Poor response to erlotinib in patients with tumors containing baseline EGFR T790M mutations found by routine clinical molecular testing

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Background: EGFR T790M is the most common mutation associated with acquired resistance to EGFR tyrosine kinase inhibitors (TKIs). Baseline EGFR T790M mutations in EGFR TKI-naïve patients have been reported, but the frequency and their association with response to EGFR TKIs remain unclear.

Patients and methods: The frequency of baseline EGFR T790M as detected by routine molecular genotyping was determined by reviewing clinical results obtained at our institution from 2009 to 2013. We also collected outcome data for treatment with EGFR TKIs.

Results: To define the incidence of EGFR T790M, we reviewed 2774 sequentially tested patients with lung cancer who underwent molecular testing using a mass spectrometry-based assay, and 11 (0.5%) had baseline EGFR T790M. Compiling results from several molecular techniques, we observed EGFR T790M in tumors from 20 patients who had not previously been treated with an EGFR TKI. In all cases, EGFR T790M occurred concurrently with another EGFR mutation, L858R (80%, 16/20), or exon 19 deletion (20%, 4/20). Two percent of all pre-treatment EGFR-mutant lung cancers harbored an EGFR T790M mutation. Thirteen patients received erlotinib monotherapy as treatment for metastatic disease. The response rate was 8% (1/13, 95% confidence interval 0%–35%). For the patients who received erlotinib, the median progression-free survival was 2 months and the median overall survival was 16 months.

Conclusions: De novo EGFR T790M mutations are rare (<1%) when identified by standard sensitivity methods. TKI therapy for patients with baseline EGFR T790M detected by standard molecular analysis has limited benefit.

Key words: lung cancer, EGFR, EGFR T790M, resistance to targeted therapies, biomarker

Introduction

EGFR mutations are identified in 20% of all lung adenocarcinomas [1] and >90% of identified mutations confer sensitivity to treatment with EGFR tyrosine kinase inhibitors (TKIs) [2, 3]. In patients with sensitizing EGFR mutations such as exon 19 deletions or EGFR L858R point mutations, treatment with EGFR TKIs leads to longer progression-free survival (PFS) compared with cytotoxic chemotherapy [4–6]. After an initial response, all EGFR-mutant tumors become resistant to erlotinib therapy. The most common mechanism of resistance to erlotinib is acquisition of the EGFR T790M point mutation in exon 20 [7].

Rarely, EGFR T790M mutations are identified in tumors before exposure to EGFR TKIs and are found concurrently with other sensitizing mutations such as EGFR L858R point mutation or EGFR exon 19 deletions [8]. The reported frequency of baseline EGFR T790M mutations varies widely in the literature, ranging from <1% of all lung cancers [9] and 1% of all EGFR-mutant lung cancers [10] to 25% of lung cancers [11] and up to 79% of all EGFR-mutant lung cancers [12]. This wide range of reported frequencies is dependent on the detection method used and the population tested. Using direct sequencing, the lowest frequency of EGFR T790M is seen [9, 10] and the highest prevalence is seen with colony hybridization method [12] and a TaqMan assay that utilizes a peptide-nucleic acid analog that inhibits wild-type amplification [13]. With more sensitive assays, small subclones within a larger population of tumor cells can be identified. The clinical significance of a mutation present within a small clone in a heterogeneous tumor is unknown. Ultra-high-sensitivity methods are also intrinsically more prone to false-positive artifacts.

The clinical implication of baseline EGFR T790M mutations varies among published studies (Table 1). There are no reports of partial responses to gefitinib or erlotinib in patients whose tumors harbor baseline EGFR T790M identified by standard sequencing. Treatment with EGFR TKI has previously been
### Table 1. Reports of EGFR T790M: prevalence and response to EGFR TKI

