

# Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond

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**Somatic mutations in certain tyrosine kinases have emerged as central ‘drivers’ of specific cancers and these mutant proteins are proving to be excellent substrates for targeted therapies. This is the case for mutant EGFR-dependent lung adenocarcinomas, where *EGFR* mutation testing is already being used to help guide treatment decisions. Here, we provide an overview of the biology of EGFR-targeted therapies and the clinical experience to date, the positive and negative predictors of response, pathologic correlates of EGFR-mutant status, testing methods to establish patient eligibility for these agents, and the basis for primary and secondary resistance.** *Modern Pathology* (2008) 21, S16–S22; doi:10.1038/modpathol.3801018

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## Overview of EGFR biology and clinical correlations

Lung cancer is the leading cause of cancer deaths in both men and women in the United States. Over the past two decades, adenocarcinoma has replaced squamous cell carcinoma as the most common subtype of non-small cell lung cancer (NSCLC) in the United States.

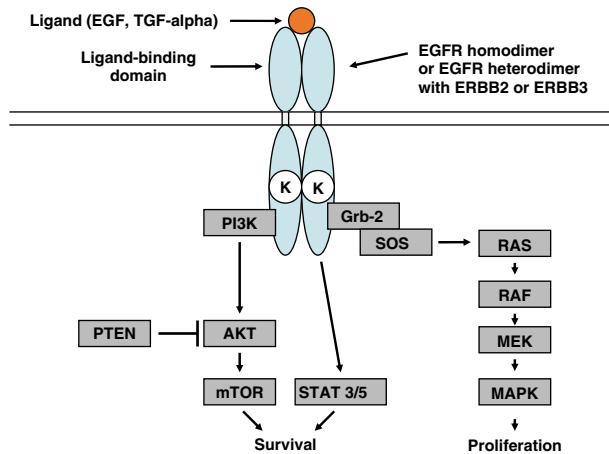
The epidermal growth factor receptor (EGFR, HER-1/ErbB1) is a receptor tyrosine kinase (TK) of the ErbB family, which consists of four closely related receptors: HER-1/ErbB1, HER-2/neu/ErbB2, HER-3/ErbB3 and HER-4/ErbB4. Upon ligand binding and receptor homo- or hetero-dimerization and activation (phosphorylation), activated EGFR signals downstream to the PI3K/AKT and RAS/RAF/MAPK pathways (Figure 1). These intracellular signaling pathways regulate key cellular processes such as proliferation and apoptosis.

The expression of EGFR by some lung cancers, the limited therapeutic options for advanced lung cancer, and the availability of new EGFR-targeted drugs led, in the early part of the decade, to clinical trials of small molecule EGFR TK inhibitors in unselected NSCLC patients. Although tumors in the vast majority of patients failed to respond, a minority showed dramatic tumor shrinkage accompanied by symptomatic improvement. Such responses were

noted to be more common in East Asians, in women, and in patients with adenocarcinomas, especially those with areas of bronchioalveolar carcinoma (BAC).<sup>1</sup> These observations then led to three landmark 2004 studies that showed that the tumors that responded to the EGFR TK inhibitors (TKIs) gefitinib and erlotinib contain somatic mutations in the *EGFR* TK domain.<sup>2–4</sup> The two most common *EGFR* mutations are short in-frame deletions of exon 19 and a point mutation (CTG to CCG) in exon 21 at nucleotide 2573, that results in substitution of leucine by arginine at codon 858 (L858R). Together, these two types of mutations account for ~90% of all *EGFR* mutations in NSCLC. Other recurrent but far less common *EGFR* mutations known to be associated with sensitivity to EGFR TKIs include the G719 mutations in exon 18 and the L861 mutations in exon 21. Screening for common *EGFR* mutations in patients with lung adenocarcinomas can now be performed in clinical molecular diagnostic laboratories to predict which patients will respond to EGFR TKIs.<sup>5</sup> It can be performed on archival material as well as on fine-needle biopsies.<sup>6</sup>

