Activity of Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors in Patients with Non-small Cell Lung Cancer Harboring Rare Epidermal Growth Factor Receptor Mutations

Tommaso De Pas, MD,* Francesca Toffalorio, MD,* Michela Manzotti, PhD,† Caterina Fumagalli, PhD,† Gianluca Spitaleri, MD,‡ Chiara Catania, MD,‡ Angelo Delmonte, MD,‡ Monica Giovannini, MD,* Lorenzo Spaggiari, MD,§ Filippo de Braud, MD,‡ and Massimo Barberis, MD†||

Introduction: Mutations of the epidermal growth factor receptor (EGFR) have been proven to predict activity of the EGFR-tyrosine kinase inhibitors (EGFR-TKIs), gefitinib and erlotinib. Although the “common” EGFR mutations, such as the L858R point mutation in exon 21 and the in-frame deletional mutation in exon 19, have been definitively associated with response to EGFR-TKIs, the correlation with response to treatment for many other rarer mutations is still unclear. In this study, we report the results of treating patients with advanced non-small cell lung cancer harboring rare EGFR mutations treated with EGFR-TKIs.

Methods: The frequency of rare mutations has been investigated in 681 cases of non-small cell lung cancer screened between 2006 and 2010. Mutations in exons 18 and 20, uncommon mutations in exons 19 and 21, and/or the presence of different mutations in a single tumor (complex mutations) were considered rare.

Results: EGFR mutations were detected in 99 tumors (14.5%). Eighteen cases carried rare mutations, and 10 of these patients were treated with erlotinib or gefitinib. The clinical outcome was described case by case with references to the literature. Of note, we found two EGFR mutations never identified before and one of unknown response to EGFR-TKIs.

Conclusions: Gefitinib and erlotinib have different antitumor activity according to the type of the EGFR mutation borne. Report of cases harboring rare mutations can support the decision-making process in this subset of patients.

Key Words: Rare EGFR mutation, Erlotinib, Gefitinib.

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TKIs.9 Nevertheless, mutations in exon 20 are relatively rare, suggesting that other mechanisms probably contribute to EGFR-TKI primary resistance in metastatic NSCLC. For many of the rare mutations, the effect on responsiveness remains unknown. Thus, it is of extreme importance for the clinical decision-making process to share information of patients harboring such mutations, particularly when the outcome of EGFR-TKI treatment is available.

We report herein our single institute’s experience in the treatment of patients with rare EGFR mutations. Mutations in exons 18 and 20, uncommon mutations in exons 19 and 21, and/or the presence of different mutations in a single tumor (complex mutations) were all considered rare. From 2006 to 2010, we screened 681 cases of NSCLC for $EGFR$ mutations and found 18 cases with rare mutations. Ten of these patients were treated with gefitinib or erlotinib, and we discuss their clinical outcome case by case with references to the literature, if available. Of note, two novel mutations were identified and in a third case, this is the first report of EGFR-TKI efficacy with such a mutation. The other mutations have been previously described in only a handful of case reports.

**MATERIALS AND METHODS**

**Sample Collection and EGFR Mutation Analysis**

$EGFR$ mutational analyses were performed in 681 patients who underwent curative surgery or surgical biopsy between 2006 and 2010 at the European Institute of Oncology, Milan, Italy. All the patients were Caucasian, and none had received any EGFR-TKI before DNA sequencing. Each patient signed a written informed consent for the use of tissue for molecular analysis.

Rare mutations were defined as mutations in exons 18 and 20, unusual ones occurring in exons 19 and 21, and/or complex mutations (different mutations co-occurring within the same tumor).

Microscopically guided dissections were performed by a pathologist in 545 of the 681 cases analyzed (80%). The percentage of tumor content was above 50 in all the cases. Cases with lower tumor content were excluded from this series. All the mutations, mainly the rare or “new mutations” (never reported in the Catalogue of Somatic Mutations in Cancer Database, www.sanger.ac.uk/genetics/CGP/cosmic/) were confirmed by a further analysis of the same specimen.

