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Genetic profiling and EGFR-directed therapy in NSCLC: evidence and clinical implications

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Abstract:

The principal of preferentially selecting patients most likely to benefit from therapy according to their genetic profile has led to substantial clinical benefit in some tumour types, and has potential to considerably refine treatment in advanced non-small cell lung cancer (NSCLC). Effective, reliable use of molecular biomarkers to inform clinical practice requires the standardization of testing methods and careful assessment of biomarkers' predictive and prognostic value. Although a number of studies show that patients with activating mutations in exons 18–21 respond particularly well to gefitinib and erlotinib, a prospective, randomized study was needed to differentiate between the prognostic and predictive value of epidermal growth factor receptor (*EGFR)* mutations. From one such study, it appears that mutational testing should become standard at diagnosis, at least for adenocarcinoma patients with a never or low smoking history, as clinical predictors are insufficient to optimize treatment. However, outstanding questions remain: what are the treatment options for patients with tumours resistant to erlotinib/gefitinib? What conclusions about treatment can we draw from EGFR copy number or KRAS mutation status? What role should anti-EGFR antibodies play in NSCLC treatment, and in which patients? This review considers current evidence linking biomarker profile to efficacy of EGFR-targeted therapy in NSCLC, and clinical implications of recent findings.

Establishing the principle of mutation testing: lessons from other tumour types

Therapies tailored to specific genetic lesions and diagnostic tests that assay for their respective molecular targets are now an established part of clinical practice across various tumour types, including chronic myeloid leukaemia (CML)[1], gastrointestinal stromal tumours, and epithelial tumours such as breast and colon cancer[2].

Clinically relevant improvements in survival have been attained by administering targeted therapy to the appropriate patient population – for example, the addition of trastuzumab to standard first-line chemotherapy in patients with human epidermal growth factor receptor-positive (HER2+) metastatic breast cancer[3]. A *HER2* amplification diagnostic test is now required in breast cancer before patients are treated with trastuzumab[2]. Clinical practice in colon cancer also reflects the need for mutational testing to identify patients most likely to benefit from cetuximab: patients whose tumours lack a *KRAS* mutation (also called wild-type) show significantly increased overall survival (OS) (median: 9.5 vs. 4.8 months) with cetuximab, whereas those with *KRAS* mutations do not benefit from therapy[4].

These successful examples validate the concept of understanding the genetic profile of patients most likely to benefit from a targeted agent and preferentially selecting those patients for therapy. However, the use of molecular biomarkers to optimize clinical outcomes requires careful assessment of their role in terms of both prognosis and treatment decision-making. Specifically, it is becoming increasingly important to accurately distinguish biomarkers as ‘prognostic’ or ‘predictive’, or define them as both. Prognostic biomarkers can be thought of as a measure of the natural history of a disease that is independent of therapeutic intervention (or lack of it)[5]. A population-based register or a placebo/control group from a randomized clinical study is appropriate for evaluating the prognostic value of a biomarker[5]. In contrast, a predictive biomarker differentiates a group with a particular response or lack of response to a therapeutic intervention. In order to establish which patients will benefit most from a treatment, and by how much, the predictive value of a biomarker must be separated from its prognostic value. To do this, experimental and control arms can be stratified by biomarker status and an interaction test performed. It is recommended, for most cases, that biomarkers should be evaluated prospectively rather than retrospectively.

This review considers the current evidence linking biomarker profile to efficacy of epidermal growth factor receptor (EGFR)-targeted therapy in advanced non-small cell lung cancer (NSCLC), and the clinical implications of recent findings.

EGFR tyrosine kinase inhibitor: the first targeted therapy in NSCLC

In NSCLC, activation of the EGFR/HER1 pathway results in a signalling cascade that promotes tumour growth and progression[6]. EGFR is expressed in a large proportion of NSCLC tumours[7], and its associated signalling pathways are frequently dysregulated. These observations provided the rationale for developing small-molecule tyrosine kinase inhibitors (TKIs) targeting EGFR, erlotinib and gefitinib, and EGFR-targeted antibodies such as cetuximab.

Gefitinib is currently the most widely used EGFR TKI worldwide. It has single-agent activity in patients previously treated with chemotherapy[8, 9], but did not prolong survival compared with placebo in the Iressa Survival Evaluation in Lung cancer (ISEL) randomized Phase III trial in the second- and third-line setting[10]. When compared with single-agent chemotherapy, it has been shown to be non-inferior to docetaxel, with improved quality of life in a large Phase III study of previously treated patients[11]; in a smaller randomized Phase II study of chemotherapy-naïve elderly patients, gefitinib improved quality of life without progression-free survival (PFS) or overall survival (OS) decrement compared with vinorelbine[12]. First-line addition of gefitinib to cisplatin and gemcitabine (INTACT-1)[13] or carboplatin and paclitaxel (INTACT-2)[14] showed no significant difference in response rate (RR) or survival compared with chemotherapy alone.

Erlotinib is the most widely used EGFR TKI in the US and EU, and has also shown single-agent antitumour activity and symptom improvement in previously treated NSCLC patients[15]. In contrast to gefitinib, second-/third-line erlotinib significantly improved OS compared with placebo in the BR.21 Phase III trial (6.7 months vs. 4.7 months; hazard ratio [HR]: 0.70, $p<0.001$)[16]. Like the combination trials with gefitinib, Phase III trials combining erlotinib with first-line chemotherapy (Tarceva Lung Cancer Investigation [TALENT] and Tarceva Responses in Conjunction with Taxol and Carboplatin [TRIBUTE]) showed no significant difference in survival between erlotinib and control arms[17, 18]. Finally, the Phase III Sequential Tarceva in Unresectable NSCLC (SATURN) trial assessed the efficacy of maintenance erlotinib compared with placebo in patients with advanced NSCLC who did not show disease

progression after first-line platinum-based doublet chemotherapy. This trial demonstrated a significant improvement in progression-free survival (PFS) for the 437 patients receiving erlotinib compared with the 447 patients receiving placebo (PFS at 24 weeks: 31% vs. 17%, hazard ratio [HR]: 0.71 [95% confidence interval (CI): 0.62–0.82], log-rank p<0.0001)[19].

To summarize, both gefitinib and erlotinib are considered to be active single-agent therapies in NSCLC patients previously treated with chemotherapy. Reasons for the discrepancy in the BR.21 and ISEL trial outcomes when both drugs are chemically and preclinically quite similar may be due to dosing (erlotinib is dosed at 150 mg/day, its maximum tolerated dose [MTD], whereas gefitinib is dosed at 250 mg/day, about 1/3–1/2 its MTD)[20]. Other contributing factors could be differences in the populations studied in the two trials, including divergent representation of patients most likely to respond, and difference in the definition of second-line patients, either as those with progressive or those with stable disease after first-line treatment.