Many other rare *EGFR* mutations have been reported in lung cancers, but the majority of such rare mutations are of much lesser clinical significance for three reasons. First, their association with response to EGFR TKIs has not been established. Second, many mutations that have been reported only once (ie, non-recurrent) may be technical artefacts arising from PCR and sequencing of DNA extracted from FFPE samples<sup>7</sup> (such artefacts can be guarded against by performing the PCR and sequencing in duplicate). Third, rare sequence alterations may actually represent rare germline polymorphisms

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**Figure 1** Schematic of EGFR signaling pathway. Erlotinib and gefitinib block signaling at the level of the kinase domain (K). They are ineffective if the signaling pathway is activated by mutations downstream of this point, such as in *KRAS* or *BRAF*.

because this possibility has not been excluded in all cases (to exclude this possibility, a normal DNA sample from the patient is sequenced).

In multiple recent prospective clinical trials of gefitinib or erlotinib, response rates of *EGFR* mutated cases range from 65 to 90%.<sup>8–13</sup> Survival data from these studies are not yet mature, but in many instances, the median overall survival rates were ‘not reached’ at the time the data were reported. In most retrospective studies, patients with *EGFR* mutations and a response to gefitinib or erlotinib show a longer progression-free survival and a trend toward improved overall survival vs patients whose tumors have wild-type *EGFR*. Whether the longer survival observed in these studies is due to an intrinsically more favorable biology of lung cancers with *EGFR* mutations, or to the treatment with *EGFR* TKIs, is still somewhat controversial, but there is an emerging consensus that both factors are contributing to this longer overall survival. Indeed, a recent study found that *EGFR* mutations are associated with a modest but statistically significant survival benefit in patients with resectable lung adenocarcinoma (not treated with *EGFR* TKIs).<sup>14</sup> Thus, *EGFR* mutations may have both predictive and prognostic value.

## Pathologic correlates of *EGFR* mutations

Historically, the practical utility of NSCLC as a clinical entity reflected the lack of distinct treatment regimens for the different histologic types encompassed by this term. With the advent of targeted therapy based on *EGFR* mutations in lung adenocarcinomas, it is evident that the histologic subtype of NSCLC should be explicitly reported. *EGFR* mutations are extremely rare in large cell carcinomas, small cell carcinomas, and pure squamous carcinomas, but have been reported in adenosquamous carcinomas.<sup>15</sup> Indeed, it may be that the very

rare squamous carcinomas reported to harbor mutations were inadequately sampled adenosquamous carcinomas. There have also been two cases reported of *EGFR*-mutant small cell carcinoma preceded by an adenocarcinoma with the same mutation.<sup>16,17</sup> Incidentally, another recent development that supports the retirement of the term ‘NSCLC’ is that bevacizumab (Avastin) is associated with a high risk of bleeding in squamous lung cancers<sup>18</sup> and therefore, has been approved only for use in ‘non-squamous’ NSCLCs.

Among adenocarcinomas, *EGFR* mutations are more prevalent in cases with BAC features.<sup>19</sup> Interestingly, the observation that BAC features are clinically significant was made before the discovery of *EGFR* mutations, based on the association of adenocarcinomas with BAC features with responses to *EGFR* TKIs.<sup>1,20</sup> It should be noted that lung adenocarcinomas with a minor or major BAC component are not well delineated in the current WHO classification and therefore strict adherence to WHO categories may reduce the likelihood of making such observations. Microdissection studies have shown that *EGFR* mutations are present in BAC-like areas as well as more solid areas, suggesting that these are early events in these tumors.<sup>19</sup> This notion is reinforced by a recent report describing *EGFR* mutations in a third of lesions diagnosed as atypical adenomatous hyperplasia.<sup>21</sup>

## *EGFR* mutation detection vs *EGFR* FISH/CISH in the determination of *EGFR* status

There has been an ongoing debate regarding the most appropriate testing approach for establishing ‘*EGFR* status’ in lung adenocarcinomas, mutation analysis or gene copy number analysis (by FISH or CISH).<sup>22–24</sup> The relative importance of these two types of genetic alterations has been difficult to tease out because of their frequent co-occurrence. In our experience, among *EGFR*-mutated cases, approximately 50% also show increased *EGFR* gene copy number, and conversely, approximately 75% of cases with increased *EGFR* gene copy number show mutations.<sup>25</sup> These numbers vary in different series due to different scoring systems for gene copy number alterations and mutation detection techniques of different sensitivity (see below). There is now mounting evidence from multiple types of analyses supporting *EGFR* mutation status as a more rational and biologically relevant marker for treatment selection.