Ten of the 18 patients harboring rare mutations were treated with gefitinib or erlotinib, and we discuss their clinical outcome case by case with references to the literature, if available. Of note, two novel mutations were identified and in a third case, this is the first report of EGFR-TKI efficacy with such a mutation. The other mutations have been previously described in only a handful of case reports.

**Evaluation of EGFR-TKI Efficacy**

Ten of the 18 patients harboring rare mutations were treated with gefitinib or erlotinib. The patients’ characteristics are summarized in Table 1. Gefitinib was taken orally at the dose of 250 mg daily, and erlotinib was taken at the dose of 150 mg daily, until tumor progression, death, or patient refusal. All patients had a pretreatment tumor assessment by

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<sup>a</sup> Overall partial response but progression at a single lymph node station and at bone metastases.

<sup>b</sup> Non-RECIST response followed by rapid tumor progression.

His, histology; adk, adenocarcinoma; undiff, undifferentiated NSCLC; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; del, deletional mutation; ins, insertion; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer.
Response to EGFR-TKI in Patients with Rare Mutations

Exon 18 Mutations

E709A+G719C

Case report. The E709A+G719C double mutation was found in an 80-year-old female never smoker with advanced adenocarcinoma of the lung with acinar and BAC characteristics. Other molecular features were absence of EGFR amplification, high EGFR chromosome 7 polysomy, and absence of K-Ras mutations.

After pulmonary and mediastinal tumor progression, the patient was treated with single-line monochemotherapy (weekly intravenous vinorelbine), which was interrupted early due to toxicity. Erlotinib was subsequently given at the starting dose of 150 mg daily, reduced to 100 and 75 mg due to G3 skin toxicity. The tumor assessment, performed 2 months after the beginning of the treatment, showed a partial response (PR), still maintained after 10 months.

Review of the literature. The complex mutation has been previously described in a few reports. In vitro data suggest that the coexistence of the E709A mutation confers resistance to the gefitinib-sensitive G719C mutation. Nevertheless, the low sensitivity to EGFR-TKIs of the double mutant has not been confirmed by Hijiya et al. Indeed, in the single case reported by these authors, a female never smoker of Asian origin, with an adenocarcinoma of the lung, had a major tumor response on treatment with gefitinib.

Conclusion. According to the cases described so far, the E709A+G719C complex mutation seems to be associated with sensitivity to EGFR-TKI.

G719S

Case report. The G719S mutation was found in a 50-year-old female never smoker with advanced adenocarcinoma of the lung. Other molecular features were absence of EGFR amplification, low EGFR chromosome 7 trisomy, and absence of K-Ras mutations.

Initially, the patient underwent six cycles of cisplatin and gemcitabine with minimal tumor response for locally advanced disease. One month after the end of chemotherapy, because of the appearance of brain and bone metastases, she started erlotinib at 150 mg/d. At the first computer tomography scan evaluation, performed 2 months later, disease reduction of multiple primary lung and nodal lesions was evident. Brain metastases were stable. Only a single mediastinal lymph node and the acetabular bone metastases (already evident. Brain metastases were stable. Only a single mediastinal lymph node and the acetabular bone metastases (already subjected to radiotherapy) were increased in dimension. The patient is still on treatment after 4 months.

Review of the literature. In vitro studies suggest that the EGFR-G719S mutation is oncogenic but is significantly less sensitive to gefitinib than the more common L858R. Three patients, all of Asian origin, have been reported with the G719S mutation: two patients showed no tumor response to gefitinib treatment, whereas only one had a PR.
Conclusion. In the literature, the G719S is not associated with a clear resistance to EGFR-TKIs, as suggested by in vitro data. Notably, in our experience, the patient had an overall major tumor response but progressed at a single lymph node station and at the bone level.

Exon 19 Mutations

del I744_K745insKIPVAI

Case report. The del I744_K745insKIPVAI mutation was found in a 36-year-old former female smoker, with an adenocarcinoma of the lung. Other molecular features included the presence of EGFR amplification (fluorescence in situ hybridization), high EGFR chromosome 7 polysomy, and absence of K-Ras mutations (codon 12, 13 e 61).