EGFR mutations in NSCLC: implications for first-line treatment with EGFR TKIs

A subset of patients responds particularly well to EGFR TKIs. Even in early studies, it was apparent that gefitinib and erlotinib were associated with higher responses in those with adenocarcinoma, never-smoking patients, those from East Asia and in women[21]. Somatic activating mutations of the *EGFR* gene have now been identified; these mutations confer an increased susceptibility to EGFR TKI-mediated cell death, and probably underlie the increased responses observed in these clinically defined groups[22-24]. Two *EGFR* mutations – the exon 19 deletion and the exon 21 L858R substitution – account for approximately 90% of all known *EGFR* kinase domain mutations[25].

A substantial body of evidence verifies the importance of *EGFR* mutational status in determining which patients are most likely to respond to treatment with erlotinib/gefitinib. Both retrospective studies of second-/third-line EGFR TKIs in unselected populations, and also prospective studies of first-line EGFR TKI treatment in enriched populations, have been published (Table 1). Over a number of studies, the weighted average RR to EGFR TKI treatment in mutation-positive cases was 78%, with most series reporting a RR of more than 60%. In mutation-negative cases, in contrast, the average RR was 10%[26]. This is evidence that EGFR mutations are clearly associated with response to EGFR TKI therapy.

The studies mentioned above include an evaluation of the impact of *EGFR* mutations on survival after gefitinib approval, compared with historical controls (*EGFR* mutants diagnosed and treated before gefitinib approval). A significant association between *EGFR* mutations and prolonged survival was shown with gefitinib[27]. Taken together, these studies suggest that *EGFR* mutational status may be a predictive biomarker. Furthermore, patients with the exon 19 deletion mutation have significantly prolonged time to progression and increased survival rate compared with those with the exon 21 L858R point mutation[28, 29]. In addition to evidence that *EGFR* mutational status may have predictive value, retrospective data from randomized, controlled trials, including INTACT and TRIBUTE study results, suggest that *EGFR* mutational status also has prognostic value, with patients harbouring *EGFR* mutations demonstrating prolonged survival compared with those who do not, regardless of treatment group assignment[30, 31].

Prospective studies have assessed the efficacy of first-line EGFR TKIs in patients harbouring *EGFR* mutations. One example is the iTARGET trial, in which patients with advanced NSCLC harbouring *EGFR* mutations (including, but not restricted to, the L858R and del19 mutations) received first-line gefitinib[32]. Of 98 patients screened, 34 had *EGFR* mutations and 31 received gefitinib. Response rate, the primary endpoint, was 55%; median progression-free survival (PFS) was 9.2 months (95% CI: 6.2, 11.8)[32]. This study used clinical characteristics to enrich the patient population for those likely to be *EGFR* mutation-positive, demonstrating that genotype-directed therapy with EGFR-TKIs is feasible in a US population, where the overall frequency of *EGFR* mutations is relatively low compared with Asian populations.

Another prospective study in advanced NSCLC was carried out by the Spanish Lung Cancer Group, in which patients with *EGFR* mutations were selected to receive first-line treatment with erlotinib. Lung tumours from 2105 patients were screened; *EGFR* mutations were found in 350 (16.6%) of these and 217 received erlotinib, among them 113 in first-line treatment. In these patients, median PFS was 14.0 months (95% CI: 11.3, 16.7) and median OS was 27 months. This study cohort demonstrates that large-scale screening of patients for *EGFR* mutations and customized treatment with EGFR TKIs is feasible[33].

Taken together, the studies described above demonstrate that EGFR TKIs are highly effective in selected patients, with treatment producing improved response rates and

PFS compared with chemotherapy. Results from these studies also support the concept that, in a particular patient subgroup, first-line treatment with EGFR TKIs may be the most effective option. A prospective, randomized study to differentiate between the prognostic and predictive value of *EGFR* mutations and to determine the optimal treatment strategy for different subgroups of NSCLC patients was needed. This past year, the first such study was completed and published.

The results from the Asian IPASS study of first-line gefitinib versus carboplatin/paclitaxel in 1217 clinically selected patients with advanced NSCLC[34] have considerable implications for clinical practice. Eligible patients were never- or light ex-smokers with adenocarcinoma histology; the overall rate of *EGFR* mutations in the 437 evaluable patients with available tissue was 59.7%. Overall, gefitinib had a superior PFS compared to chemotherapy, exceeding the primary endpoint of the trial, which was to show non-inferiority. The molecular subgroup analysis demonstrated that patients with *EGFR* mutations had superior PFS in the gefitinib arm compared with those in the chemotherapy arm (HR: 0. 48; 95% CI: 0.36, 0.64; p<0.001; treatment by *EGFR* mutations status interaction test, p<0.0001)[34]. A crucial observation from this study is taken from the patients whose tumours were *EGFR*-wild-type. In these patients, all of whom had clinical characteristics typical of gefitinib responders, those receiving gefitinib had a marked decline in PFS compared with those who received chemotherapy (HR: 2.85; 95% CI: 2.05, 3.98; p<0.001)[34]. This argues strongly that mutational testing should become standard practice at diagnosis, at least for adenocarcinoma patients with a never- or low smoking history, as clinical predictors are insufficient to optimize treatment. Such patients should be treated with EGFR TKI therapy in the first-line if their tumours harbour activating *EGFR* mutations, given the demonstrated PFS benefit, and chemotherapy should be the preferred therapy for those patients with wild-type *EGFR*. OS analysis on the IPASS trial is not yet mature. However, other studies support its conclusions: in a smaller Phase III study comparing first-line gefitinib with carboplatin/paclitaxel in patients known to have EGFR mutation-positive advanced NSCLC, PFS was significantly prolonged in the gefitinib group in an interim analysis (10.4 vs. 5.5 months; HR: 0.4, log rank p<0.001)[35]. This was also confirmed by another more recently published Phase III trial comparing first-line gefitinib with cisplatin plus docetaxel in NSCLC patients harbouring *EGFR* mutations. The gefitinib group had significantly prolonged median PFS compared with the patients receiving cisplatin plus docetaxel (9.2 months vs. 6.3 months; HR: 0.489, log rank p<0·0001)[36].