- (1) Mutant *EGFR* is biologically linked to ligand-independent increased downstream signaling, unlike simple overexpression of native *EGFR*.
- (2) When both alterations are present, it is the mutated *EGFR* allele that is amplified preferentially.<sup>25,26</sup> This suggests that in cases with both mutation and amplification, it is the biological advantage provided by the mutation that drives selection for copy number gains.

- (3) *EGFR* mutations are more closely linked to known risk factors (negative association with smoking) and other biological features (female sex, Asian origin) than is *EGFR* amplification.<sup>25</sup>
- (4) Response rates to *EGFR* TKIs are high in *EGFR*-mutated cases regardless of *EGFR* copy number. In contrast, *EGFR*-amplified cases that lack mutations have very low response rates, in the order of 8%.<sup>27</sup>
- (5) In several recent studies, it has been shown that *EGFR* mutation status is a better predictor of outcome in patients treated with *EGFR* TKIs than *EGFR* copy number.<sup>28–30</sup> The failure of some earlier studies to detect survival benefits may have been due to the fact that they were statistically underpowered with respect to *EGFR*-mutant cases.

Part of the explanation for the survival benefit seen in *EGFR* amplified tumors apparently lacking *EGFR* mutations may be the following. Because FISH or CISH for *EGFR* amplification is a morphologic method (ie, non-neoplastic cells are not counted) whereas sequencing for mutation analysis is prone to false negatives due to excess non-neoplastic cells, it is likely that at least some *EGFR*-amplified cases reported to be mutation-negative based on direct sequencing have harbored mutations that would have been detected by more sensitive methods. Indeed, direct sequencing is prone to miss *EGFR* mutations (see below). Nonetheless, the situation remains complex because the subset of patients with tumors that lack detectable *EGFR* mutations but show increased *EGFR* copy number do show improved survival on *EGFR* TKIs despite the lack of radiologic response, compared to *EGFR* non-mutant tumors without *EGFR* amplification.<sup>27,29,31</sup>

### Clinical heterogeneity of *EGFR* mutations

As mentioned above, some mutations are clearly associated with favorable responses to *EGFR* TKIs, whereas others are associated with poor responses or outright resistance to these same drugs. Thus, the relatively uncommon in-frame insertions in exon 20 are associated with primary resistance to *EGFR* TKIs. Interestingly, there may be differences even between the two main sensitivity mutations. Two retrospective studies have compared the outcome of *EGFR* TKI-treated patients based on the type of mutation present, namely exon 19 deletions or exon 21 L858R point mutation, and both found that the former group had a significantly better overall survival.<sup>32,33</sup>

### Criteria for selecting cases for *EGFR* testing and the pathologist's role

On the basis of the above considerations, the surgical pathologist's role in *EGFR* testing is to ensure that the

testing is performed only in lung adenocarcinomas (or adenosquamous carcinomas), unless specifically requested by the oncologist. Among adenocarcinomas, some would suggest to exclude the mucinous subtype of BAC, as this special subtype is associated with *KRAS* mutations and not *EGFR* mutations, unlike non-mucinous BAC.<sup>34,35</sup> Blocks or unstained sections submitted for DNA extraction and mutation analysis should be the most cellular available, preferably restricted to areas with >50% tumor cells. To ensure this, in many cases, further microdissection of the sections needs to be performed in the molecular diagnostic laboratory.

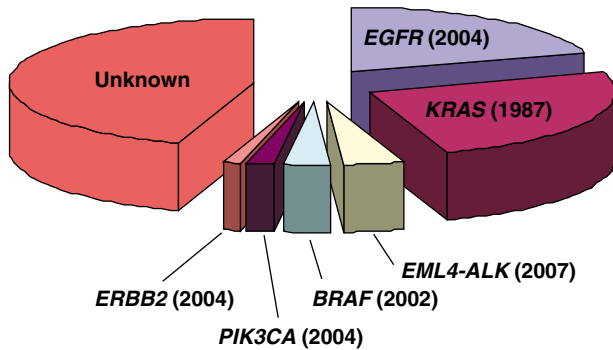
Importantly, *EGFR* IHC plays little or no role in selecting patients for *EGFR* mutation testing as it is only weakly correlated with the presence of mutations, if at all. Given this, it is not surprising that *EGFR* IHC positivity has generally been found of limited use in predicting response to *EGFR* TKIs.<sup>36</sup> Ironically, despite multiple publications questioning the significance of *EGFR* IHC, the Tarceva (erlotinib) product insert still emphasizes its importance.