At diagnosis, the patient also had synchronous metastases in lungs, liver, and bone. The patient was treated as first-line chemotherapy with carboplatinum and paclitaxel combined with a vascular disrupting agent/placebo (double-blinded randomized phase III study) for four cycles with liver, brain, and lung disease progression. After a whole brain radiation, she received erlotinib at a 150 mg daily dose. A clinical meaningful improvement of symptoms was obtained within a few days, and a tumor assessment after 8 weeks showed a PR. After 7 months, the patient experienced disease progression and died 2 months after treatment interruption.

Review of the literature. The presence of the del I744_K745insKIPVAI has already been reported in Asian and Caucasian patients by Kosaka et al.,20 Okami et al.,21 and Ilie et al.,22 but no information on the efficacy of EGFR-TKI is mentioned. The only evidence of treatment outcome relies on a single case of an Asian patient treated with gefitinib. The patient experienced tumor progression.

Conclusion. Differently from the single case reported in literature showing progression following gefitinib, the patient treated in our institution had a rapid symptomatic improvement and a RECIST PR.

Exon 20 Mutations

S784F

Case report. The S784F EGFR mutation was found in a 55-year-old current heavy male smoker, with pulmonary undifferentiated NSCLC not otherwise specified. Other molecular features included absence of EGFR amplification and absence of K-Ras mutations (codon 12, 13 e 61).

The tumor was diagnosed as stage IIIb pN3 with synchronous adrenal metastasis and seemed to be refractory to the cisplatinum and gemcitabine combination. The patient was subsequently treated with docetaxel, interrupted early because of an allergic reaction, and then received erlotinib 150 mg/d. The patient was taken off erlotinib after 2 weeks due to a lung infection and then started treatment again after 2 months. A tumor progression was observed after 10 weeks at the first treatment assessment.

Review of the literature. The S784F mutation was previously described in a single patient, of the 325 analyzed by Tsao et al.24 Nevertheless, no data dealing with EGFR-TKI activity are reported. The same mutation has been found by Ludovini et al.,25 but in association with the V786M in exon 20, in a never-smoking patient with an adenocarcinoma with BAC features. In this case, the patient had a complete and long-lasting remission after gefitinib treatment.

Conclusion. Differently from the case reported by Ludovini et al. where the S784F was linked to the V786M, in our experience the S784F EGFR mutation is associated with resistance to erlotinib.

S768_V769insVAS

Case report. S768_V769insVAS was detected in a 72-year-old woman with unknown smoking habits. Other molecular features were absence of EGFR amplification and absence of K-Ras mutations (codon 12, 13 e 61).

After diagnosis of pulmonary adenocarcinoma with synchronous bone metastases, the patient received gefitinib 250 mg/d upfront. Despite this treatment, she had a clinical progression after 3 months.

Review of the literature. A number of alterations within the EGFR S768 region, such as single nucleotide substitutions, in-frame deletions, and insertions, have been observed in a Japanese population.26 Nevertheless, no information about the responsiveness to EGFR-TKIs was reported.

Conclusion. Herein, we describe an insertion of the S768 region, the S768_V769insVAS, in a female of Caucasian origin. In our experience, this alteration confers resistance to gefitinib.

770_771insVDSVDNP

Case report. The 770_771insVDSVDNP EGFR mutation was found in a 55-year-old female never smoker with a pulmonary adenocarcinoma. Other molecular features were the absence of EGFR amplifications and the absence of K-Ras mutations (codon 12, 13 e 61).

The tumor was diagnosed as stage IV with synchronous liver, bone, and lung metastases. After a partial tumor response obtained with a combination of carboplatinum and paclitaxel, she received maintenance therapy with gefitinib 250 mg/d, with an immediate progressive disease (4 weeks). The patient received further treatment with cisplatinum plus gemcitabine, without neither clinical benefit nor tumor response. She received third-line chemotherapy with vinorelbine in another institution. The patient died 3 months later.