<table>
<thead>
<tr>
<th>Paper</th>
<th>Method</th>
<th>Baseline EGFR T790M Among EGFR+</th>
<th>Among NSCLC</th>
<th>T790M + treated with EGFR TKI</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu et al. 2013</td>
<td>Mass spectrometry (MALDI-TOF)</td>
<td>11/579 (2%)</td>
<td>11/2274 (0.5%)</td>
<td>n = 13</td>
<td>T790M+ RR 8% mPFS 1.5 months</td>
</tr>
<tr>
<td>Maheswaran et al. [18]</td>
<td>Mutant-enriched PCR (SARMs)</td>
<td>10/26 (38%)</td>
<td></td>
<td>n = 10</td>
<td>T790M+ RR 70% mPFS 8 months, HR 11.5 (95% CI 3–45), P &lt; 0.001</td>
</tr>
<tr>
<td>Rosell et al. [13]</td>
<td>Mutant-enriched PCR (Taq-Man)</td>
<td>45/129 (35%)</td>
<td>45 T790M+/129 with tissue for EGFR T790M testing/2105 NSCLC</td>
<td>n = 45</td>
<td>T790M+ RR 64% mPFS 12 months (95% CI 8–16 months), P = 0.05</td>
</tr>
<tr>
<td>Su et al. [11]</td>
<td>Direct sequencing</td>
<td>3/40 (8%)</td>
<td>3/107 (3%)</td>
<td>n = 2 (direct sequencing) n = 23 (MALDI-TOF)</td>
<td>T790M+ RR 57% mPFS 7 months, HR 1.9 (95% CI 1.0–3.3), P &lt; 0.05</td>
</tr>
<tr>
<td>Fujita et al. [12]</td>
<td>Mutant-enriched PCR (SARMs)</td>
<td>15/48 (31%)</td>
<td>27/107 (25%)</td>
<td></td>
<td>T790M– RR 73% mPFS 10 months</td>
</tr>
<tr>
<td>Inukai et al. [9]</td>
<td>Colony hybridization</td>
<td>0/38 (0%)</td>
<td></td>
<td>n = 30</td>
<td>T790M+ RR 0% mPFS 8 months, P = 0.44</td>
</tr>
<tr>
<td>Sequist et al. [16]</td>
<td>Direct sequencing</td>
<td>1/280 (0.4%)</td>
<td>10/280 (4%)</td>
<td>n = 9</td>
<td>T790M+ RR 0% mPFS 10 months</td>
</tr>
<tr>
<td>Wu et al. [10]</td>
<td>Direct sequencing</td>
<td>6/627 (1%)</td>
<td>6/1261 (0.5%)</td>
<td>n = 4</td>
<td>T790M+ RR 74% mPFS 8.5 months</td>
</tr>
</tbody>
</table>

EGFR+, EGFR mutant; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor; RR, response rate; mPFS, median progression-free survival; CI, confidence interval.
reported in nine patients with baseline EGFR T790M mutations found by standard sequencing, and zero of nine had a response to EGFR TKI [8, 10, 14–17]. In contrast, when baseline EGFR T790M is identified by other, more sensitive molecular assays, the response rate to EGFR TKI ranges from 57% to 70% [11, 13, 18] and PFS is between 7 and 12 months [11–13, 18]. The proportion of pre-treatment EGFR T790M-mutant alleles within a tumor may range from a small subclone to clonally dominant (on most alleles containing the coexistent sensitizing mutations), and the proportion present may impact responsiveness to EGFR TKI therapy. We sought to identify patients with baseline EGFR T790M-mutant lung cancers found by routine molecular testing and review their outcomes after treatment with erlotinib.

methods

patient identification

Patients with lung cancers harboring EGFR T790M in pre-treatment tumor specimens were identified using programatically abstracted elements from diagnostic molecular pathology reports and tumor registrar data available via a web-based application. We also identified additional patients whose tumors harbored baseline EGFR T790M identified at other testing sites but who received care at our institution. A subset of these patients was described previously [19]. The results were manually reviewed to exclude patients with acquired EGFR T790M after treatment with an EGFR TKI. Data collection was approved by the MSKCC Institutional Review Board/Privacy Board. Clinical characteristics, radiographic response data to EGFR TKI treatment, PFS on EGFR TKI treatment, and overall survival (OS) were obtained from the medical record. As these patients were not on an official protocol, imaging frequency varied, but was on average every 2–3 months. Response was determined on interval CT scans using RECIST 1.1.

