Antitumour Treatment

EGFR-mutated oncogene-addicted non-small cell lung cancer: Current trends and future prospects

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Introduction

Lung cancer causes a fifth of all cancer-related deaths.1 Non-small cell lung cancer (NSCLC) accounts for most cases of lung cancer and is usually diagnosed in advanced stages. Platinum-based chemotherapy, the recommended standard first-line systemic treatment for advanced NSCLC,2 has limited efficacy3 and significant toxicity. The identification of oncogenic mutations that contribute to the pathogenesis of NSCLC has recently led to novel approaches to diagnosis, classification, and management of this disease. The epidermal growth factor receptor (EGFR) tyrosine kinase has been a particularly important target for new therapies. EGFR (also known as ErbB1 or human epidermal growth factor receptor [HER1]) belongs to the Erb family of transmembrane receptor tyrosine kinases.4 Two EGFR tyrosine kinase inhibitors (TKIs), erlotinib (Tarceva®; Roche)5 and gefitinib (Iressa®; AstraZeneca),6 are currently approved for the treatment of NSCLC. EGFR–TKIs bind reversibly to the EGFR tyrosine kinase, competing with the substrate, adenosine triphosphate (ATP), and thereby blocking the catalytic activity of the enzyme. First-line EGFR–TKI therapy when given as monotherapy or in combination with chemotherapy,7–12 had disappointing efficacy in Phase III trials performed in populations of patients with NSCLC who were not selected according to any molecular analysis of EGFR. It is now known that the efficacy of EGFR–TKI is influenced by somatic mutations in EGFR. More fundamentally, there is accumulating evidence that EGFR-mutated NSCLC represents one of several ‘oncogene-addicted’ forms of NSCLC that differ from classical NSCLC in terms of the patient groups affected, the prognosis, and the optimal diagnostic and therapeutic approaches. Focusing on EGFR, this paper reviews these developments and discusses their important implications for molecular testing and screening, treatment selection and sequencing, and the evaluation of future treatments for NSCLC.

EGFR TKI in unselected NSCLC populations

Early Phase III trials showed little benefit of EGFR TKI when these agents were used as monotherapy after the failure of standard chemotherapy in patients with advanced NSCLC. In the
BR.21 trial, erlotinib monotherapy significantly improved overall survival (OS) and progression-free survival (PFS) after the failure of standard chemotherapy, as compared with placebo. Gefitinib did not improve survival or other outcomes compared with placebo when used as second- or third-line therapy in the Iressa\textsuperscript{a} Survival Evaluation in Lung cancer (ISEL) trial; however, eligibility criteria in this trial restricted the study population to one with a very poor prognosis (patients with advanced NSCLC, refractory or intolerant to their first or second line of therapy and not suitable for further chemotherapy), which may have contributed to the outcomes.\textsuperscript{14} Subgroup analysis did show a significant survival benefit in never smokers and patients of Asian origin. In two other studies – the Iressa\textsuperscript{a} NSCLC Trial Evaluating Response and Survival versus Taxotere (INTEREST) and the V15–32 trial – gefitinib had efficacy similar to docetaxel when used in patients previously treated with platinum-based chemotherapy.\textsuperscript{15,16} More recently, in the Iressa\textsuperscript{a} as Second-line Therapy in Advanced NSCLC–Korea (ISTANA) study, second-line gefitinib significantly prolonged PFS (as compared with docetaxel) in Korean patients.\textsuperscript{17} Maintenance therapy with EGFR–TKIs has also significantly improved PFS following platinum-based chemotherapy in unselected patients.\textsuperscript{18,19}

EGFR–TKI also had disappointing efficacy when added to first-line combination chemotherapy regimens. Gefitinib did not significantly improve survival or time to progression (TTP) when added to first-line paclitaxel plus carboplatin or gemcitabine plus cis-platin in the Iressa\textsuperscript{a} NSCLC Trial Assessing Combination Treatment (INTACT) trials.\textsuperscript{7,8} Erlotinib did not confer a significant overall benefit on these outcomes when it was added to first-line chemotherapy with carboplatin–paclitaxel\textsuperscript{9} in the TRIBUTE study or cisplatin–gemcitabine in the TALENT study.\textsuperscript{10} In both studies, erlotinib provided significant survival benefits in the subsets of patients who reported having never smoked.\textsuperscript{9,10}

More recently, the Tarceva\textsuperscript{a} or Chemotherapy (TOCH) study was discontinued when an interim analysis revealed that first-line erlotinib followed on progression by second-line cisplatin–gemcitabine was inferior in unselected patients to the reverse, standard strategy of first-line cisplatin–gemcitabine followed by second-line erlotinib.\textsuperscript{11} The Tarceva\textsuperscript{a} Or Placebo In Clinically Advanced Lung cancer (TOPICAL) study showed that erlotinib did not significantly improve OS when added to best supportive care (as compared with best supportive care alone) in a population of chemotherapy-naïve patients who had a poor performance status (ECOG 2/3) or who were unfit for platinum chemotherapy, although there was a trend for a prolongation of PFS and a significant prolongation of both OS and PFS in the subset of female patients.\textsuperscript{12}

**EGFR abnormalities in NSCLC**

EGFR is activated by the binding of various ligands of the extracellular growth factor (EGF) family of peptides.\textsuperscript{22} Upon ligand binding, the enzyme homo- or hetero-dimerizes with other EGFR units or other Erb receptor subtypes, e.g. ErbB2 (HER2). The intracellular domain undergoes autophosphorylation and increased kinase activity. These changes result in the transduction of oncogenic signals via downstream pathways that mediate cell survival, proliferation and invasion, including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K) pathways.\textsuperscript{23}

Abnormalities in EGFR in NSCLC tumours include over-expression, amplification and mutation. Although wild-type EGFR is commonly over-expressed in NSCLC tumours (30–75% of samples),\textsuperscript{10,15,22–24} this does not reliably correlate with a poor prognosis\textsuperscript{25–28} or predict response to EGFR–TKIs.\textsuperscript{29} EGFR over-expression correlates with a high EGFR gene copy number, as measured by fluorescence in situ hybridization (FISH).\textsuperscript{31} According to criteria developed by the University of Colorado Cancer Center (UCCC), EGFR FISH-positivity is defined as either $\geq 4$ copies of $\text{EGFR}$ in $\geq 40\%$ of cells or the presence of gene amplification (an $\text{EGFR}$ gene to centromere 7 ratio of $\geq 2$ or $\geq 10\%$ of tumour cells demonstrating either $\geq 15$ copies of $\text{EGFR}$, the presence of small $\text{EGFR}$ gene cluster [4–10 copies], or an $\text{EGFR}$ signal larger and brighter than centromere 7).\textsuperscript{32} Approximately 30–45% of tested NSCLC tumours are FISH-positive.\textsuperscript{33,34} High $\text{EGFR}$ gene copy number (including amplification of the gene) has also shown conflicting results as a predictor of survival after EGFR–TKIs,\textsuperscript{15,22–24,33,35} although it was the only predictor of survival in trials without the confounding effect of chemotherapy and without crossover between arms. Interpretation of studies that have attempted to correlate prognosis or response with the over-expression of wild-type EGFR is hindered by differences in the methods and definitions used, and the lack of differentiation between wild-type and mutated EGFR.\textsuperscript{37}