Review of the literature. To our knowledge, the 770_771insVDSVDNP mutation has never been reported before. Other, more frequent insertions within the same region, i.e., D770_N771insNPG, D770insSVQ, and D770_N771insG, occurring in almost 5% of NSCLC, have been widely studied. Greulich et al.9 demonstrated in vitro that in comparison with exon 19 deletions and with the L858R mutation, D770_N771insNPG is insensitive to EGFR-TKI. In keeping,
Wu et al. described a case of a Japanese never smoker female with an adenocarcinoma harboring an insertion at the D770_N771 level (D770_N771insD) who was resistant to gefitinib.

**Conclusion.** Herein, we report for the first time the L861R mutation; the patient bearing the alteration was resistant to treatment with gefitinib.

### Exon 21 Mutations

**L861R**

**Case report.** The L861R EGFR mutation was detected in a 34-year-old female never smoker, with a pulmonary adenocarcinoma with prevalent BAC characteristics. Other molecular features included the absence of EGFR amplification and the absence of K-Ras mutations (codon 12, 13 e 61).

The tumor was diagnosed as stage IV with synchronous liver, bone, and lung metastases. The patient received cisplatinum, gemcitabine, and bevacizumab as front-line treatment, with a partial tumor response after the first three cycles and tumor progression after three additional cycles. She was subsequently treated with erlotinib 150 mg/d but showed tumor progression after 2 months and died 1 month later due to brain and leptomeningeal progression.

**Review of the literature.** Data dealing with this EGFR mutation and response to EGFR-TKI were firstly reported by Yang et al. The mutation was found in a single case out of 109 NSCLCs analyzed. After gefitinib treatment, the patient had stable disease, according to RECIST criteria, for an unknown duration. Interestingly, in a different patient with a lung adenocarcinoma, the L861R mutation was found coexisting with an EGFR germline mutation (R831C). After a few months of gefitinib treatment, the patient displayed a PR. No information about the sensitivity of R831C to gefitinib is available.

**Conclusion.** Literature data and our experience suggest that the L861R EGFR mutation confers resistance to gefitinib treatment. Indeed, the only response to gefitinib observed in the presence of the L861R reported by Chung et al. could be attributed to the coexisting EGFR mutation, R831C.

**L861Q**

**Case report.** The L861Q EGFR mutation was detected in a 57-year-old female former smoker with a lung adenocarcinoma with prevalent BAC features. Other molecular features were the absence of EGFR amplification and chromosome 7 polysomy. No K-Ras mutations at codons 12, 13 e 61 were found.

The tumor was diagnosed with a multifocal omo-lobar disease and was treated with surgery followed by adjuvant chemotherapy (platinum and gemcitabine), which consented 2 years of disease-free survival. When the disease progressed, the patient received erlotinib 150 mg/d, but at the first tumor assessment (3 months), there was disease progression. The patient subsequently received a pemetrexed and gemcitabine combination resulting in a tumor response, followed by vinflunine with disease progression. She was further treated with vinorelbine and with paclitaxel in another institution, without clinical benefit.

**Review of the literature.** The L861Q EGFR mutation has been extensively investigated and is considered as one of the major drug-sensitive mutations.

**Conclusion.** In our experience, the L861Q EGFR mutation was associated with tumor progression, despite treatment with active doses of erlotinib and the presence of chromosome 7 polysomy, a positive predictive factor.

**L862V**

**Case report.** The L862V EGFR mutation was detected in a 54-year-old male light smoker (3 packs/y) with a pulmonary adenocarcinoma. Other molecular features were the absence of K-Ras mutations (codon 12, 13 e 61) and EGFR amplification. The tumor was diagnosed as stage IV with synchronous brain metastasis, and the patient was treated with induction chemotherapy (carboplatinum and paclitaxel, gamma knife on the brain lesion, and radical surgery for the primary tumor). One year later, because of disease progression, he received gefitinib, 250 mg/d, with further tumor progression in 2 months.

**Review of the literature.** To the authors’ knowledge, this mutation has never been previously reported.

**Conclusion.** This is the first description of L862V EGFR mutation. In our experience, this alteration is associated with gefitinib resistance.