molecular analysis

The molecular diagnostic modalities included in the search for all patients with lung cancers harboring EGFR T790M included standard sequencing, PCR-based restriction fragment length analysis, locked nucleic acid-based PCR sequencing, and a mass spectrometry-based mutation profiling assay [20, 21]. The fragment length analysis identifies deletions or insertions in EGFR exons 19 and 20, the mass spectrometry-based assay identifies hotspot point mutations in EGFR exons 18–21, and LNA-based sequencing suppresses wild-type amplification resulting in more sensitive EGFR T790M detection.

estimate of EGFR T790M frequency

To ascertain the frequency of baseline EGFR T790M mutations, we carried out an additional electronic medical record search to identify all patients with a diagnosis of lung cancer by ICD-0 code that had a mass spectrometry-based mutation profiling assay carried out between January 2009 and April 2013. All positive tests for EGFR T790M were reviewed to identify patients with tumors harboring EGFR T790M before treatment with an EGFR TKI. Patients whose tumors acquired EGFR T790M after treatment with an EGFR TKI were excluded from this analysis.

statistical analysis

PFS was measured from the start of EGFR TKI therapy to date of progression by RECIST 1.1. Overall survival was measured from the date of diagnosis of metastatic or recurrent disease. Patients alive were censored at the time of the last follow-up.

results

estimated frequency of EGFR T790M

To estimate the frequency of baseline EGFR T790M detected using standard molecular testing, we identified 2744 unique patients with lung cancers that had mass spectrometry-based genotyping carried out as part of routine clinical care between January 2009 and April 2013. Five hundred and seventy-nine patients had an EGFR mutation identified in their tumor: 300 EGFR exon 19 deletions, 227 EGFR L858R, 52 other (non-T790M), and 64 EGFR T790M mutations. Fifty-three tumor samples with EGFR T790M identified were from patients previously treated with EGFR TKI. Eleven patients were identified to have baseline EGFR T790M mutations, for a frequency of 0.5% [95% confidence interval (CI) 0.22%–0.71%], amounting to 2% of all EGFR-mutant tumors.

outcome of patients with baseline EGFR T790M

Including the above 11 patients identified by a mass spectrometry-based assay between 2004 and 2013, we identified a total of 20 TKI-naïve patients with lung tumors harboring EGFR T790M. The remaining nine patients were identified out of the above date range or by alternative molecular methods. Of the 20 lung samples identified, EGFR T790M was identified by direct sequencing in 4, by LNA-based enhanced sequencing in 3, and by mass spectrometry-based genotyping in the remaining 13. Patient characteristics are noted in Table 2. All tumors specimens with baseline EGFR T790M also harbored a sensitizing EGFR mutation. Compared with a contemporary cohort of 593 patients with EGFR mutations, in these patients with baseline EGFR T790M, L858R was more frequent than exon 19 deletion (P = 0.003) [1].

Thirteen patients received erlotinib for metastatic or recurrent lung cancer. Seven patients did not receive erlotinib...
monotherapy or any other EGFR-directed therapy. Two patients had clinical progression and died without repeat radiographic evaluation and were considered to have progressive disease as their best response. The response rate (complete response + partial response) was 8% (1/13, 95% CI 0%–35%). The one patient with a partial response had a concurrent EGFR 858R mutation and received erlotinib as first-line treatment for 5 months before disease progression. Stable disease was observed in 31% (4/13, 95% CI 12%–58%). Eight patients had progressive disease as their best response to erlotinib. The median PFS on erlotinib monotherapy was 1.5 months (Figure 1). Of those patients with metastatic disease treated with erlotinib, the median overall survival from the diagnosis of stage IV disease was 16 months (Figure 1).

discussion

Erlotinib and afatinib were recently approved for the treatment of lung cancers harboring EGFR exon 19 deletions or EGFR L858R point mutations, but reported responses to EGFR TKIs in patients with less common EGFR mutations have been mixed. As genotyping for EGFR mutations has become standard of care, understanding the significance of less common EGFR variants has taken on greater importance. EGFR T790M is typically included in the panel of mutations of both laboratory-developed and commercial diagnostic tests for EGFR, and given the association of EGFR T790M with resistance to EGFR TKIs, its identification has considerable clinical relevance.