**Activating EGFR mutations**

Analysis of trial data revealed that EGFR–TKIs were most effective in patients with somatic mutations in the $\text{EGFR}$ gene.\textsuperscript{38–40} Larger studies recently confirmed that EGFR–TKIs significantly improve PFS in patients with these mutations.\textsuperscript{22,41–43} EGFR mutations are known as activating mutations because (in most cases) they induce a ligand-independent activation of the tyrosine kinase,\textsuperscript{44} resulting in upregulation of oncogenic cell signalling.\textsuperscript{37}

Activating mutations in the $\text{EGFR}$ gene are found in around 10–20% of all patients with advanced NSCLC\textsuperscript{65} and in more than 50% of adenocarcinomas and tumours from East Asians, never smokers and women.\textsuperscript{37,45–48} These mutations occur in exons 18–21 of $\text{EGFR}$ and result in mutations within the active site of the $\text{EGFR}$ kinase domain (Fig. 1).\textsuperscript{49} The substitution of arginine for leucine at codon 858 (L858R) accounts for approximately 40% of mutations, with in-frame deletions at exon 19 (del E746_A750 being the most common) accounting for another 40–45%.\textsuperscript{37,45} Less common mutations ($\leq 5\%$) include insertions of residues in exon 20 and substitutions at the glycine residue at position 719 in exon 18 (e.g. G719S). All of these mutations occur close to the shared binding site of ATP and EGFR–TKI.\textsuperscript{59}

L858R and exon 19 deletions render EGFR highly sensitive to gefitinib and erlotinib.\textsuperscript{44} Kinetic analysis of the L858R mutation has revealed that it reduces the affinity of the tyrosine kinase for ATP while increasing its affinity for EGFR–TKI.\textsuperscript{44} In contrast, the G719S point mutation decreases the affinity of EGFR for both ATP and gefitinib.\textsuperscript{49} Thus, G719L mutants are sensitive to erlotinib (and the newer Erb family TKIs, e.g. afatinib (BIBW 2992)\textsuperscript{50} and neratinib\textsuperscript{51}) but may be less sensitive in vitro to gefitinib (50% inhibitory concentration: 68 mmol/L for gefitinib compared with 16 mmol/L for erlotinib).\textsuperscript{44} Other mutations, most notably the T790M mutation in exon 20, are associated with resistance to EGFR–TKIs, as discussed below.

Importantly, EGFR-mutated NSCLC tumours have been termed ‘oncogene addicted’ to reflect their dependence on EGFR-mediated pro-survival signalling and their high susceptibility to EGFR–TKI-induced apoptosis.\textsuperscript{52} The acute inactivation of EGFR is thought to shift the balance of cell signalling, with a rapid deterioration of pro-survival signals resulting in the predominance of more sustained pro-apoptotic signals.\textsuperscript{37,52}

**Testing for EGFR mutations**

EGFR mutations are usually detected in DNA from tumour samples using gene sequencing (Sanger sequencing) of exon sequences amplified by polymerase chain reaction (PCR).\textsuperscript{52} While this method has been regarded as the gold standard, it is associated with various disadvantages, including limited sensitivity, a dependence on
Biomarkers for other genomic mutations in NSCLC

Various molecular markers and targets are being evaluated as part of efforts to improve NSCLC care (Table 1). The KRAS gene codes for the Kras protein, a membrane-bound guanosine triphosphatase that mediates cell signalling via various pathways. KRAS mutations that trigger over-activity of Kras are an important pathogenic step in NSCLC. According to a recent meta-analysis of 22 studies, KRAS mutations were found in 16% of patients with NSCLC treated with EGFR TKIs in clinical studies, and a quarter of smokers and patients with adenocarcinomas. Some studies have correlated KRAS mutations with lower tumour response rates and progressive disease in patients treated with EGFR–TKIs.64–66 According to the aforementioned meta-analysis, the odds ratio of tumour response in patients with KRAS mutations was 0.29 (p < 0.01) compared with patients with wild-type KRAS.64 Some retrospective studies have suggested that KRAS mutations also predict survival in patients treated with EGFR–TKIs.36,65,67 However, the predictive value of this marker has not been fully established.55,64,68,69 Furthermore, different KRAS mutations may vary in their predictive value, as suggested by the recent finding that the glycine-to-aspartate transition mutation at codon 13 of KRAS (p.G13D) predicts survival in patients treated with EGFR–TKIs.36,64,67

Additional molecular markers under evaluation in NSCLC include HER2, the echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) fusion gene (EML4–ALK), PI3K and BRAF, fibroblast growth factor receptor 1 (FGFR1) and insulin-like growth factor receptor 1 (IGF1R). HER2 is over-expressed in up to 35% of patients with NSCLC (especially in adenocarcinomas) and is associated with poor prognosis.71,72 Increased copy number of the HER2 gene (determined by FISH) is associated with gefitinib sensitivity in patients with EGFR over-expression, gene amplification or mutations.73 Activating insertion mutations in HER2 are found in around 2% of all patients. They are most common (~3%) in women, never smokers and in adenocarcinomas and

Table 1
Approximate prevalence rates for selected biomarkers in non-small cell lung cancer tumours.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Frequency</th>
<th>Sub-populations</th>
<th>Effect on EGFR–TKI efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR activating mutation</td>
<td>10–20%</td>
<td>&gt;50% In adenocarcinomas females, East Asian, never smokers</td>
<td>⊥</td>
</tr>
<tr>
<td>EGFR T790M</td>
<td>Up to 35%</td>
<td>50% In patients who progress on EGFR–TKI</td>
<td>⊥</td>
</tr>
<tr>
<td>MET amplification</td>
<td>Up to 20%, but rarely high enough to cause resistance (&lt;2%)</td>
<td>⊥</td>
<td></td>
</tr>
<tr>
<td>KRAS mutation</td>
<td>~16%</td>
<td>~25% In adenocarcinomas and smokers</td>
<td>⊥</td>
</tr>
<tr>
<td>HER2 mutation</td>
<td>~2%</td>
<td>~3% In women, never smokers and adenocarcinomas</td>
<td>(but potentiate agents active against HER2)</td>
</tr>
<tr>
<td>BRAF mutation</td>
<td>ND</td>
<td>2–3% In adenocarcinomas</td>
<td>⊥</td>
</tr>
<tr>
<td>EML4–ALK mutation</td>
<td>5–13%</td>
<td></td>
<td>⊥</td>
</tr>
<tr>
<td>PIK3CA mutation</td>
<td>~1.5%</td>
<td>5% In patients who progress on EGFR–TKI</td>
<td>⊥</td>
</tr>
</tbody>
</table>