**L858R+H870R**

**Case report.** The double L858R, H870R EGFR mutation were detected in a 56-year-old male never smoker with a pulmonary adenocarcinoma. Other molecular features were the presence of EGFR amplification and the absence of K-Ras mutations (codon 12, 13 e 61). The tumor was diagnosed as stage IV with synchronous brain and bone metastases. The patient received first-line chemotherapy with cisplatinum and gemcitabine in another Institution and then, at the time of progression, was started on erlotinib, 150 mg/d. At the first tumor assessment, 2 months before the beginning of treatment, a non-RECIST response was observed, but, during the following 3 months, the disease rapidly progressed, with brain metastases, leading to death.

**Review of the literature.** The L858R, H870R EGFR mutation was extensively studied by Tam et al. In vitro experiments demonstrated that both the single H870R and the double L858R/H870R mutations show a higher resistance to gefitinib compared with the single L858R mutation. Moreover, in one case of a tumor harboring the double mutation, there was an initial response to gefitinib but leptomeningeal metastases developed after 6 months. The authors speculate that the cerebral disease progression could be ascribed to dosage, suggesting that the double L858R/H870R mutation...
may require higher gefitinib administration to overcome the brain barrier effect.

Conclusions. Notably, similar to the case reported by Tam et al., in our experience, the L858R H870R mutation is associated to a short-term response to gefitinib followed by immediate and rapid tumor progression, in particular to the brain. These finding suggest that the additional presence of the H870R mutation could interfere with the extreme gefitinib sensitivity of the common L858R mutation indicated by in vitro experiments.

DISCUSSION

In this study, we report our single, institutional experience of the treatment of patients with NSCLC harboring various rare EGFR mutations.

The overall frequency of EGFR mutations in NSCLC is pretty high, ranging from 5 to 20%, depending on the population studied. Gefitinib and erlotinib, two small molecules that target EGFR, have proven their efficacy in the treatment of patients with this subset of tumors, showing a response rate of approximately 75%. The two most common mutations that account for 90% of all cases are termed “classical” activating mutations. One is a short in-frame deletion of exon 19 and the other a point mutation (CTG to CGG) in exon 21 at nucleotide 2573 resulting in the substitution of leucine by arginine at codon 858 (L858R). Nevertheless, not all activating mutations necessarily cause a full blown of the EGFR tyrosin-kinase activity. Indeed, it is widely accepted that in NSCLC, response rates to EGFR-TKIs are higher in patients with tumors carrying exon 19 mutations than in those with exon 21 mutations.

Moreover, the T790M mutation, as well as other point mutations, such as the D761Y (aspartic acid-761 to tyrosine) have been reported to be strongly associated with drug resistance probably by weakening the interaction of EGFR-TKI with its target. This type of mutation could, therefore, influence the sensitivity of the tumor to EGFR-TKIs. Things become even more complicated when rare mutations are encountered, as there is very little information in the literature concerning the efficacy of gefitinib and erlotinib in such cases.

For this reason, starting from an EGFR mutation screen, performed in our institute, we selected tumors with rare EGFR mutations and illustrated the response rate after gefitinib or erlotinib treatment. In detail, we analyzed 681 tumors and identified 18 cases, with rare mutations, defined as mutations in exons 18 and 20, uncommon mutations in exons 19 and 21, and/or complex mutations (different mutations present in a single tumor). These mutations accounted for approximately 2% of all the cases screened. Ten of these patients were treated with EGFR-TKIs, and we report herein the individual case reports with reference to the literature.

The various tumor responses observed supported the different predictive value of the single EGFR mutations in terms of treatment efficacy. Of note, in our experience, exon 20 mutations were associated with gefitinib and erlotinib resistance; however, there are only a few reports in the literature of cases responding to EGFR-TKIs. Therefore, we believe that the evaluation of the single mutations case by case could be useful also in the presence of exon 20 mutations, as in the presence of other uncommon EGFR mutations.

In conclusion, we have provided additional information regarding the efficacy of gefitinib and erlotinib for several rare EGFR mutations not previously or only rarely reported and have also identified two new mutations. We think that this data will be useful for the treatment of patients harboring these mutations, and we encourage the publication of similar studies to support the decision-making process in such subsets of patients.

REFERENCES


