analysis evaluating NSCLC patients with activating EGFR mutations pooled 12 trials evaluating erlotinib (365 patients), 39 trials evaluating gefitinib (1069 patients) and 9 evaluating chemotherapy (375 patients) in weighted pooled analysis. The overall median PFS was 13.2 months with erlotinib, 9.8 months with gefitinib and 5.9 months with chemotherapy.(8)

Development of Resistance

The latest guidelines recommend testing all advanced NSCLC tumors for EGFR activating mutations and treating positive tumors with EGFR TKIs in the first line setting. Management of EGFR tumor resistance has become the next challenge in order to lengthen these patients' overall survival.

Secondary Mutations: EGFR resistant mutations

One of the first identified mechanisms of acquired resistance to EGFR TKI was the discovery of an acquired point mutation in exon 20. This mutation is in the tyrosine kinase domain (TKD) of the EGFR gene rendering the 1st generation TKIs (erlotinib and gefitinib) inactive.(9) The threonine-790 to methionine (T790M) point mutation represents approximately 50% of all acquired resistance in NSCLC.(10) The development of this "gatekeeper" mutation is analogous to the ABL T315I in chronic myelogenous leukemia.(11) The development of a T790M restores the EGFR tyrosine kinase domain affinity to ATP.(12) Improved sequencing techniques for EGFR mutations have now shown that some patients harbor the T790M in a small number of tumor cells at diagnosis.(13) During treatment of these tumors with 1st generation TKI, clonal selection allows for an increasing number of tumor cells bearing the T790M and therefore becomes the larger percentage of cells in the tumor mass.(14)

Other secondary resistant mutations have been described at a much lower incidence (<5%). To date these mutations are mainly case reports of an acquired D761Y, L747S, in known L858R mutated lung cancer.(15) The D761Y, L747S and T854A mutations have also been identified in pretreatment tumors and similarly do not respond to the 1st generation TKIs. (14, 16)

MET amplification

Another form of resistance in EGFR mutated tumors develops through amplification of the mesenchymal-epithelial transition factor (MET). Upon initial diagnosis of NSCLC, MET gene amplification is uncommon, however, acquired MET amplification has been noted in up to 20% of EGFR mutated tumors that have been pretreated with an EGFR TKI (Figure 1).(17) Bean et al. reported that MET amplification occurs in 21% of tumors treated with a TKI as compared to only 3% in untreated patients with NSCLC.(18) Conversely, a study of 37 patients that underwent repeat biopsying at the time of acquired EGFR TKI resistance found the MET amplification only occurring at a rate of only 5%.(19) In tumors that contain the MET gene amplification, stimulation of the tumor occurs via the co-receptor Human

Epidermal Receptor-3 (HER-3) resulting in activation of the phosphatidylinositol-3-kinase (PI3K) signaling pathway, thereby circumventing the effects of an EGFR TKIs.(20)

Increased Signaling in Other Pathways

Other parallel signaling pathways may contribute to the development of resistance to EGFR TKIs, such as the vascular endothelial growth factor (VEGF) receptor and insulin-like growth factor-1 receptor (IGF-1R). Exposing NSCLC cell lines to anti-EGFR antibodies results in a 4 fold up regulation of VEGF.(21) Activation of the VEGF pathway can co-stimulate the tumor cells. Similarly, IGF-1R can activate downstream targets in the EGFR pathway and thereby bypassing cell dependency on the EGFR receptor.(22) In NSCLC cell lines that have been continuously exposed to a 1st generation TKIs, an increased activation of the IGF-1R has led to cell growth.(23) NSCLC patients with increased IGF-1R gene copy number has been associated with decreased survival.(24)