See text for references.
EGFR, epidermal growth factor receptor; EML4–ALK, echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase; HER2, human epidermal growth factor receptor 2; PIK3, phosphatidylinositol 3-kinase.

a Rates of 35–38% in recent studies. Prevalence highly dependent on test methods, with some studies reporting rates of <5%. sample preparation methods, and the requirement for a sufficient tumour sample (five to ten 5 μm sections being recommended for molecular diagnosis). The need for direct sequencing has been avoided by the development of rapid, simple and highly sensitive PCR assays for tumour samples or blood. The Amplification Refractory Mutation System™ (ARMS™) PCR system has been used advantageously in some recent trials of EGFR–TKIs.36,41

Fig. 1. Structure of the epidermal growth factor receptor kinase domain, highlighting the sites of oncogenic mutations. The kinase domain fold consists of a smaller N-terminal lobe and a larger C-terminal lobe. The active site lies in the cleft between the two lobes. The kinase is shown in the active conformation. Locations of activating mutations are indicated in red. The regulatory C-helix is coloured pink, the phosphate coordinating P-loop is shown in magenta, and the activation loop (A-loop) is coloured orange. Reproduced from Eck MJ and Yun CH.

mutations have been detected with high specificity but somewhat lower sensitivity by employing mutation-specific antibodies with immune histochemistry analyses. In the future, the development of platforms that can simultaneously screen for multiple mutations in various genes, including EGFR, should further facilitate gene-directed therapy. However, these measures will only detect known mutations and will not identify novel mutations.
do not occur in conjunction with EGFR or KRAS mutations. As the signalling activity of EGFR and HER2 are interlinked (owing to their heterodimerization during activation), the dual inhibition of both proteins is an attractive target for new treatments for NSCLC. HER2 mutations confer resistance to EGFR–TKIs, but sensitivity to novel agents that inhibit both HER2 and EGFR.

Caused by a small chromosome 2p inversion, EML4–ALK causes the highly oncogenic expression of a chimeric tyrosine kinase in which the N-terminal portion of EML4 is fused to the intracellular portion of ALK. Although this may be related to the absence of EGFR mutations in these patients. EML4–ALK-positive patients are younger than those with EGFR-mutations or wild-type EGFR, and are more likely to be male and non-smokers. Several ALK inhibitors are in clinical development and have shown promising efficacy so far in EML4–ALK-positive patients, while crizotinib (Xalkori®; Pfizer) has recently been approved in the US for use in patients with ALK-positive, locally advanced or metastatic NSCLC. The ongoing development of immunohistochemical assays should facilitate the routine testing for ALK gene rearrangement and hence the identification of patients who may benefit from these therapies.

PI3K are lipid kinases that act downstream of tyrosine kinases to promote cell survival and proliferation via signalling mediators, including the serine/threonine kinase Akt and mammalian target of rapamycin (mTOR). Amplification and mutation of the PIK3CA gene, which encodes the principal catalytic unit of PI3K, have been implicated in NSCLC in a minority of patients, as have abnormalities in the downstream mediators. Mutations in PIK3CA were found in 1.6% of an international sample of NSCLC tumours. B-Raf is another serine/threonine kinase, coded for by the BRAF gene. Activating mutations in the BRAF gene have been found in 2–5% of NSCLC adenocarcinomas. Approximately half of these mutations involve the amino acid substitution V600E, and this is associated with a particularly aggressive phenotype. Inhibitors of PI3K, mTOR and BRAF are in clinical development.

So far, targeted treatment approaches for NSCLC have been directed at molecular lesions that occur principally in adenocarcinomas and not in squamous cell cancer associated with smoking. Recently, the FGFR1 kinase was identified as a promising therapeutic target for squamous cell lung cancer. Amplification of the FGFR1 gene was found in 22% of squamous lung tumours, compared with only 1% of non-squamous tumours. Furthermore, tumours with FGFR1 amplification were dependent on FGFR1 and were susceptible in vivo to a developmental FGFR inhibitor. Mutations in the gene encoding the discoidin domain receptor (DDR2) kinase have been detected in approximately 3% of squamous cell lung tumours tested, which suggests that such tumours may be susceptible to treatment with multikinase inhibitors in development. IGF1R is a novel marker expressed more commonly in squamous cell NSCLC than in other histologies. Although IGF1R expression does not correlate with survival in patients with resectable NSCLC, a high IGF1R gene copy number (present in 27% of resected NSCLC tumours in one study) appears to be prognostic in this group.

Clinical selection of patients

Several trials have prospectively compared first-line EGFR–TKIs against chemotherapy in patient populations defined clinically according to characteristics associated with activating mutations. The largest trial, the Iressa® Pan-Asia Study (IPASS), randomized 1217 East Asian non-smokers (or former light smokers) with previously untreated advanced adenocarcinomas (79% of whom were female) to gefitinib or carboplatin–paclitaxel. Overall, gefitinib significantly prolonged the median PFS (Table 2), and increased the objective response rate (ORR), compared with chemotherapy. However, the benefit of gefitinib on PFS was limited to patients with EGFR mutations. EGFR mutation data were available for 437 patients, of whom 261 (59.7%) had mutations (mostly exon 19 deletions and L858R mutations). Gefitinib significantly prolonged PFS (compared with chemotherapy) in patients with EGFR mutations, but was associated with a significantly shorter PFS among patients with wild-type EGFR (Fig. 2). There was no difference in OS between the two treatment arms either in the overall analysis or the analysis according to EGFR status (Table 2). These IPASS data indicate that clinical criteria are not sufficient alone to predict benefit from EGFR–TKIs.

Similar findings were observed in the smaller First-SIGNAL trial (First-line Single agent Iressa® versus Gemcitabine and cisplatin Trial in Never-smokers with Adenocarcinoma of the Lung). This study randomized 313 Korean never-smokers with adenocarcinoma to first-line treatment with gefitinib or cisplatin–gemcitabine. Overall, there were no significant differences between the arms in OS or PFS (Table 2). As in IPASS, gefitinib therapy improved PFS versus chemotherapy in mutation-positive patients (although this did not reach statistical significance), but worsened PFS in mutation-negative patients (Table 2). OS did not differ between the groups regardless of EGFR status.

In the United States, the Phase II Cancer and Leukaemia Group B (CALGB) 30406 study randomized 181 mostly Caucasian never or light smokers with adenocarcinoma to first-line therapy with erlotinib administered either alone or in combination with carboplatin–paclitaxel. According to preliminary data (reported in abstract form), these two regimens had similar efficacy and in both arms PFS and OS were significantly prolonged in the subset of patients with EGFR mutations compared with those with wild-type EGFR (Table 2). A similar Phase II study also investigating first-line erlotinib given alone or intercalated with carboplatin–paclitaxel chemotherapy in EGFR-positive advanced NSCLC recently reported that patients with EGFR activating mutations did better on erlotinib alone (median PFS 18.2 months vs. 4.9 months for EGFR-mutant patients on erlotinib and chemotherapy). However, in this study the 6-month PFS rates in both treatment arms were significantly less than historical controls.