It is important to note that IPASS was an Asian study, and that activating *EGFR* mutations occur at a lower frequency in Caucasian populations (~40% and ~10%, respectively). Some feel that this may play a role in determining the uptake of mutational testing before first-line therapy, although it could be argued that it is more important to perform the definitive test in a population with a lesser chance of mutation. In addition to its implications for therapy choice, IPASS also set a new standard for the collection and analysis of biomarker data within large-scale clinical studies; this has an important bearing on tissue collection and analysis in future studies. Further prospective clinical trials are needed to confirm these findings in a study population that is not entirely Asian, validate that the same trend is seen with other chemotherapeutics (such as pemetrexed), and to examine whether the sequence of chemotherapy and EGFR TKI therapy in patients with mutations influences survival and other outcomes. For example, in a recently published retrospective study including 152 NSCLC patients with exon 19 deletions or L858R, those receiving first-line gefitinib had a significantly higher response rate than chemotherapy-treated patients (76% vs. 54%; p=0.005). However, OS and PFS did not differ significantly between chemo-naïve and chemotherapy-pretreated groups (p=0.207 and p=0.804, respectively)[37]. It is also important to note that patients with EGFR mutations also have a higher response rate to chemotherapy compared to patients with EGFR wild-type. This was demonstrated by a Phase III open-label study investigating the efficacy of gefitinib compared with carboplatin plus paclitaxel in patients with NSCLC. During this study *EGFR* mutation positive patients were shown to have a higher objective response rate to carboplatin/paclitaxel chemotherapy than EGFR wild-type patients (47.3% vs. 23.5%)[34]. Physicians need to consider this information alongside data from mutational testing and the overall state of health of the patient when deciding on first- and second-line therapy, until more conclusive evidence is available; in the long term, data on patient selection may also have an impact on social security reimbursement in European countries. In addition to these considerations, the time to initiation of therapy with EGFR TKIs requires clarification in cases of aggressive disease in which it may not be appropriate to wait for the results of EGFR mutation testing. Furthermore, as it is likely that most patients will at some point receive treatment with an EGFR TKI, the risk of patients with EGFR mutations experiencing side effects from first-line chemotherapy which preclude further treatment, or of new metastases occurring at progression, should always be considered when selecting a first-line treatment.

Resistance to EGFR TKIs: need for a new generation of targeted therapy

Although patients with *EGFR* mutations initially tend to have a good therapeutic response to erlotinib or gefitinib, prolonged administration of either drug invariably leads to secondary resistance, with patients experiencing relapse or tumour progression[25].

So far, two principal mechanisms have been identified that underlie secondary resistance (Figure 1). One is a resistance mutation in the *EGFR* gene, T790M[38, 39], which impairs the binding of the reversible TKIs erlotinib or gefitinib to the ATP binding pocket of the EGFR tyrosine kinase, rendering them ineffective[40]. T790M occurs in ~50% of patients with acquired resistance to gefitinib/erlotinib[38, 41]. Some studies have suggested that, rather than causing the mutation to arise, treatment with TKIs simply selects for the resistant clones. Molecular characterization of tumour tissue from 27 patients with metastatic NSCLC using an ultra-sensitive allele-specific assay revealed that low levels of T790M were present in 38% of patients. The presence of the T790M mutation was associated with a significantly shorter PFS with EGFR TKI therapy compared with patients who did not have detectable levels of T790M at baseline, although it did not preclude response[42]. Although other mutations in exons 19–21 have been identified that also confer resistance to EGFR TKIs[43], T790M is the most common.

Irreversible TKIs that bind covalently with the catalytic pocket of EGFR are believed to provide a sustained blockade of EGFR signalling and may also retain activity against tumours that harbour resistant mutations such as EGFR T790M. Several such agents are under clinical development for the treatment of various tumour types, including EKB-569[44], CI-1033[45], PF-00299804[46] and BIBW 2992[47] (Table 2). In NSCLC, it is crucial to perform studies of these drugs in patients with EGFR mutations, both in those naïve to therapy with first-generation TKIs such as gefitinib and erlotinib, and in those who have progressed through prior TKI therapy. Preliminary Phase II results from 67 patients with *EGFR* mutations receiving BIBW 2992 as second-line treatment show that 66% achieved a PR, with 51% of patients remaining progression-free at 12 months[48]. If irreversible EGFR TKIs prove to be as effective or superior to gefitinib and erlotinib, then defining their role in treating or preventing acquired resistance are questions of great interest.

The second major mechanism of acquired resistance is MET amplification, observed in ~20% of patients with NSCLC who develop resistance to EGFR TKIs[49]. MET

amplification activates PI3K signalling via erbB3, independently of EGFR. This allows signalling downstream of EGFR to continue despite the presence of EGFR inhibitors[50]. MET amplification occurs independently of the T790M mutation, although both can occur simultaneously in the same patient[49, 51]. A number of therapeutic strategies for the inhibition of c-MET or its ligand, hepatocyte growth factor, are currently under investigation in early-phase clinical trials (Table 2)[52].

In general, combination treatment with EGFR TKIs and other agents targeting downstream or redundant pathways may have considerable clinical potential; combination treatment with the mTOR inhibitor rapamycin and irreversible EGFR TKIs has shown activity in preclinical *in vivo* experiments in EGFR L858R/T790M mouse models[53].

With increasing knowledge about the molecular mechanisms of acquired resistance to EGFR TKIs, the clinical implications should be considered: will repeat mutational testing be required during the course of a patient's treatment; if so, are repeat biopsies needed or can sensitive methods be devised that allow mutations to be tested from blood samples? Which samples are most informative – those from the primary tumour or those from metastases? In which order should treatments be administered to optimize response? And which agents are effective once the first-generation EGFR TKIs erlotinib and gefitinib are no longer effective?

EGFR copy number in NSCLC: a more open question than _EGFR_ mutation

In addition to *EGFR* mutations, other biomarkers for identifying patients who may benefit from treatment with EGFR TKIs have been studied. The most notable of these is *EGFR* fluorescence *in situ* hybridization (FISH) status, which indicates whether there is an overall increase in *EGFR* gene copy number[54]. FISH has been shown to correlate with increased sensitivity to gefitinib or erlotinib and increased survival[54-56].

Results from both the BR.21 and ISEL trials suggested that patients with increased gene copy number by FISH had improved survival with EGFR TKI therapy compared with placebo (BR.21: HR, 0.43; 95% CI: 0.23–0.78; p=0.004. ISEL: HR, 0.61; 95% CI: 0.36–1.04; p=0.067)[57, 58]. However, biomarker analyses of the SATURN study indicate that increased *EGFR* copy number by FISH does not have adequate predictive power to enable selection of patients for early second-line treatment with erlotinib over placebo[59]. Furthermore, in randomized trials comparing an EGFR TKI

to chemotherapy, EGFR gene copy number by FISH has not always been associated with improved results on the TKI arm (Table 3). Finally, in the INTEREST study, no significant difference in OS between treatment arms was detected for any of the biomarkers assessed, including *EGFR*-FISH, and *EGFR*-mutation was more powerful than EGFR-FISH analysis in predicting objective response and PFS in patients receiving gefitinib[60].

To date, one prospective clinical trial has selected patients for gefitinib therapy based on *EGFR* copy number by FISH. Results from the Phase II ONCOBELL study show that of 37 patients with sufficient tumour tissue for analysis, 25 (69.4%) were *EGFR* FISH-positive. Patients who had *EGFR* FISH-positive status had a significantly higher RR than *EGFR* FISH-negative patients (68.0% vs. 9.1%; $p<0.001$). *EGFR* FISH-positive patients also had a significantly longer time to progression than *EGFR* FISH-negative patients (7.6 vs. 2.7 months, respectively; $p=0.02$). These data suggest that *EGFR* FISH analysis may, indeed, predict response to gefitinib[61].