KRAS and BRAF mutations

Ras genes encode a family of membrane-bound 21-kD guanosine triphosphate (GTP)-binding proteins that regulate cell growth, differentiation, and apoptosis. Point mutations of one of the three *Ras* genes (*H-Ras*, *K-Ras*, *N-Ras*) result in impaired GTP-ase activity and constitutive activation of Ras-Raf-MEK-ERK cytoplasmic kinase cascade. In lung adenocarcinoma, *Ras* is mutated in approximately 30% of cases and *K-Ras* mutations account for more than 90% of those mutations. Patients with *K-Ras* mutant tumors are more likely to be former/current smokers, present with locally advanced disease, are more likely to have adenocarcinomas and are unlikely to harbor *EGFR* mutations.(25–28) Patients with *K-Ras* mutations usually do not respond to EGFR TKIs.(29) *B-Raf* mutations are detected in only 2–3% of NSCLC, are mutually exclusive of *Ras* and *EGFR* mutations and are seen predominantly in current or former smokers. Unlike the V600E substitution, which accounts for the majority of the *B-Raf* mutations in other tumor types, approximately 90% *B-Raf* mutations in NSCLC are non-V600E.(30, 31)

PI3K/AKT mutations and activation

The phosphoinositide 3-kinase (PI3K) family of lipid kinases and its downstream mediators, PIP3 and the serine-threonine protein kinase, AKT, form a growth and survival signaling pathway, which may be constitutively activated by several mechanisms including somatic mutations of its components and activation of RTKs.(32) *PIK3CA*, which encodes p110 α isoform, the main catalytic subunit of PI3K, is mutated in 3–4% and amplified in 12–20% (with a higher incidence in squamous cell cancers) of NSCLC. *PIK3CA* mutations are not mutually exclusive of *EGFR* or *KRAS* mutations.(33–36) AKT activation is found in 30–75% of NSCLC and in 2% of cases (limited to squamous cell subtype), an E17K point mutation of *Akt1* leads to its PI3K-independent activation.(37, 38) AKT activation is a poor prognostic factor and has been implicated in resistance to chemotherapy and radiation.(39, 40)

Histological Transformation

Sequist et al. recently reported on a cohort of 37 patients with advanced NSCLC that underwent repeat biopsying at the time of progression on an EGFR TKI. Five of the 37 patients underwent a histological transformation into a small cell lung cancer phenotype.(19) These transformed cancers responded to traditional SCLC chemotherapy regimens.

Strategies for overcoming resistance to EGFR inhibitors

Identification of the molecular resistance mechanisms will allow for the treatment of EGFR TKI resistant tumors.

Second generation TKIs

Second generation TKIs targeting EGFR have a higher affinity for the ATP binding domain and form an irreversible covalent bond to the ATP binding site. Three of these agents have undergone testing in advanced NSCLC.

Neratinib(HKI-272) (Wyeth Pharmaceuticals, Pfizer; New York, NY; US) is an irreversible TKI with activity against both EGFR and HER2 receptors. Neratinib has been tested in 167 patients with advanced NSCLC after failure of a 1st generation TKI. The best response rate (RR) was 3% and no patients with known T790M responded.(41) Further development in NSCLC has been halted.

Afatinib (BIBW 2992) (Boehringer Ingelheim, Ridgefield, CT; US) is another 2nd generation irreversible TKI that has kinase activity against EGFR and HER-2. Preclinical results demonstrated afatinib is effective in lung cancer models, including T790M (EGFR) mutations. Afatinib is being investigated as part of the LUX-Lung program, which will evaluate afatinib as a first-line treatment in patients with EGFR-activating mutations (LUX-Lung 2, 3 and 6) and in the second or third line treatment in patients that have acquired resistance to gefitinib or erlotinib (LUX-Lung 1, 4 and 5). LUX-Lung 1 and 2 have demonstrated an increase in the disease control rate of 58% and 86%, and a prolongation of PFS.(42, 43)

Dacomitinib (PF-00299804) (Pfizer; New York, NY, US USA) is another oral, irreversible, TKI that targets the kinase activity of all active HER (-1, -2, and -4) tyrosine kinase domains. Dacomitinib has shown activity in NSCLC cell lines harboring T790M.(44) Dacomitinib has been evaluated in an open-label, single-stage, phase II trial evaluating patients with wild-type *KRAS* advanced NSCLC after failure of 1 or 2 chemotherapy regimens and failure on erlotinib. Sixty-five patients were enrolled with 3 partial responses and a disease control rate.(45) Dacomitinib has also been evaluated in another phase II trial comparing it to erlotinib in the second and third line setting in 188 patients with advanced NSCLC and was associated with improvement in PFS (HR 0.681; 95% CI, 0.490–0.945; $p = 0.019$) and objective response rate (17% vs. 4.3%). However, the percentage of tumors harboring EGFR mutations was unbalanced (dacomitinib 20.2% vs. erlotinib 11.7%).(46) Further development of this drug will focus its use in upfront therapy over first generation TKIs and in resistant EGFR mutated tumors.