Molecular selection of patients

Other trials have specifically selected patients according to the molecular identification of EGFR mutations, rather than by clinical characteristics. The West Japan Thoracic Oncology Group (WJTOG) 3405 trial compared gefitinib against cisplatin–docetaxel in 177 Japanese patients with adenocarcinomas and exon 19 or L858R EGFR mutations. In this population, gefitinib therapy significantly prolonged PFS (Table 2) and improved the ORR compared with chemotherapy. There was no significant difference in median OS between the arms, but the reported data are immature and require further follow-up. Similar data were obtained when the North East Japan Study Group compared gefitinib with carboplatin–paclitaxel in patients with EGFR mutations.

Two phase III trials have compared first-line erlotinib with platinum-based chemotherapy in patients with NSCLC and activating

The role of EGFR–TKIs in patients with EGFR mutation

Small studies in 2004 first showed that NSCLC tumours with activating EGFR mutations were more likely to respond to EGFR–TKIs. Subsequent single-arm studies showed that EGFR mutations predicted survival or progression in patients treated with gefitinib.

A pooled analysis of five trials showed that the presence of EGFR mutations predicted these outcomes more accurately than did the clinical predictors of ethnicity, sex, histology and smoking status.

Overall, small studies in 2004 first showed that NSCLC tumours with activating EGFR mutations were more likely to respond to EGFR–TKIs. Subsequent single-arm studies showed that EGFR mutations predicted survival or progression in patients treated with gefitinib. A pooled analysis of five trials showed that the presence of EGFR mutations predicted these outcomes more accurately than did the clinical predictors of ethnicity, sex, histology and smoking status.
Table 2
Results of selected randomized Phase III trials comparing first-line EGFR tyrosine kinase inhibitors and chemotherapy in previously untreated patients with advanced non-small cell lung cancer, showing effects on survival measures according to EGFR mutational status.

<table>
<thead>
<tr>
<th>Trial/ref</th>
<th>Population</th>
<th>Regimens</th>
<th>Median OS, m (rate, %)</th>
<th>Median PFS, m (rate, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical selection</strong></td>
<td></td>
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<tr>
<td>IPASS</td>
<td>East Asian never smokers or former light smokers with adenocarcinoma</td>
<td>GEF (n = 609)</td>
<td>18.6 HR vs. chemo: 0.91 (95% CI 0.76–1.10)</td>
<td>ND HR vs. chemo: 0.78 (95% CI 0.50–1.20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PAC + CAR (n = 608)</td>
<td>20.3 (12 m 73.7) HR vs. chemo: 1.029 (95% CI 0.756–1.401; p = 0.427)</td>
<td>19.6 (80% CI 14.7–24.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GEF (n = ND)</td>
<td>23.1 (12 m 76.2)</td>
<td>39.0 (80% CI 39.0–NR)</td>
</tr>
<tr>
<td>FIRST-SIGNAL</td>
<td>Korean never smokers with adenocarcinoma</td>
<td>ERL (n = 82)</td>
<td>24.0 (80% CI 18.4–27.6)</td>
<td>19.6 (80% CI 14.7–24.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIS + GEM (n = ND)</td>
<td>30.9 (95% CI 24.1–NR) HR vs. chemo: 1.638 (95% CI 0.749–3.582; p = 0.211)</td>
<td>30.5 (2 y: 61.4%)</td>
</tr>
<tr>
<td></td>
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<td>CIS + DOC, (n = 89)</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>GEF, (n = 115)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CAR + PAC, (n = 115)</td>
<td>30.5 (2 y: 61.4%) HR vs. chemo: 0.31 (95% CI 0.22–0.41; p &lt; 0.001)</td>
<td>23.6 (2 y: 46.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ERL, n = 82</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>CAR + GEM, (n = 72)</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>ERL, n = 77</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>Platinum-based chemotherapy, (n = 76)</td>
<td>–</td>
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</tr>
</tbody>
</table>

**Mutational selection**

| WJTOG3405 | Japanese patients with adenocarcinoma and EGFR mutations (exon 19 deletions or L858R) | GEF, (n = 88) | 30.9 (95% CI 24.1–NR) HR vs. chemo: 1.638 (95% CI 0.749–3.582; p = 0.211) | 30.5 (2 y: 61.4%) |
| | | CIS + DOC, (n = 89) | – | – |
| | | GEF, (n = 115) | – | – |
| | | CAR + PAC, (n = 115) | 30.5 (2 y: 61.4%) HR vs. chemo: 0.31 (95% CI 0.22–0.41; p < 0.001) | 23.6 (2 y: 46.7%) |
| | | ERL, n = 82 | – | – |
| | | CAR + GEM, (n = 72) | – | – |
| | | ERL, n = 77 | – | – |
| | | Platinum-based chemotherapy, (n = 76) | – | – |

**CALGB 30406**

| Never smokers or former light smokers with adenocarcinoma (>75% Caucasian) | | ERL (n = 82) | 24.0 (80% CI 18.4–27.6) | 19.6 (80% CI 14.7–24.6) |
| | | ERL + CAR + PAC (n = 100) | 30.9 (95% CI 24.1–NR) HR vs. chemo: 1.638 (95% CI 0.749–3.582; p = 0.211) | 30.5 (2 y: 61.4%) |
| | | ERL + CAR + PAC (n = 115) | – | – |
| | | ERL, n = 82 | – | – |
| | | CAR + GEM, (n = 72) | – | – |
| | | ERL, n = 77 | – | – |
| | | Platinum-based chemotherapy, (n = 76) | – | – |

**NEJSG**

| Japanese patients with EGFR mutations (and no T790M) | | ERL, n = 82 | – | – |
| | | CAR + PAC, (n = 115) | 23.6 (2 y: 46.7%) | – |
| | | ERL, n = 82 | – | – |
| | | CAR + GEM, (n = 72) | – | – |
| | | ERL, n = 77 | – | – |
| | | Platinum-based chemotherapy, (n = 76) | – | – |

**Europe Randomised Trial of Tarceva® versus Chemotherapy**

| Patients with activating EGFR mutations | | ERL, n = 77 | 22.9 HR vs. chemo: 0.80; p = 0.42 | 22.9 HR vs. chemo: 0.80; p = 0.42 |
| | | Platinum-based chemotherapy, (n = 76) | – | – |