In this study, we demonstrate that before treatment with an EGFR TKI, EGFR T790M occurs in <1% of all lung cancers and 2% of all EGFR-mutant lung cancers. Tumors with baseline EGFR T790M in our series always had a concurrent sensitizing EGFR mutation. The clinical characteristics of these patients are similar to patients harboring only sensitizing EGFR mutations. Yet, the presence of baseline EGFR T790M is associated with low response rate to erlotinib, short PFS, and an overall survival similar to what is seen with EGFR wild-type patients. Our conclusions are limited by the retrospective, single-center nature of our study.

The variable frequency of baseline EGFR T790M is the result of the sensitivity of the assays used and their ability to identify minor clones within a tumor. Several groups have proposed that EGFR-mutant tumors before exposure to EGFR TKIs contain a small proportion of EGFR T790M subclones [10, 15, 16, 22]. Using Sanger sequencing, a method of relatively low sensitivity, studies report an incidence of baseline EGFR T790M of 0.4%–3%, and have only been reported in conjunction with a sensitizing EGFR mutation [10, 11, 15, 16, 22]. When molecular methods with greater sensitivity are utilized, the reported incidence of baseline EGFR T790M is greater, 4%–25% of all lung adenocarcinomas [9, 11]. Sensitive methods include mass spectrometry-based mutation assays and several mutant-enriched PCR-based sequencing methods including restriction enzyme digestion [23], Locked Nucleic Acid (LNA)-based PCR [13, 24], and Scorpion Amplification Refractory Mutation System (SARMS) technology. Within tumors that harbor known sensitizing EGFR mutations, the EGFR T790M prevalence has been reported at 0%–38% [13, 18, 25], and as high as 79% using colony hybridization [12]. These highly sensitive methods detect EGFR T790M at low concentrations, as low as 0.1% of the total DNA present. Multiple rounds of amplification are required to detect these small clones, as only 1 EGFR T790M-mutant allele may be found among 500 EGFR alleles [18].

Among EGFR-mutant tumor samples, the frequency of baseline EGFR T790M varies considerably, which may be a result of small sample size and the exact patient population tested. With the same assay, such as the mass spectrometry-based assay used in our series, the EGFR T790M prevalence varies from <1% to 25% [11]. At our institution, where lung cancers all undergo multiplex molecular testing at diagnosis, <1% of patients had EGFR T790M identified in their tumor specimen. In contrast, Su et al. [11] using the same assay in an Asian population of primarily (77%) never-smokers found baseline EGFR T790M in 25%. There may be differences based on the stage or other clinical characteristics as well. Using 454 (next-generation) sequencing with a sensitivity of 0.2%, 0 of 16 untreated early-stage resected EGFR-mutant samples harbored EGFR T790M (95% CI 0%–23%) [25].

With more sensitive assays, in addition to the detection of minor EGFR T790M-containing clones, there is also a potential

![Figure 1. Outcome of patients with lung cancers harboring baseline EGFR T790M. (A) Progression-free survival on EGFR TKI. (B) Overall survival from stage IV disease.](https://academic.oup.com/annonc/article-abstract/25/2/423/193379)
for false-positive results. Using mass spectrometry-based assays, low DNA content and quality can lead to the generation of ‘mutant peaks’ that can be indistinguishable from a true mutation. With mutant-enriched PCR assays, the amplification of Taq errors can occur in very low template DNA samples leading to false-positive mutation calls as well. In addition, the use of formalin-fixed specimens for molecular testing may result in artificial mutation calls when using these sensitive assays [26]. Thirty-six TKI-naïve tumors harboring known sensitizing EGFR mutations with both frozen and formalin-fixed paraffin-embedded (FFPE) samples were analyzed for EGFR T790M using a mutant-enriched PCR assay. In the FFPE samples, the EGFR T790M-positive rate was 42% (15/36) in the tumor tissue, and 49% (16/33) in the adjacent normal tissue as well. When using frozen tissue, only 1 of 36 samples (3%) was EGFR T790M positive, and none of the 35 adjacent normal tissue samples harbored EGFR T790M.