In conclusion, *EGFR* gene amplification together with *EGFR* mutation is a common finding and usually affects the mutant allele[62]. It is probable that the predictive value of *EGFR* FISH for EGFR TKI effectiveness is more likely a result of its association with *EGFR* mutations. In some cases, EGFR protein overexpression may result from EGFR amplification alone, but its impact on response to EGFR TKIs remain debatable.

KRAS mutations in NSCLC: do they have predictive or prognostic value? A matter for debate

Somatic mutations in the oncogene *KRAS* have been associated with lack of primary response to EGFR TKIs in several studies. It is thought that mutations in codons 2, 12, 13 and 61 lead to constitutive activation of the RAS protein, which may allow tumour cells to grow independently of EGFR signalling and thus render them resistant to EGFR TKIs[63]. Mutations in *KRAS* occur in approximately 25% of European patients with adenocarcinoma, although they are less common in Asian patients[64]. Increased frequency of *KRAS* mutations have been shown to be not significantly associated with age, gender or smoking history[63]. Using clinical characteristics only to identify those patients who have a very limited chance of responding to treatment with EGFR TKIs is therefore not the best option, and molecular testing will be required.

Analysis of 206 tumours from the BR.21 study showed that 15% had mutations in codons 12 or 13 of *KRAS*. These patients did not appear to derive any benefit from erlotinib therapy, whereas patients with wild-type *KRAS* did appear to gain a survival benefit (HR: 0.69, p=0.03)[58]. In the TRIBUTE study, 55 of 264 patients (21%) had *KRAS* mutations, and those with *KRAS* mutations in the erlotinib arm exhibited significantly shorter OS than those in the chemotherapy-only arm (HR: 2.1; 95% CI: 1.1–3.8, p=0.019)[31]. Preliminary results from 246 patients with sequenced tumour specimens receiving erlotinib in the prospective ERMETIC cohort show that *KRAS* mutations have no significant impact on PFS but negatively affect survival, whereas *EGFR* mutations strongly predict prolonged PFS compared with wild-type *EGFR* but did not affect survival in multivariate analyses including all clinical and molecular markers[65].

As *EGFR* and *KRAS* mutations appear to be mutually exclusive[66-68], the possibility of defining these two biomarkers as predictors of response and resistance to EGFR-TKIs, respectively, is generally accepted by many physicians, although simultaneously occurring mutations in *EGFR* and *KRAS* have been observed very rarely in some tumours[31].

Antibodies to EGFR in NSCLC: waiting for a predictive biomarker?

Cetuximab, a humanized monoclonal antibody that prevents ligand binding in the extracellular domain of EGFR, has shown encouraging results in NSCLC in combination with standard chemotherapy, in both the first- and second-line setting[69-73]. In the FLEX study, a randomized, Phase III study of cetuximab combined with cisplatin/vinorelbine (CV) versus CV alone in the first-line treatment of patients with EGFR immunohistochemistry (IHC)-positive advanced NSCLC, patients receiving cetuximab had statistically longer OS (primary endpoint) than those receiving CV alone (11.3 months vs. 10.1 months; HR: 0.871, p=0.044). There was no significant difference in PFS between treatment groups[72]. The role of *EGFR* copy number, *KRAS* mutation status and *EGFR* IHC values in the FLEX study have recently been reported[74]. A benefit from cetuximab treatment was seen regardless of either *EGFR* copy number by FISH nor *KRAS* mutation status[74]. Currently, only a clinical characteristic is associated with increased PFS with cetuximab in FLEX: the early occurrence of skin rash. However, it is not thought that *EGFR* mutations play a crucial role in cetuximab activity as they do in EGFR TKI treatment, and cross-resistance with EGFR TKIs is unlikely to occur.

Mutation testing: the need for standardization

Standardization of sampling and test methodologies is essential to remove bias, allow comparison across trials and further our understanding of which patients may benefit from specific treatments. However, such efforts are hampered by a lack of consensus on optimal methods between various centres and practical limitations, including tissue availability. Going forward, it is crucial to identify, standardize and validate methods of sampling and testing that are practicable across a wide number of hospital laboratories and create evidence-based practice guidelines, to facilitate comparison of test results between studies. The mutation status of *EGFR* is generally determined from samples taken at surgical resection, biopsy, or fine-needle aspiration, before treatment begins[75-79]. Although minimally invasive fine-needle aspiration procedures have safety advantages for the patient, larger tissue samples, such as those provided by core biopsies, may allow more informative and reliable mutation testing. A further consideration is tumour heterogeneity: it remains unclear whether isolated biopsy samples are truly representative of the overall tumour and whether samples taken from a primary tumour may have a different profile than metastatic sites.

If a high fraction of neoplastic cells are present in a biopsy sample, direct sequencing to determine EGFR mutation status has been regarded as the gold standard[80]. Limitations in the feasibility of genomic DNA sequencing arise when tumour material available for polymerase chain reaction (PCR) or reverse transcriptase PCR is limited. In addition, direct sequencing techniques are relatively costly and time-consuming.

The fixative used in pathologic preservation and the age of the samples can also affect the quality of sequencing test results. Formalin fixation can cause nucleic acid degradation, decreased amplicon length and PCR artifacts[81]. For example, in the molecular analysis of samples from the BR.21 trial of second- and third-line erlotinib, a large proportion of *EGFR* mutations were misidentified as uncommon novel transitions, an error caused by post-mortem deamination of cytosine or adenine. These small aberrations can be artifactually amplified from low concentrations of tumoral DNA and interpreted as significant when a small or antiquated sample is analyzed, whereas such deaminated sites are diluted and not detected when larger amounts of tumoral DNA are used[80, 82].

Biopsy samples with a large proportion of non-neoplastic cells are more suited to allele-specific assays, although these can only be used to assess the presence of a small number of predefined mutations. PCR-based assays are often the preferred

choice here, due to their sensitivity, specificity, robustness and relative cost-effectiveness compared with direct sequencing. Because PCR-based assays look for predefined variants, they avoid the time-consuming steps of tissue microdissection and multiple rounds of DNA extraction, thus enabling their routine use in the clinical setting at acceptable cost. However, allele-specific PCR-based tests can only amplify known mutations in the selected EGFR regions. There are a plethora of different methods that have been published to identify *EGFR* mutations[42, 83-85].

Novel techniques are being developed to improve the feasibility of *EGFR* mutation testing from non-tissue-based samples. Non-invasive testing of *EGFR* mutation status using serum samples and captured circulating tumour cells are under investigation[42, 86]. For example, the SMart Amplification Process (SMAP) is a single nucleotide polymorphism-based diagnostic assay that can be used to detect *EGFR* alterations from blood samples. Hoshi *et al.* adapted the SMAP technology to target three known hotspots for activating *EGFR* mutations, identifying the mutations with a high sensitivity within 30 minutes directly from blood samples[87]. In addition, mutation-specific antibodies which detect deletions in exon 19 and the L858R mutation in exon 21 have been developed and have shown high sensitivity and specificity when tested in paraffin-embedded tumour samples from NSCLC patients[88]. To simplify *EGFR* mutation testing and ease patient selection, one option is inclusion of a standardized, registered companion diagnostic test.