### Common methods for *EGFR* and *KRAS* mutation analysis

Initially, most large series used direct sequencing to detect these mutations. It is known that direct sequencing is likely to miss mutations when the tumor cells make up less than 25% of the sample. In some cases with *EGFR* mutations, this limit is somewhat lower because the mutant allele may be present in multiple copies per tumor cell (concurrent amplification). More recently, a number of studies of *EGFR* in lung cancer have employed more sensitive mutation detection techniques, including mutation-specific PCR assays,<sup>37,38</sup> PCR with hybridization in real-time with mutation-specific fluorescent probes,<sup>39,40</sup> single-strand conformational polymorphism,<sup>35</sup> denaturing high-performance liquid chromatography,<sup>41,42</sup> peptide nucleic acid-locked nucleic acid PCR clamp<sup>11</sup> and more experimental techniques such as mass spectrometry and single molecule sequencing.<sup>43</sup> These have been recently reviewed.<sup>44</sup> Almost all such studies find a few cases with mutations that were missed by direct sequencing. The technical concern of detection sensitivity may be heightened for the secondary resistance mutations (see below).

### Mutations in *KRAS*, *BRAF* and *ERBB2* as predictors of primary resistance to *EGFR* inhibitors

It is now clear that mutations in *EGFR*, *KRAS*, *BRAF* and *ERBB2* are essentially mutually exclusive in lung adenocarcinoma<sup>19,45–49</sup> (Figure 2). This reflects the fact that they represent alternative ways of oncogenically activating some of the same downstream pathways, including the PI3K–AKT pathway



**Figure 2** Pie chart of known activating mutations in signaling molecules in lung adenocarcinoma. The dates the mutations were discovered in human lung cancer are indicated. These mutations are mutually exclusive with the exception of *PIK3CA*. Data on *EML4-ALK* prevalence and relationship to other mutations are preliminary.<sup>73</sup>

and the MAPK pathway (Figure 1). *ERBB2* mutations (in-frame insertions in exon 20; not amplification) occur in about 1–2% of adenocarcinomas and interestingly these, like *EGFR*-mutant tumors, often show BAC features, and occur preferentially in never smokers and women.<sup>46,47,50</sup>

The presence of a *KRAS* mutation is both an adverse prognostic factor<sup>14</sup> and a predictor of failure to benefit from *EGFR* TKI therapy.<sup>51–53</sup> Indeed, in one study this went beyond a simple lack of response in that tumors with *KRAS* mutations did significantly worse when an *EGFR* TKI was added to their standard chemotherapy.<sup>52</sup> Most *KRAS* mutations, in contrast to *EGFR* mutations, appear smoking-related.<sup>54</sup>

From these considerations, it should be evident that obtaining a comprehensive mutational profile of will eventually become clinically highly desirable, as each of these mutations has some impact on treatment selection. Aside from the fact that *KRAS* mutations are known, and *BRAF* and *ERBB2* mutations are likely, to be associated with lack of response to *EGFR* TKIs, *BRAF* and *ERBB2* mutations may also predict sensitivity to other targeted therapies. For instance, *BRAF*-mutant tumors may be especially sensitive to MEK inhibitors.<sup>55</sup> Finally, it should be noted that some rare primary *EGFR* mutations, such as exon 20 insertions, show poor responses to *EGFR* TKIs.