More sensitive molecular assays have identified EGFR T790M mutations without co-incident sensitizing EGFR mutations. In 27 samples where EGFR T790M mutations were identified by a mass spectrometry-based assay, 12 (44%) had no co-incident EGFR mutation [11]. In another series, EGFR T790M was identified in 10 of 280 samples: four had co-incident EGFR mutations, two had concurrent KRAS mutations, and four had no identifiable additional mutation [9]. The clinical significance of identifying EGFR T790M without concurrent mutations in EGFR is unknown.

If EGFR T790M was only identified through a more sensitive assay like SARMs, its presence did not predict resistance with response rates to erlotinib of 57%–70% [11, 13, 18] and PFS between 7 and 12 months [11–13, 18]. These responses to erlotinib are inferior to that reported in patients with only sensitizing EGFR-mutant tumors, but are superior to responses to standard cytotoxic chemotherapy. Maheswaran et al. [18] reported a PFS of 8 months with erlotinib for EGFR T790M-containing tumors compared with 17 months for EGFR-mutant tumors without T790M (P < 0.001). Another series described a shorter median PFS with erlotinib in patients with EGFR-mutant lung cancer with concurrent T790M (P = 0.05) [13]. In distinction to the presence of EGFR T790M detected by standard sequencing, the presence of baseline EGFR T790M found at a low frequency using sensitive assays does not preclude a response to EGFR TKIs, although the responses appear to be of shorter duration.

The prognostic significance of baseline EGFR T790M has not been reported. In the acquired resistance setting, patients with second-site mutations in EGFR T790M have longer median post-progression survival compared with EGFR T790M-negative tumors (1.9 versus 1.6 years, P = 0.015) [7]. In this series, patients with baseline EGFR T790M-mutant tumors have a shorter median overall survival, only 16 months, similar to what is seen in patients with EGFR wild-type tumors and half what is seen in patients with sensitizing EGFR-mutant tumors [27].

We have reported that up to 50% of patients with baseline EGFR T790M-mutant tumors will have germline EGFR T790M mutations [19]. If a germline mutation is present, one would expect the mutation to be in 50% of the alleles of interest which would be detected by routine molecular assays such as direct sequencing. If a mutant allele is present at a low frequency, detectable only by a more sensitive assay, it is unlikely to be a germline mutation. We continue to recommend that all patients with baseline EGFR T790M-mutant tumors identified through routine molecular testing be referred for germline testing with appropriate patient education and consent. In our series, three of five patients consented to clinical genetic testing for germline EGFR T790M had germline mutations identified.

**conclusions**

Based on our series of patients identified by routine clinical molecular testing, baseline EGFR T790M mutations are rare, seen in <1% of pre-treatment tumors. When EGFR T790M is detected by assays such as SARMs, TaqMan, or colony hybridization, we recommend confirming positive results by direct sequencing as mutation allele frequency has clinical significance. When identified by routine molecular testing, the presence of EGFR T790M predicts the lack of response to EGFR TKI therapy and we recommend initial treatment with cytotoxic chemotherapy or on a clinical trial. In all cases, we found EGFR T790M coincident with a sensitizing EGFR mutation. Baseline EGFR T790M if present in a large proportion of a pre-treatment tumor sample is a negative prognostic factor associated with a shorter overall survival. New therapis being assessed in the EGFR TKI acquired resistance setting, including CO-1686 and AZD9291 should be tested in this population with primary resistance. Patients with baseline EGFR T790M-mutant tumors found by routine clinical assays should be sent for genetic evaluation and germline testing. In patients with baseline EGFR T790M mutations found by standard molecular assays, erlotinib is ineffective and cytotoxic chemotherapy is recommended. Individuals with baseline EGFR T790M represent a group of patients with a distinct clinical course, and should be addressed separately from other EGFR-mutant lung cancers.

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**disclosure**

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**references**