It remains a challenge to ensure that testing methods are used consistently and to encourage the realization of biomarker-directed treatment in NSCLC. Efforts are ongoing: for example, the French National Cancer Institute has implemented a 2-year, multicentre, prospective study (ERMETIC). The primary objective of this study is to evaluate the ability of each of the participating 15 centres to perform biomarker assays, including *EGFR* exons 18–21 and *KRAS* exon 2 sequencing in paraffin-embedded tissues, as determined by the concordance of results between centres with those of an external molecular reference laboratory. After a pilot phase, during which all centres become familiar with the sequencing techniques involved, a prospective analysis has been undertaken of tumour samples from 521 EGFR-TKI-naïve patients with stage IV NSCLC who received erlotinib at these centres. The objective of this part of the study was to assess the effectiveness of *EGFR* sequencing in identifying patients who are likely to benefit from treatment with erlotinib[65].

Conclusions

Having established the current state of evidence regarding genetic profiling and targeted therapy in NSCLC, what clinical implications can we draw? For now, EGFR TKIs should not be given as first-line treatment in the absence of an *EGFR* mutation test. However, we can now realistically envisage *EGFR* mutational testing becoming standard practice in NSCLC diagnostics, especially in patients with appropriate clinical predictors, such as never- but also former smoking patients. As this practice becomes increasingly common, important considerations include the timing of testing and standardization of the methodology used; future efforts should be directed at developing a more practical test for *EGFR* mutations. For patients with *EGFR* mutations, the issue of secondary resistance must be addressed, and the sequence of chemotherapy in treatment paradigms that include EGFR TKIs must be more clearly defined. For patients with *KRAS* mutations, alternative targeted therapies may be more appropriate than EGFR TKIs and should be investigated further. For patients with neither *EGFR* nor *KRAS* mutations, representing the largest proportion of NSCLC patients, further studies to establish the best treatment options are still needed. However, it is likely that because of what we have learned about *EGFR* mutations and EGFR TKIs over the past decade, development of future targeted therapies will include earlier investigation into the genotype of good responders and efforts will be focused on defining particular populations that benefit the most from treatment.

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Tables and Figures

Table 1: Studies evaluating the predictive value of *EGFR* mutations and responses to erlotinib/gefitinib

Study	Number screened	*EGFR* mutations	Treated patients	Treatment line	Drug	RR (%) [95% CI] in *EGFR* mutation-positive patients
Asahina et al.[89]	82	20	16	First-line	Gefitinib	75 (48–93)
Yoshida et al.[79]	66	27	21	Mixed	Gefitinib	90.5 (69.6–98.8)
Sunaga et al.[90]	33	21	21	Mixed	Gefitinib	76 (53–92)
Mok et al.[34]	683	261	NR	First-line	Gefitinib	71.2 (NR)
Sequist et al.[32]	98	34	31	First-line	Gefitinib	55 (33–70)
Inoue et al.[91]	99	25	16	First-line	Gefitinib	75 (54–96)
Rosell et al.[33]	2105	350	217	Not specified	Erlotinib	70.6 (NR)
Rosell et al.[92]	NR	123	12	First-line	Erlotinib	90 (NR)
Tamura et al.[93]	118	32	28	Mixed	Gefitinib	75 (58–93)
Sutani et al.[84]	109	38	27	Second-line	Gefitinib	78 (62–94)
Mitsudomi et al.[36]	337	189	175	First-line	Gefitinib	62.1 (NR)

Costa et al. (pooled analysis)[94]	NA	101	101	Mixed	Gefitinib	80.8 (80–99)

EGFR, epidermal growth factor receptor; RR, response rate; CI, confidence interval; NR, not reported

Table 2: Irreversible EGFR inhibitors and c-MET inhibitors in clinical development in NSCLC

Agent	Target	Development phase	Ongoing Phase II/III studies
Irreversible EGFR inhibitors			
EKB-569	EGFR	Phase II	• Second-/subsequent-line EKB-569 in platinum- and docetaxel-refractory patients with advanced NSCLC (study completed)
CI-1033	EGFR HER2 HER4	Phase II	• Second-/subsequent-line CI01933 in patients with advanced/metastatic NSCLC who have failed prior platinum-based combination chemotherapy (study completed)
BIBW 2992	EGFR HER2	Phase II/III	• Phase II: single-arm study of BIBW 2992 monotherapy in *EGFR* FISH-positive patients • Phase II: single-arm study of BIBW 2992 monotherapy in *EGFR* mutation-positive patients • Phase II single-arm study of BIBW 2992 monotherapy in patients with *EGFR* mutations, HER2/neu mutations or *EGFR* FISH-positive tumours with no *EGFR* mutations • Phase IIB/III: BIBW 2992 in patients with NSCLC who have received 1–2 chemotherapy regimens (including one platinum-containing regimen) and either gefitinib or erlotinib for a period of at least 12 weeks • Phase III: First-line BIBW 2992 versus pemetrexed/cisplatin in patients with lung adenocarcinoma bearing activating *EGFR* mutations
XL647	EGFR HER2 VEGFR2	Phase II	• Open-label study of XL647 monotherapy in previously untreated NSCLC patients • Open-label study of XL647 monotherapy in NSCLC patients who have progressed after previously responding to gefitinib/erlotinib
PF-00299804	Pan-HER	Phase II/III	• Open-label study of PF-00299804 monotherapy in NSCLC patients who have progressed after chemotherapy and erlotinib • Open-label study of PF-00299804 monotherapy in patients with adenocarcinoma who are either non-smokers or former light smokers • PF-00299804 vs. erlotinib in patients with advanced NSCLC who have progressed after 1 or 2 prior chemotherapy regimens • PF-00299804 in patients with advanced NSCLC that has not responded to standard therapy
c-MET inhibitors			
MET-Mab (antibody)	MET	Phase II	• METMab plus erlotinib versus erlotinib plus placebo in second-/third-line NSCLC
ARQ197 (small	MET	Phase I/II	• Randomized study of ARQ 197 plus erlotinib versus erlotinib plus placebo in patients with

molecule; only non-ATP inhibitor)			advanced/metastatic NSCLC who have progressed after one chemotherapy regimen
XL184 (small molecule)	MET, VEGFR2, RET	Phase I/II	• XL184 with or without erlotinib in patients with NSCLC who have progressed after previously responding to erlotinib

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; HER, human epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor

Information on ongoing studies is current as per http://clinicaltrials.gov/. Accessed 4th February 2010. Agents and targets as per authors' own knowledge.