## Secondary (acquired) resistance to *EGFR* inhibitors

In spite of dramatic tumor shrinkage in many patients with *EGFR* mutations, durable complete responses to *EGFR* TKIs remain uncommon. Acquired resistance to *EGFR* TKIs has been associated in at least 50% of cases with selection for a second *EGFR* mutation, most often T790M in exon 20 (rarely D761Y in exon 19).<sup>13,56–59</sup> The T790M secondary mutation may be underestimated for

technical reasons, especially in cases with amplification of the *EGFR* allele with the first mutation.<sup>23,60,61</sup> A minority of patients whose tumors contain a T790M have not been exposed to *EGFR* TKIs, so this mutation may also be detectable at diagnosis. A family with multiple cases of NSCLC has been associated with germline transmission of this mutation,<sup>62</sup> but a subsequent screen of 237 lung cancer families for germline T790M mutations failed to find any additional cases.<sup>63</sup>

In about 20% of patients with acquired resistance to gefitinib or erlotinib, tumor cells display amplification (thus far without mutations) of the *MET* oncogene at chromosome position 7q31.<sup>64,65</sup> *MET* encodes a receptor TK implicated in the development, maintenance and progression of cancers in both animals and humans (reviewed in Birchmeier *et al*<sup>66</sup>). Interestingly, *MET* amplification appears to occur with or without T790M mutations.<sup>65</sup> As *MET* inhibitors and ‘second-generation’ *EGFR* inhibitors that can target T790M-bearing tumors enter into clinical use, pathologists may also need to optimize rapid testing methods for the T790M *EGFR* mutation and for *MET* amplification.

## Prospects for IHC markers of *EGFR* and *KRAS* status

As discussed above, *EGFR* expression alone correlates poorly with the presence of activating *EGFR* mutations. In spite of this, the Tarceva package only mentions *EGFR* IHC for determining treatment eligibility. There has been intense interest in developing a better IHC-based marker for *EGFR* TKI treatment selection, that is, a marker that would have both good sensitivity and specificity for *EGFR* mutations. However, such a marker has so far proven elusive. The following have been investigated: phosphorylated *EGFR* or phosphorylated downstream signaling proteins such as phospho-AKT, phospho-ERK/MAPK and phospho-S6.<sup>29,67–70</sup> In some of these studies, expression of phospho-AKT appeared to correlate with response to *EGFR* TKIs. Adenocarcinomas without evidence of epithelial-to-mesenchymal transition (ie, better differentiated ones) have also been reported to be more likely to respond and they can be identified by staining for E-cadherin.<sup>71,72</sup>

## Conclusion

The clinical development of *EGFR* TKIs in lung cancer and the subsequent discovery of activating mutations in *EGFR* as the molecular basis for increased tumor sensitivity to these drugs have revolutionized lung cancer management. However, the field continues to rapidly evolve. At present, *EGFR* mutation analysis represents the most accurate available test for prediction of response to *EGFR* TKIs, and many studies support its usefulness in

**Table 1** Comparison of targeted therapies in breast and lung cancers

Target	HER2 (ERBB2)	EGFR
Cancer	Breast carcinoma	Lung adenocarcinoma
Targeted agent	Trastuzumab	Gefitinib/erlotinib
Approximate prevalence of target	15% have <i>HER2</i> amplification	20% have <i>EGFR</i> mutations
Typical response rate to targeted agent	~15%	>75%
Approximate cost (in USA)	>\$10k per month	\$2.0–2.5k per month

predicting survival benefit as well. Combining mutation analysis with EGFR FISH or CISH may provide additional clinically useful information. Some key areas of further work in the practical molecular pathology of *EGFR*-mutant tumors include defining optimal mutation detection techniques, identifying the key mutations that need to be tested for selection of first or second generation EGFR TKIs, developing cost-effective approaches to testing for a growing number of clinically relevant mutations in EGFR and associated genes, and identifying robust surrogate IHC markers of EGFR status. If other subtypes of adenocarcinoma prove to have molecular aberrations that could direct targeted therapy, the current paradigm of *EGFR*-mutant lung cancers will be extended. Finally, it should be noted that, in several respects, the targeted therapy of lung adenocarcinoma compares favorably with that of breast carcinoma (Table 1).

## Web resources

The Sanger Institute (UK) maintains the COSMIC database (Catalogue of Somatic Mutations in Cancer) that includes a listing of all reported *EGFR* mutations, as well as mutations in *KRAS* and other kinases: <http://www.sanger.ac.uk/genetics/CGP/cosmic/>

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