CALGB, Cancer and Leukaemia Group B; CAR, carboplatin; CI, confidence interval; CIS, cisplatin; CTONG, Chinese Thoracic Oncology Group; DOC, docetaxel; EGFR, epidermal growth factor receptor; ERL, erlotinib; EURTAC, European Randomised Trial of Tarceva® versus Chemotherapy; GEF, gefitinib; GEM, gemcitabine; HR, hazard ratio; INTEREST, Iressa NSCLC Trial Evaluating Response and Survival versus Taxotere; IPASS, Iressa Pan-Asia Study; IV, intravenous; m, month; ND, not disclosed; NEJSG, North East Japan Study Group; n, number of patients randomized; NR, not reached; PAC, paclitaxel; OS, overall survival; PO, orally; PFS, progression-free survival; TTP, time to progression; WJTOG, West Japan Thoracic Oncology Group; WT, wild type; y, year.
EGFR mutations (Table 2). According to preliminary abstract data from the Chinese Thoracic Oncology Group (CTONG) 0802 (OPTIMAL) trial, erlotinib significantly improved PFS among Chinese patients, as compared with carboplatin–gemcitabine.106 Interim analysis of the European Randomised Trial of Tarceva/C210 versus Chemotherapy (EURTAC) also showed a significant benefit of erlotinib versus platinum-based chemotherapy in this setting,107 and the EMA has recently amended the label for erlotinib to include the first-line treatment of patients with locally advanced or metastatic NSCLC with EGFR activating mutations. EGFR mutations may vary in their prognostic significance. In the IPASS study, gefitinib achieved a higher tumour response rate against tumours with exon 19 mutations as compared with exon 21 deletions (Mok TS, personal communication). There are limited, mostly retrospective, data suggesting that exon 19 deletions may predict a longer OS and/or PFS after EGFR–TKI therapy than the L858R point mutation.47,9,101,108 For example, according to a pooled analysis of five trials in the United States, patients with exon 19 deletions had a longer median TTP (14.6 months vs. 9.7 months; p = 0.02) and OS (30.8 months vs. 14.8 months; p < 0.001) than those with L858R mutations. However, the prospective, randomized WJTOG3405 and North East Japan Study Group trials did not find a significant difference in PFS between patients with these mutations, regardless of the treatment arm.42,43 Suggested explanations
for this discrepancy include inter-trial differences in patient ethnicity and use of EGFR–TKIs. A potential biological basis for a greater efficacy of EGFR–TKI against exon 19-mutated tumors is suggested by evidence that in untreated patients, EGFR amplification occurs invariably in exon 19 mutants (rather than in wild-type or other mutants) and confers an advantage in these cells. This suggests that exon 19 mutants may be particularly dependent on EGFR signalling, and hence especially sensitive to its inhibition by EGFR–TKI. In addition, biochemical studies suggest that erlotinib is a much better inhibitor of EGFR in the presence of the exon 19 deletion compared to L858R, which may also contribute to the clinical observations.

**EGFR mutation as a prognostic biomarker**

Recent trials have confirmed earlier evidence that EGFR mutations are themselves associated with improved prognosis independently of treatment (Table 2). In the placebo arm of the BR.21 study, the median survival time was 9.1 months among patients with activating EGFR mutations and 3.5 months in those with wild-type EGFR. Combined data from patients treated with carboplatin–paclitaxel with or without erlotinib in the TRIBUTE (Tarc-etova) responses in conjunction with carboplatin and paclitaxel study revealed significant differences between EGFR-mutated and EGFR wild-type in TTP (8 months vs. 5 months; p < 0.001) and OS (not reached vs. 10 months; p < 0.001). Similarly, in the more recent CALGB 3406 trial, PFS and OS were higher in EGFR-mutated patients following treatment with erlotinib with or without carboplatin–paclitaxel (Table 2). In the INTEREST trial, OS was significantly longer among patients with EGFR mutations treated with either gefitinib (median 14.2 months) or docetaxel (16.6 months) than it was in the overall populations treated with these two agents (7.6 months vs. 8.0 months, respectively) or in those with wild-type EGFR (6.4 months vs. 6.0 months, respectively). Therefore, EGFR-mutated NSCLC is now thought to represent a distinct form of NSCLC that has a more indolent prognosis than classical NSCLC and which responds readily to EGFR–TKIs owing to their ‘oncogene-addicted’ pathogenesis. However, further prospective data are required to confirm overcome limitations in the existing data. For example, most of the existing trials involved patients with advanced disease and it is possible that some patients in the control had in fact received EGFR–TKI as salvage therapy. Pertinently, studies in Japan have shown that EGFR mutations do not independently predict survival among patients who undergo surgical resection of early NSCLC. For example, although one univariate analysis of 397 patients found that patients with EGFR mutations survived longer than those without mutations, this association disappeared on multivariate analysis.

The predictive value of EGFR mutations in patients treated with chemotherapy alone is unclear. According to a retrospective analysis, EGFR mutation was not associated with improved survival (as compared with wild-type EGFR) among East Asian patients treated with chemotherapy, despite a non-statistically significant trend toward a higher response rate. Among the predominantly white population in the NCIC Clinical Trials Group JBR.10 study, the presence of exon 19 deletions or L858R mutations was associated with a non-significant trend towards a survival benefit following adjuvant chemotherapy with vinorelbine/cisplatin.

**Clinical trial endpoints: OS versus PFS**

Conventionally, OS is regarded as the gold-standard measure of oncological efficacy. The assessment of OS is easy, objective, highly accurate and of clear significance to patients. It is not dependent on predetermined clinic visits and can be assessed in all randomized patients using an intention-to-treat approach. Many Phase III trials have failed to show a significant benefit of EGFR–TKIs on OS in patients with EGFR-mutated NSCLC, despite their significant prolongation of PFS (Table 2). There are several reasons that might contribute to this finding. Crucially, the indolent prognosis of EGFR-mutated NSCLC, regardless of therapy, is likely to confound the demonstration of a significant difference between treatment arms. Long survival durations necessitate increasingly long follow-up periods, thereby increasing the risk that patients will be lost to follow-up. Furthermore, larger and larger sample sizes are required to provide adequate statistical power to differentiate between increasingly effective comparator regimens. Finally, the assessment of a first-line regimen is confounded by the effects of...
second- and third-line therapies administered upon disease progression especially when those therapies are commercially available, as in the case of gefitinib and erlotinib. In many recent trials, patients who show disease progression after first-line chemotherapy are crossed over onto the comparator EGFR–TKI, skewing the response in the chemotherapy arm. Thus, the endpoint of OS has important limitations in the evaluation of novel NSCLC treatments. The PFS endpoint avoids many of these problems. PFS events occur before OS events and hence can be assessed using shorter follow-up periods and fewer patients. PFS directly measures drug effects on the tumour burden (while OS incorporates non-cancer causes of death), capturing both tumour shrinkage and stabilization. As PFS is documented at the point of progression, it is not influenced by subsequent lines of therapy. However, PFS also has drawbacks and it remains to be validated as a surrogate endpoint against OS. Measures are also required to minimize confounding effects due to variations and in the timing and nature of assessments, and in the subjective evaluation of progression.

Resistance to EGFR–TKI

The long-term efficacy of current EGFR–TKIs in patients with activating EGFR mutations is limited by the invariable development of resistance.