Table 3: Impact of *EGFR* gene copy number and *EGFR* mutations in NSCLC treated by EGFR TKIs

Study	Drug (dose)	Sample number (gene copy evaluation/gene mutation evaluation)	Endpoints analyzed by biomarker	HR for FISH-positive patients (95% CI)	HR for mutation-positive patients (95% CI)
IDEAL/INTACT[30]	Gefitinib (250 mg and 500 mg); gefitinib 250/500 mg/day plus gemcitabine/cisplatin or plus carboplatin/paclitaxel	821	For INTACT: OS Others: NR	For INTACT: 2.03 (0.67–6.13) Others: NR	For INTACT: 1.77 (0.50–6.23) Others: NR
TRIBUTE[95]	Erlotinib (150 mg) plus carboplatin/paclitaxel vs. carboplatin/paclitaxel plus placebo	245	OS	1.52 (0.94–2.46)	NR
BR.21[56]	Erlotinib (150 mg) vs. placebo	125/110	OS	0.44 (0.23–0.82)	0.77 (0.40–1.50)
ISEL[57]	Gefitinib (250 mg) vs. placebo	370/215	OS	0.61 (0.36–1.04)	Evaluation limited owing to low number of deaths in *EGFR*-mutation positive patients
IPASS[34]	Gefitinib (250 mg) vs. carboplatin/paclitaxel	NR	PFS	NR	0.78 (0.50–1.20)
INTEREST[60]	Gefitinib (250 mg) vs. docetaxel	374/297	OS, PFS, RR	For OS: 1.00 (0.80–1.25)	For OS: 0.97 (0.76–1.25)
INVITE[12]	Gefitinib (250 mg) vs. vinorelbine	158/65	PFS, OS	For OS: 2.88 (1.21–6.83)	For OS: NR owing to low patient numbers
SATURN[59]	Erlotinib (150 mg) vs. placebo (maintenance therapy after first-line chemotherapy)	488/437	PFS	0.68 (0.51–0.90)	0.10 (0.04–0.25)
ONCOBELL[61]	Gefitinib 250 mg/day	37	TTP	NR	NR

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; TTP, time to progression; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction; FISH, florescence *in situ* hybridization; NR, not reported

Figure 1: A: The T790M mutation prevents erlotinib/gefitinib from effectively inhibiting phosphorylation of EGFR. B: MET amplification activates PI3K signalling via erbB3, independently of EGFR [55]

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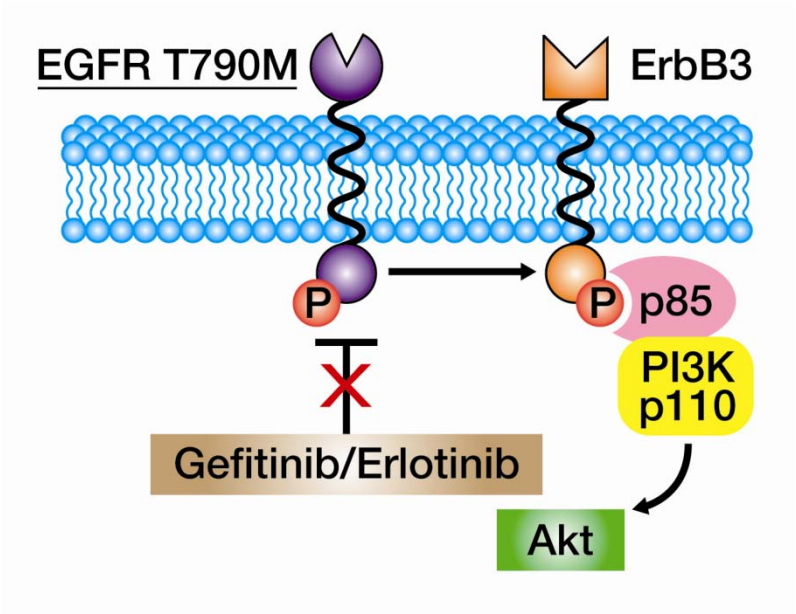
[Note: Ensure hyperlinked in final article]

A



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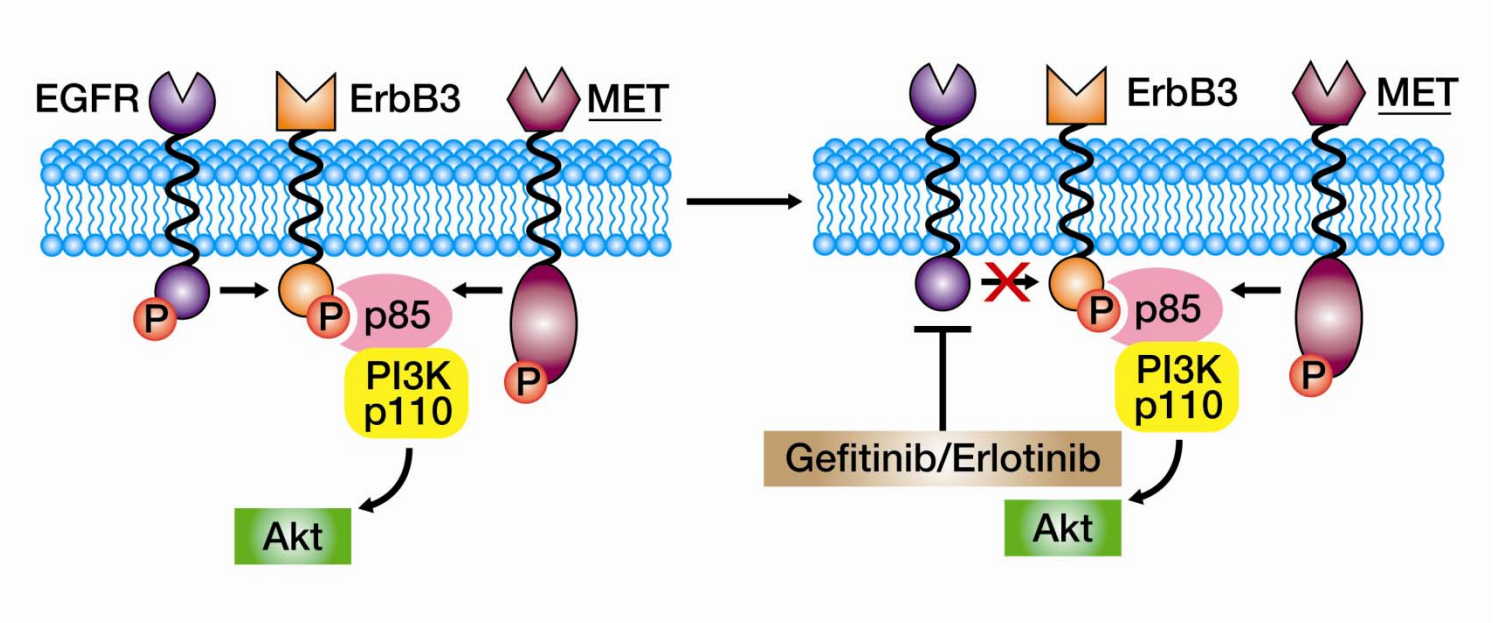