MET inhibitors

MET receptor activation is associated with a poor prognosis in NSCLC and with EGFR TKI resistance in NSCLC.(47) The targeting of the MET pathway has been attempted with small molecules and with monoclonal antibodies.

Tivantinib (ARQ197) (ArQule; Woburn, MA; USA) is an oral, small molecule inhibitor of c-Met. Tivantinib binds to the c-Met protein and disrupts c-Met signal transduction pathways in a non-ATP-competitive manner causing cell death in tumor cells overexpressing c-Met protein. Tivantinib has been evaluated in a randomized phase II trial examining erlotinib plus tivantinib versus placebo. This trial enrolled previously treated patients with EGFR TKI-naïve advanced NSCLC. Patients were randomly assigned to receive erlotinib plus oral tivantinib (ET) or erlotinib plus placebo (EP). The primary end point was PFS and at time of progression and allowing cross-over from the placebo group. Of the 167 patients enrolled the median PFS was 3.8 months for ET and 2.3 months for EP (hazard ratio [HR], 0.81; 95% CI, 0.57–1.16; $P=.24$). Objective responses were seen in 10% of patients on ET, 7% of patients on EP, and in two patients who crossed over, including one with EGFR mutation and MET gene copy number greater than 5.(48) The study did not

meet its primary end point, however in subgroup analysis in patients harboring mutated KRAS tumors had an unexplained benefit from the combination.

Cabozantinib (XL 184) (Exelixis, Inc; San Francisco, CA, US) is an ATP-competitive small molecule inhibitor that targets both MET and vascular endothelial growth factor receptor 2 (VEGFR2). Early observations of clinical benefit were observed in phase I studies.(49) Cabozantinib is currently being investigated in combination with erlotinib to overcome EGFR TKI resistance.(50)

Crizotinib (PF-02341066) (Pfizer; New York, NY, USA) is an oral, ATP-competitive inhibitor of ALK and c-Met/Hepatocyte Growth Factor Receptor (HGFR) tyrosine kinases. Crizotinib was recently approved by the United States Federal Drug Administration (FDA) for the treatment of NSCLC adenocarcinoma bearing EML-4-ALK translocations after a phase I and phase II testing demonstrated significant activity.(51) Crizotinib has been examined for its antitumor action in lung cancer cells that are both positive and negative for MET amplification or mutations. Inhibition of MET signaling by crizotinib has demonstrated the inhibition of the AKT pathway as well as extracellular signal-regulated kinase phosphorylation activity resulting in apoptosis of lung cancer cells that are MET amplified.(52)

MetMAB (OAM4558g) (Roche; Basel, Switzerland) is a humanized monoclonal antibody that targets c-MET. MetMAB has been evaluated in combination with erlotinib in a phase II trial of unselected NSCLC patients that have undergone previous treatment. The combination of MetMAB to erlotinib did not show a statistically significant improvement in PFS compared to erlotinib alone. Unfortunately, the trial failed to select patients based on EGFR mutational status and by overexpression of MET.(53) Subset analysis demonstrated the addition of MetMAB to erlotinib as second or third line therapy improved PFS and OS in patients with MET overexpression.(54)

HSP-90 inhibitors

Sequist et al. evaluated retaspimycin (IPI-504) (Infinity Pharmaceuticals, Cambridge, MA, US) an inhibitor of heat-shock protein 90 (Hsp90) in NSCLC. Seventy-six patients with advanced NSCLC were enrolled after the failure of an EGFR TKI. The ORR was 7%, 4 tumors were EGFR wild-type and 1 had an EGFR mutation. Restapimycin did not demonstrate clinical activity in patients with resistant EGFR mutated NSCLC.(55)