Mechanisms of resistance

In approximately half of cases, acquired resistance is caused by the selection of the secondary T790M mutation in EGFR, wherein threonine is replaced by methionine at position 790 in exon 20. The T790M mutation is thought to confer resistance to current EGFR–TKI therapy by increasing the affinity of EGFR for its substrate, ATP. Rarer secondary point mutations causing EGFR–TKI resistance include the replacement of aspartic acid with tyrosine at position 761 (D761Y) and a threonine-to-alanine switch at position 854 (T854A). T790M may also be a rare cause of primary resistance to EGFR–TKIs. One prospective study reported one patient with a T790M mutation – detected in combination with an L858R mutation using direct sequencing – among tumour samples from 98 screened patients (i.e. 1% prevalence). However, direct sequencing might under-estimate the prevalence of T790M. Researchers in Japan analyzed the mutation profiles among samples from 280 patients with NSCLC, 185 of whom (66%) were not treated with EGFR–TKIs. Direct sequencing revealed the T790M mutation in 0.4% of patients, while mutant-enriched PCR assay detected the mutation in 3.6%. In the IPASS study, T790M was found in 11 of 437 (2.5%) tested samples using PCR analysis. Using PCR, Maheswaran et al. found low pretreatment levels of T790M, likely to be present only in a small number of cells, in 10 of 26 (38%) patients with EGFR-mutated NSCLC. Moreover, PFS following EGFR–TKI therapy was significantly shorter among patients with pretreatment T790M (7.7 months) than in those without it (16.5 months; \( p = 0.001 \)). More recently, Rosell et al. tested 129 NSCLC tumour samples from EGFR–TKI naive patients using the highly specific TaqMan assay in conjunction with a peptide nucleic acid designed to inhibit amplification of the wild-type allele. The T790M was detected in 35% of patients and, on multivariate analysis, was independently and significantly associated with a shorter PFS (HR 4.35; 95% CI 1.85–10.17; \( p = 0.001 \)).

The higher frequencies of T790M mutations detected in these last studies suggest that in some patients the T790M mutation may exist in a fraction of cells before treatment, and that the selection of cells by EGFR–TKI therapy can result in a worse clinical outcome.

While the presence of T790M is associated with EGFR–TKI treatment failure, there is emerging evidence that patients who fail due to the T790M mutation may have a better prognosis than those who fail without this mutation. Among 93 patients who progressed during EGFR–TKI therapy, those with T790M mutation had a significantly longer post-progression survival, were less likely to show subsequent disease progression in a previously uninvolved organ system and had a better performance status at the time of progression, as compared with those without the T790M mutation. Therefore T790M at progression may be a marker for an indolent genotype, despite being associated with EGFR–TKI failure.

In approximately 20% of patients, EGFR–TKI resistance is caused by the amplification of the gene encoding the MET tyrosine kinase. MET amplification confers resistance by triggering the alternative oncogenic signalling ErbB3 pathway and hence is sometimes referred to as ‘kinase switching’. MET amplification can occur independently of T790M mutation, but the two mutations can occasionally occur concurrently. An analysis of 15 gefitinib-resistant tumour samples from six patients revealed that T790M was present in 14/15 (93%) tumours without MET amplification, in four out of five (80%) of those with moderate MET amplification (\( \leq 4\)-fold gene copy number gain), and in only one of 13 (8%) of tumours with greater MET amplification (\( > 4\)-fold).

Although primary MET amplification has been reported in 20% of EGFR–TKI-naïve patients, recent data suggest that levels of amplification conferring EGFR–TKI resistance are rare in untreated patients. As a result, treatment strategies against MET-amplified tumours will be principally relevant to EGFR–TKI-resistant patients. MET amplification can be detected by measuring the MET gene copy number using FISH or quantitative DNA analysis. No clear consensus for defining amplification of MET has yet emerged; many studies employ the same criteria used for defining EGFR amplification, whereas others define amplification based on the copy number of the MET gene normalised to a control gene. Overexpression of the MET protein can also be evaluated by immunohistochemistry, and the optimum methodology for assessing MET status in clinical specimens has yet to be determined. The relationship between gene copy number and clinical outcome, and hence the threshold for resistance, is not well characterized.

Hepatocyte growth factor (HGF), which mediates pro-survival effects through activation of the MET kinase, can induce EGFR–TKI resistance by restoring PIK3-Akt signalling. HGF is mainly expressed by tumour-associated stromal cells and predominantly activates MET kinase in a paracrine manner. High levels of HGF expression were found in samples from two patients with EGFR-mutated adenocarcinomas that were intrinsically resistant to EGFR–TKI and in one patient with a tumour showing acquired resistance, but without T790M mutation or MET amplification. In contrast, HGF immunoreactivity was low among EGFR–TKI responders and patients with T790M-mediated resistance. The developmental anti–MET monoclonal antibody, MetMb, blocks HGF-mediated MET activation. According to preliminary Phase II data, MetMb improved PFS and OS when added to erlotinib in the second- or third-line treatment of patients with MET-positive NSCLC (defined as \( > 50\% \) of tumour cells staining moderately or strongly for MET by immunohistochemistry), but worsened these outcomes in those with MET-negative disease.

A novel acquired resistance mechanism is the acquisition of mutations in PIK3CA, recently reported for the first time in 2 of 37 tested patients (5%). Interestingly, this study also found that tumours in five patients (14%) underwent a phenotypic change – from NSCLC to small cell lung cancer (SCLC) – upon the development of EGFR–TKI resistance. Whether this represented a fundamental histological change following treatment with EGFR–TKIs
was unclear. Alternatively, this may reflect the selection of a population of resistant SCLC cells from a histologically heterogeneous original tumour following eradication of the majority NSCLC cells. The EGFR mutations present in the original NSCLC were maintained in each case. A PIK3CA mutation was acquired by one of these SCLC tumours, but none had T790M or MET amplification. This type of histological change was not observed in patients with tumours resistant to chemotherapy and hence may be associated only with EGFR–TKI resistance.136

Further in vitro studies have revealed other mechanisms underlying the intrinsic or acquired resistance to EGFR–TKI among EGFR-mutated tumours. More recent in vitro data have revealed that the responsiveness of EGFR-mutated NSCLC to EGFR–TKI is mediated, at least in part, by the activation of the pro-survival pathway mediated by FAS and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells).137 Resistance to erlotinib can be generated in EGFR-mutant NSCLC cells by inhibiting the IκB, an NF-κB inhibitor, and thereby enhancing NF-κB activity. Conversely, inhibition of NF-κB activity (by inhibiting IκB kinase) restores erlotinib sensitivity. According to preliminary clinical data, high-level NF-κB activity (measured by low levels of IκB) is associated with shorter survival among patients with EGFR-mutant NSCLC treated with EGFR–TKI. These data suggest that NF-κB inhibition could be a future therapeutic target for use in combination with EGFR–TKI.137

**EGFR–TKI resistance in practice**

Ideally, the mechanism of EGFR–TKI resistance should be identified upon disease progression in order to guide subsequent therapy. Criteria for the definition of acquired resistance to EGFR–TKIs have recently been proposed to aid investigators and physicians (Table 3).138 However, there are important challenges to assessment of resistance in clinical practice.139 First, patients rarely undergo a repeated biopsy upon treatment failure and hence samples for resistance testing are not often available. T790M mutations can be detected non-invasively using PCR-based tests on plasma,140 but these tests require further validation. A second problem is that tests for T790M need to be highly sensitive, as resistance-conferring alleles may represent only a small fraction of the total number of alleles. High-performance liquid chromatography has been used to improve the sensitivity of resistance mutation testing.139 Recently, a molecular beacon designed to detect T790M has been used with PCR and in situ fluorescence analysis to provide a rapid, simple and highly sensitive assay.141 Other challenges include the potential heterogeneity between tumours within the same patient (which may mean that different metastatic sites express different resistance mutations) and the current lack of knowledge concerning the mechanisms of resistance in the fraction of patients who show disease progression without known resistance-conferring point mutations or MET amplification.