EGFR, epidermal growth factor receptor

References

1. Jobbagy Z, van Atta R, Murphy KM, Eshleman JR, Gocke CD. Evaluation of the Cepheid GeneXpert BCR-ABL assay. J Mol Diagn 2007;9:220-7.
2. Papadopoulos N, Kinzler KW, Vogelstein B. The role of companion diagnostics in the development and use of mutation-targeted cancer therapies. Nat Biotechnol 2006;24:985-95.
3. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783-92.
4. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757-65.
5. Clark G. Prognostic factors versus predictive factors: Examples from a clinical trial of erlotinib. Mol Oncol 2008;1:406-412.
6. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008;358:1160-74.
7. Rusch V, Baselga J, Cordon-Cardo C, et al. Differential expression of the epidermal growth factor receptor and its ligands in primary non-small cell lung cancers and adjacent benign lung. Cancer Res 1993;53:2379-85.
8. Fukuoka M, Yano S, Giaccone G, et al. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial). J Clin Oncol 2003;21:2237-46.
9. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. JAMA 2003;290:2149-58.
10. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527-37.
11. Kim ES, Hirsh V, Mok T, et al. Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. Lancet 2008;372:1809-18.
12. Crino L, Cappuzzo F, Zatloukal P, et al. Gefitinib versus vinorelbine in chemotherapy-naive elderly patients with advanced non-small-cell lung cancer (INVITE): a randomized, phase II study. J Clin Oncol 2008;26:4253-60.
13. Giaccone G, Herbst RS, Manegold C, et al. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. J Clin Oncol 2004;22:777-84.
14. Herbst RS, Giaccone G, Schiller JH, et al. Gefitinib in combination with paclitaxel and carboplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 2. J Clin Oncol 2004;22:785-94.

15. Perez-Soler R, Chachoua A, Hammond LA, et al. Determinants of tumor response and survival with erlotinib in patients with non--small-cell lung cancer. J Clin Oncol 2004;22:3238-47.
16. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al. Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123-32.
17. Gatzemeier U, Pluzanska A, Szczesna A, et al. Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small-cell lung cancer: the Tarceva Lung Cancer Investigation Trial. J Clin Oncol 2007;25:1545-52.
18. Herbst RS, Prager D, Hermann R, et al. TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small-cell lung cancer. J Clin Oncol 2005;23:5892-9.
19. Cappuzzo F, Ciuleanu T, Stelmakh L, et al. SATURN: A double-blind, randomized, phase III study of maintenance erlotinib versus placebo following nonprogression with first-line platinum-based chemotherapy in patients with advanced NSCLC. Journal of Clinical Oncology 2009;27 (Suppl 15):Abstract 8001.
20. Thatcher N. The ISEL and BR.21 trials - outcomes similar or different? European Journal of Cancer Supplements 2006;4:23.
21. Giaccone G, Rodriguez JA. EGFR inhibitors: what have we learned from the treatment of lung cancer? Nat Clin Pract Oncol 2005;2:554-61.
22. Cortes-Funes H, Gomez C, Rosell R, et al. Epidermal growth factor receptor activating mutations in Spanish gefitinib-treated non-small-cell lung cancer patients. Ann Oncol 2005;16:1081-6.
23. Takano T, Ohe Y, Sakamoto H, et al. Epidermal growth factor receptor gene mutations and increased copy numbers predict gefitinib sensitivity in patients with recurrent non-small-cell lung cancer. J Clin Oncol 2005;23:6829-37.
24. Han SW, Kim TY, Hwang PG, et al. Predictive and prognostic impact of epidermal growth factor receptor mutation in non-small-cell lung cancer patients treated with gefitinib. J Clin Oncol 2005;23:2493-501.
25. Sequist LV, Lynch TJ. EGFR tyrosine kinase inhibitors in lung cancer: an evolving story. Annu Rev Med 2008;59:429-42.
26. Sequist LV, Bell DW, Lynch TJ, Haber DA. Molecular predictors of response to epidermal growth factor receptor antagonists in non-small-cell lung cancer. J Clin Oncol 2007;25:587-95.
27. Takano T, Fukui T, Ohe Y, et al. EGFR Mutations Predict Survival Benefit From Gefitinib in Patients With Advanced Lung Adenocarcinoma: A Historical Comparison of Patients Treated Before and After Gefitinib Approval in Japan. J Clin Oncol 2008;26:5589-5595.
28. Jackman DM, Yeap BY, Sequist LV, et al. Exon 19 deletion mutations of epidermal growth factor receptor are associated with prolonged survival in non-small cell lung cancer patients treated with gefitinib or erlotinib. Clin Cancer Res 2006;12:3908-14.
29. Riely GJ, Pao W, Pham D, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19

and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res 2006;12:839-44.
30. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol 2005;23:8081-92.
31. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005;23:5900-9.
32. Sequist LV, Martins RG, Spigel D, et al. First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations. J Clin Oncol 2008;26:2442-9.
33. Rosell R, Moran T, Queralt C, et al. Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 2009;361:958-67.
34. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009;361:947-57.
35. Inoue A, Kobayashi K, Maemondo M, et al. A randomized phase III study comparing gefitinib with carboplatin (CBDCA) plus paclitaxel (TXL) for the first-line treatment of non-small cell lung cancer (NSCLC) with sensitive EGFR mutations: NEJ002 study European Journal of Cancer Supplements 2009;7:Abstract 9 LBA.
36. Mitsudomi T, Morita S, Yatabe Y, et al. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010;11:121-128.
37. Wu JY, Yu CJ, Yang CH, et al. First- or second-line therapy with gefitinib produces equal survival in non-small cell lung cancer. Am J Respir Crit Care Med 2008;178:847-53.
38. Balak MN, Gong Y, Riely GJ, et al. Novel D761Y and common secondary T790M mutations in epidermal growth factor receptor-mutant lung adenocarcinomas with acquired resistance to kinase inhibitors. Clin Cancer Res 2006;12:6494-501.
39. Pao W, Miller VA. Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions. J Clin Oncol 2005;23:2556-68.
40. Yun CH, Mengwasser KE, Toms AV, et al. The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc Natl Acad Sci U S A 2008;105:2070-5.
41. Kosaka T, Yatabe Y, Endoh H, et al. Analysis of epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer and acquired resistance to gefitinib. Clin Cancer Res 2006;12:5764-9.
42. Maheswaran S, Sequist LV, Nagrath S, et al. Detection of mutations in EGFR in circulating lung-cancer cells. N Engl J Med 2008;359:366-77.
43. Bean J, Riely GJ, Balak M, et al. Acquired resistance to epidermal growth factor receptor kinase inhibitors associated with a novel T854A