MTOR inhibitors

Everolimus (RAD-001) (Novartis Pharmaceuticals, East Hanover, N.J.) is an oral inhibitor of the mammalian target of rapamycin (mTOR) that initially demonstrated activity in phase I trials in NSCLC patients. Everolimus has been evaluated in advanced NSCLC patients, after failure of two or fewer chemotherapy regimens, one platinum based (stratum 1) or both chemotherapy and EGFR-TKIs (stratum 2). Of the 85 patients enrolled, the ORR was 4.7% (7.1% stratum 1; 2.3% stratum 2) and a median PFS of 2.6 months.(56) Riley et al. attempted to evaluate everolimus after failure of TKI therapy. Three weeks after restarting erlotinib or gefitinib, everolimus was added to treatment. Only 10 patients completed the study and no responses were observed.(57)

PI3K/AKT inhibitors

BKM120(Novartis Pharmaceuticals, East Hanover, N.J.), a selective inhibitor of class I PI3K enzymes, and is currently being investigated in NSCLC patients with tumors that exhibit PI3K pathway activation. GDC-0941(Genentech, South San Francisco, CA), is a pan-PI3K inhibitor being studied in combination with erlotinib and conventional chemotherapy.

MK-2206(Merck, Whitehouse Station, NJ), a non-ATP competitive, allosteric AKT inhibitor that is well tolerated and has induced sustained AKT blockade in a phase I trial. (58) Preclinical studies have shown synergistic inhibition of cell proliferation with the combination of MK-2206 and EGFR-TKIs and is currently in phase II trials.

MEK inhibitors

ERK1/2 is the only known substrate of MEK (or MAPK) and, in pre-clinical studies, activating mutations of *Ras* and *B-raf* identified tumors are sensitive to MEK inhibition.(59, 60) In unselected pre-treated NSCLC patients, monotherapy with selumetinib (AZD6244) (AstraZeneca; Wilmington, DE, US), a selective non-competitive ATP inhibitor of MEK1/2, offered no advantage over standard treatment with pemetrexed.(61) Ongoing trials are now evaluating the efficacy of selumetinib, alone or in combination with EGFR inhibition, in tumors with and without *Ras* mutations (Figure 2).

IGFR-1 inhibitors

Figitumumab (CP-751871) (Pfizer; New York, NY. US) is a monoclonal antibody that targets IGF-1R. Figitumumab has undergone testing in a large multicenter randomized phase III trial which added the monoclonal antibody to standard first line chemotherapy for advanced NSCLC. This trial was stopped secondary to no improvement in overall survival with increased toxicities. Data from this trial has been further analyzed and found that free IGF-1 may be a potential predictive biomarker to predict the clinical benefit. Of the 110 patients enrolled on the trial pre-treatment circulating levels of free IGF-1 correlated inversely with IGF binding protein 1, ($p=0.005$), and the pre-treatment ratio of insulin to IGFBP-1 was predictive of clinical benefit from figitumumab.(62)

Conclusion

Sensitizing mutations occur frequently in the EGFR receptor in NSCLC and the use of 1st generation TKIs can increase initial response rates and prolong PFS. Unfortunately the PFS benefit is limited to months and inevitably resistance develops. Targeting the resistance mechanism may be the way forward and may require biopsying patients at the time of progression.

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Key Points

- Initial molecular analysis of NSCLC tumors for EGFR activating mutations is the standard of care.
- A subset of these patients will have resistant mutations at baseline molecular testing.
- Identifying the mechanisms of resistance will allow patients to become prescreened for clinical trials at progression.
- Offering repeat biopsying of patients at the time of progression with molecular profiling will allow patients to be referred, screened and ultimately treated.
- Personalized treatment trials are needed to increase the survival in advanced NSCLC EGFR mutated patients.

Mechanisms of acquired resistance to first generation EGFR TKIs

Legend
EGFR: Epidermal Growth Factor Receptor
TKIs: Tyrosine Kinase Inhibitors
T790M: threonine-790 to methionine
MET: mesenchymal-epithelial transition
SCLC: Small Cell Lung Cancer

Figure 1.

Evaluation of the MEK inhibitor (selumetinib) in combination with erlotinib

Figure 2.