**Clinical cross resistance between EGFR–TKIs**

The T790M and MET amplification mutations confer resistance to both gefitinib and erlotinib. Accordingly, uncontrolled case series have reported that erlotinib provides limited benefit in unselected patients progressed during second-line gefitinib therapy.142-145 Vasile et al. collected case series data suggesting that almost a third of patients in whom gefitinib failed achieved a partial response or stable disease to erlotinib therapy.146 These authors pointed out that T790M mutation and MET amplification do not account for all EGFR–TKI failures, suggesting that one agent may fail for reasons that do not preclude the effectiveness of another. Some evidence suggests that patients who previously obtained benefit from gefitinib may have a better clinical response to erlotinib.142,144,146 There are also limited data suggesting that a rebound effect may occur after the cessation of EGFR–TKI therapy in patients who develop acquired resistance. A study of 10 patients showed that symptomatic progression and tumour enlargement that occurred after stopping an EGFR–TKI may be reversed by the re-initiation of the agent.147

**Overcoming EGFR–TKI resistance**

Several orally administered, small-molecule second-generation TKI in late-stage clinical development bind irreversibly to EGFR and hence retain activity against T790M mutants. In addition, these inhibitors are active against multiple receptor types that have interdependent roles in oncogenic signalling.140 Afatinib (BIIB 2992; Boehringer Ingelheim) is an oral, irreversible inhibitor of the Erb family. Afatinib inhibits wild-type EGFR and variants with the T790M, L854R, and MET mutations. In preclinical models, the combination of afatinib and cetuximab against T790M-mutant NSCLC shows significantly greater in vitro and in vivo activity than existing EGFR–TKIs.121,148 According to preliminary data from the single-arm LUX-Lung 2 trial, afatinib showed promising efficacy in patients who were chemotherapy naive or with progressive disease following first-line chemotherapy, and who had EGFR-activating mutations.148 The placebo-controlled phase IIb/III LUX-Lung 1 trial evaluated afatinib in patients with NSCLC refractory to chemotherapy and at least 12 weeks treatment with gefitinib or erlotinib (n = 585). According to preliminary abstract data, afatinib did not prolong OS in the overall patient population.149 As previously discussed, this may reflect the confounding effects of the prolonged survival among patients with EGFR oncogene-addicted tumours and the associated lines of subsequent treatment. However, it is also possible that using the current dosing regimen (continuous daily oral) afatinib does not reach concentrations sufficient to inhibit T790M. A post hoc analysis of 391 patients who were most likely to have EGFR mutations (based on their response to, and duration of, prior treatment with EGFR–TKIs) showed that afatinib significantly prolonged PFS (4.4 months vs. 1 month for placebo) and showed a trend toward improved OS.151 The objective response rate in LUX-Lung 1 was 7%. Further trials are investigating the efficacy of afatinib specifically in patients with EGFR-activating mutations. In preclinical models, the combination of afatinib and the EGFR-specific antibody, cetuximab, showed enhanced activity against T790M-mutant NSCLC.152 Afatinib and cetuximab were evaluated in combination in 26 NSCLC patients with acquired resistance to erlotinib or gefitinib, with confirmed partial responses observed in 8/22 evaluable patients (including 4/13 of patients with T790M+ tumours) and no dose-limiting toxicity.153 PF299 (PF-00299804; Pfizer) is an oral irreversible EGFR/HER2/HER4 inhibitor. Preliminary data from a single-arm Phase II study

**Table 3**

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<th>Proposed criteria for the definition of patients with acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors (EGFR–TKIs).138</th>
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<tr>
<td>Previous treatment with a single-agent EGFR–TKI (e.g. gefitinib or erlotinib)</td>
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<tr>
<td>Presence of a tumour that harbours an EGFR mutation known to be associated with drug sensitivity and/or objective clinical benefit from treatment with an EGFR–TKI</td>
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<tr>
<td>Systemic progression of disease (RECIST or WHO criteria) while on continuous treatment with gefitinib or erlotinib within the last 30 days</td>
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<tr>
<td>No intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy</td>
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RECIST, Response Evaluation Criteria in Solid Tumors; WHO, World Health Organization.
(n = 39) suggest that PF299 may have promising efficacy as first-line therapy in patients with advanced NSCLC and EGFR mutations based on molecular evidence or clinical characteristics. PF299 has also shown promising efficacy in a single-arm Phase I/II study of Korean patients with KRAS wild-type adenocarcinoma after failure of platinum-based chemotherapy and EGFR–TKI therapy.

Neratinib (HKI 272; Wyeth) is an irreversible oral EGFR/HER2 inhibitor. In a Phase II study (n = 167), neratinib had little efficacy in EGFR–TKI-experienced patients (EGFR-mutated or wild type) or EGFR–TKI-naïve patients (ORR 0–3%). No patients with T790M responded. The limited effect of neratinib may have been due to the dose limitation necessitated by a high incidence of diarrhoea.51 BMS-690514 (Bristol-Myers Squibb) is a novel pan-HER/vascular endothelial growth factor (VEGF) receptor inhibitor with efficacy against H1975 cells expressing the T790M mutation. A Phase I/II trial demonstrated the activity of BMS-690514 in patients with NSCLC, although in a randomized, double-blind trial it showed no significant benefit over erlotinib.58

The limited survival benefit observed in trials of the second-generation TKIs may be a result, in some cases, of limited clinical activity of the drug itself. Alternatively, it could reflect issues with clinical trial design (as discussed above for the Lux-1 trials) or the limitations in our understanding of the biological activity of these multi-kinase inhibitors and of the patient populations most likely to obtain benefit.

In addition to these second-generation TKIs, preclinical data have been reported for irreversible, third-generation pyrimidine-based EGFR inhibitors designed with enhanced selectivity against T790M EGFR mutants. Other small molecule agents and monoclonal antibodies targeting EGFR, HER2 and VEGF receptors are also in various stages of clinical development (Table 4). As reviewed elsewhere, an array of other novel therapeutic agents are also under investigation, including those targeting abnormalities in EML4–ALK translocation, the PI3K/Akt/mTOR pathway (e.g. everolimus, sorafenib), MET kinase (e.g. ARQ 197 and MetMab), anti-IGF1R (e.g. CP-751,871), Ras and B-RAF kinases and FGFR.

**Clinical implications: optimizing current and future therapy**

**Molecular testing in practice and in research**

The optimal therapy for individuals with NSCLC depends on the molecular ‘portrait’ of their tumour (Fig. 3). Accordingly, biopsy sampling should be performed to give adequate material for genetic testing. Advances in testing methods should soon facilitate the identification of mutations, allowing the simultaneous screening for multiple mutations and the use of less invasive sampling. The key biomarkers used in clinical decision-making or with a role in future trials of molecular targeted agents are EGFR, KRAS, HER2, EML4–ALK, PI3K, Akt, BRAF and FGFR1.

At present, the systematic detection of EGFR mutations allows clinicians to identify patients most likely to benefit from available
EGFR–TKI in a manner analogous to the use of HER-2 testing to direct trastuzumab therapy for breast cancer. Recently, EGFR analysis is recommended for use principally in the subgroups of patients in whom activating mutations are most common, i.e. patients with adenocarcinomas, never smokers, Asians and women. Determining and reporting EGFR status as ‘positive’ or ‘negative’ may be insufficient given the different effects of known mutations and hence the specific mutation involved should be reported.

In principle, the use of both positive and negative predictive markers of EGFR–TKI efficacy would help optimize the cost-effectiveness of EGFR–TKI use. Thus, in addition to activating mutations, detection of the T790M EGFR mutation and mutations in the MET gene have a role in predicting the response to EGFR–TKIs, although further research is required. Although KRAS has shown potential usefulness as a negative predictor, its role in clinical practice is not clear.

Tests for HER2 and ALK/EML4–ALK may also be part of routine practice in future, but their role is currently limited to clinical trials of novel agents that target these proteins. In the case of ALK gene rearrangement tests, the positivity rate is likely to be increased by pre-selection according to factors associated with this mutation, i.e. adenocarcinoma histology, EGFR/KRAS-negative status and non-smoking. Similarly, tests for mutations in the PIK3CA and BRAF, and amplification of FGFR1, are presently employed to select patients for treatment with developmental therapies.

In the future, DNA dysfunctionality analysis may be used to identify patients who may benefit from sensitizing interventions, such as poly-ADP ribose polymerase (PARP) inhibitors.

Therapy

Histologically driven chemotherapy and radiotherapy remain appropriate for use in patients without genetic mutations associated with response to molecular targeted therapies. Thus, EGFR–TKIs should not be used as first-line therapy for patients wild-type EGFR or patients with unknown EGFR genotypes, including those in whom testing cannot be performed for whatever reason. The presence of genetic mutations should direct the selection of the relevant molecular targeted therapies (Fig. 3). At present, patients with activating mutations should receive first-line EGFR–TKI therapy, based on evidence that these agents benefit PFS, symptoms and quality of life, and because they have the advantage of oral administration. Available data suggest that EGFR–TKI monotherapy may be as efficacious as and less toxic than combination therapy with EGFR–TKIs and chemotherapy in this setting. There are no head-to-head data comparing EGFR–TKIs. However, in Europe, gefitinib is currently approved for use in all lines of therapy in patients with activating EGFR mutations, while erlotinib is only approved for use (regardless of EGFR mutations) after the failure of at least one chemotherapy regimen. This situation may change based on the results of the OPTIMAL trial and EURTAC trials.

The optimum treatment regimen for use in patients who progress during EGFR–TKI therapy is unclear. Furthermore, asymptomatic, slow progression is frequently seen in patients with EGFR-mutated NSCLC during EGFR–TKI therapy and the optimal time to switch these patients to an alternative therapy is unknown. Generally, progressing patients should be exposed to platinum-based chemotherapy. EGFR–TKIs are sometimes continued in order to maintain an EGFR inhibitory ‘pressure’ and to avoid a rebound effect associated with discontinuation. In the near future, a potential strategy in EGFR-addicted NSCLC might be to use an existing EGFR–TKI for first-line therapy, followed by platinum-based chemotherapy when resistance occurs (to reduce selection pressure), followed by a novel Erb-family inhibitor (Fig. 3). It has been suggested that tumours that possess the T790M mutation at progression might be particularly susceptible to ongoing EGFR-targeted therapy owing to dependence on EGFR signalling.

Recent evidence that EGFR–TKI resistance is associated in some cases with histological conversion to SCLC suggests that repeat biopsy may be warranted upon treatment failure, allowing SCLC-specific treatment to be chosen where appropriate.

Conclusions

Cancer management is moving away from organ-based disease definitions to molecular-based classifications. Treatment strategies for oncogene-addicted NSCLC are distinct from those for non-oncogene addicted NSCLC, and will depend on the specific genetic mutation (e.g. in EGFR, HER-2, EML4–ALK, PIK3CA/Akt and FGFR1). EGFR mutations are now known to be both prognostic (i.e. associated with indolent course) and predictive of survival benefit from EGFR–TKI therapy. In the near future the routine care for NSCLC patients is likely to involve a molecular ‘portrait’ that will inform diagnosis, prognosis and – where oncogenic molecular abnormalities are found – the selection of targeted therapy directed at the specific mutation or amplification. Crucially, EGFR-mutated NSCLC is likely to be treated increasingly as a chronic illness owing to its relatively good prognosis. This will require a paradigm shift whereby the former use of palliative chemotherapy is replaced by long-term treatment and monitoring, in a manner akin to current strategies used in HER2-positive breast cancer. A second generation of molecular targeted agents in development for NSCLC is likely to offer important benefits over current EGFR–TKIs, especially in the management of resistant tumours.

Conflict of interest statement

J.C.S. has acted as a consultant for Abbott, Amgen, AstraZeneca, Bristol-Myers Squibb, Boehringer-Ingelheim, glaxosmithkline, Lilly, Merck-Serono, Merck Sharp & Dohme, Pfizer, Roche-Genentech, Servier and sanofi-aventis. T.S.M. has acted as a consultant for AstraZeneca, Roche, Eli Lilly, Pfizer, Taiho, BMS, Merck Serono and Boelinger Ingelheim, has received honoraria from Astrazeneca, Roche, Eli Lilly, Pfizer, Merck Serono, Boehringer-Ingelheim, and has received research funding from Astrazeneca. F.C. has acted as a consultant for Roche and holds a patent for EGFR FISH testing. P.A.J. has acted as a consultant for Boehringer-Ingelheim, Roche, Genentech, Abbott, AstraZeneca and Pfizer. He owns stocks in Gatekeeper Pharmaceuticals and has received royalties from intellectual property related to EGFR mutation tests owned by the Dana-Farber Cancer Institute and licensed to Genzyme.

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