mutation in a patient with EGFR-mutant lung adenocarcinoma. Clin Cancer Res 2008;14:7519-25.
44. Yoshimura N, Kudoh S, Kimura T, et al. EKB-569, a new irreversible epidermal growth factor receptor tyrosine kinase inhibitor, with clinical activity in patients with non-small cell lung cancer with acquired resistance to gefitinib. Lung Cancer 2006;51:363-8.
45. Janne PA, von Pawel J, Cohen RB, et al. Multicenter, randomized, phase II trial of CI-1033, an irreversible pan-ERBB inhibitor, for previously treated advanced non small-cell lung cancer. J Clin Oncol 2007;25:3936-44.
46. Gonzales AJ, Hook KE, Althaus IW, et al. Antitumor activity and pharmacokinetic properties of PF-00299804, a second-generation irreversible pan-erbB receptor tyrosine kinase inhibitor. Mol Cancer Ther 2008;7:1880-9.
47. Eskens FA, Mom CH, Planting AS, et al. A phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2 (HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours. Br J Cancer 2008;98:80-5.
48. Yang CJ, Chao TJ, Shih J, et al. Use of BIBW 2992, a novel irreversible EGFR/HER2 TKI to induce regression in patients with adenocarcinoma of the lung and activating EGFR mutations: Preliminary results of a single arm phase II clinical trial. Journal of Clinical Oncology 2008;26:8026.
49. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
50. Engelman JA, Janne PA. Mechanisms of acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res 2008;14:2895-9.
51. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci U S A 2007;104:20932-7.
52. Cipriani NA, Abidoye OO, Vokes E, Salgia R. MET as a target for treatment of chest tumours. Lung Cancer 2008;Epub ahead of print.
53. Li D, Ambrogio L, Shimamura T, et al. BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 2008;27:4702-11.
54. Cappuzzo F, Hirsch FR, Rossi E, et al. Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 2005;97:643-55.
55. Hirsch FR, Varella-Garcia M, McCoy J, et al. Increased epidermal growth factor receptor gene copy number detected by fluorescence in situ hybridization associates with increased sensitivity to gefitinib in patients with bronchioloalveolar carcinoma subtypes: a Southwest Oncology Group Study. J Clin Oncol 2005;23:6838-45.
56. Tsao MS, Sakurada A, Cutz JC, et al. Erlotinib in lung cancer - molecular and clinical predictors of outcome. N Engl J Med 2005;353:133-44.

57. Hirsch FR, Varella-Garcia M, Bunn PA, Jr., et al. Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small-cell lung cancer. J Clin Oncol 2006;24:5034-42.
58. Zhu CQ, da Cunha Santos G, Ding K, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. J Clin Oncol 2008;26:4268-75.
59. Brugger W, Triller N, Blasinska-Morawiec M, et al. Biomarker analyses from the phase III placebo-controlled SATURN study of maintenance erlotinib following first-line chemotherapy for advanced NSCLC. Journal of Clinical Oncology 2009;27 (Suppl 15):Abstract 8020.
60. Douillard J, Shepherd F, Hirsh V, et al. Molecular predictors of outcome with gefitinib and docetaxel in previously treated non–small-cell lung cancer: data from the randomized Phase III INTEREST trial. J Clin Oncol 2010;28:744-752.
61. Cappuzzo F, Ligorio C, Janne PA, et al. Prospective study of gefitinib in epidermal growth factor receptor fluorescence in situ hybridization-positive/phospho-Akt-positive or never smoker patients with advanced non-small-cell lung cancer: the ONCOBELL trial. J Clin Oncol 2007;25:2248-55.
62. Li A, Chitale D, Riely G, et al. EGFR mutations in lung adenocarcinomas: clinical testing experience and relationship to the EGFR gene copy number and immunohistochemical expression. J Mol Diagn 2008;10:242-248.
63. Riely GJ, Kris MG, Rosenbaum D, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 2008;14:5731-4.
64. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med 2005;2:e17.
65. Cadranel J, Lizard S, Mauguen A, et al. Impact of clinical and biological markers on progression-free survival (PFS) and overall survival (OS) in patients (pts) with advanced non-small-cell cancer (NSCLC) treated by erlotinib: results of the ERMETIC cohort. 13th World Conference of Lung Cancer 2009;Abstract PD 7.2.1 and e-poster.
66. Kosaka T, Yatabe Y, Endoh H, et al. Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. Cancer Res 2004;64:8919-23.
67. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. J Natl Cancer Inst 2005;97:339-46.
68. Tam IY, Chung LP, Suen WS, et al. Distinct epidermal growth factor receptor and KRAS mutation patterns in non-small cell lung cancer patients with different tobacco exposure and clinicopathologic features. Clin Cancer Res 2006;12:1647-53.
69. Belani CP, Schreeder MT, Steis RG, et al. Cetuximab in combination with carboplatin and docetaxel for patients with metastatic or advanced-stage nonsmall cell lung cancer: a multicenter phase 2 study. Cancer 2008;113:2512-7.

70. Butts CA, Bodkin D, Middleman EL, et al. Randomized phase II study of gemcitabine plus cisplatin or carboplatin [corrected], with or without cetuximab, as first-line therapy for patients with advanced or metastatic non small-cell lung cancer. J Clin Oncol 2007;25:5777-84.
71. Kim ES, Mauer AM, William WN, Jr., et al. A phase 2 study of cetuximab in combination with docetaxel in chemotherapy-refractory/resistant patients with advanced nonsmall cell lung cancer. Cancer 2009;115:1713-22.
72. Pirker R, Pereira JR, Szczesna A, et al. Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. Lancet 2009;373:1525-31.
73. Rosell R, Robinet G, Szczesna A, et al. Randomized phase II study of cetuximab plus cisplatin/vinorelbine compared with cisplatin/vinorelbine alone as first-line therapy in EGFR-expressing advanced non-small-cell lung cancer. Ann Oncol 2008;19:362-9.
74. O'Byrne KA, Bondarenko I, Barrios C, et al. Molecular and clinical predictors of outcome for cetuximab in non-small cell lung cancer (NSCLC): Data from the FLEX study. Journal of Clinical Oncology 2009;27 (Suppl 15):Abstract 8007.
75. Chou TY, Chiu CH, Li LH, et al. Mutation in the tyrosine kinase domain of epidermal growth factor receptor is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res 2005;11:3750-7.
76. Nakajima T, Yasufuku K, Suzuki M, et al. Assessment of epidermal growth factor receptor mutation by endobronchial ultrasound-guided transbronchial needle aspiration. Chest 2007;132:597-602.
77. Pinter F, Papay J, Almasi A, et al. Epidermal growth factor receptor (EGFR) high gene copy number and activating mutations in lung adenocarcinomas are not consistently accompanied by positivity for EGFR protein by standard immunohistochemistry. J Mol Diagn 2008;10:160-8.
78. Savic S, Tapia C, Grilli B, et al. Comprehensive epidermal growth factor receptor gene analysis from cytological specimens of non-small-cell lung cancers. Br J Cancer 2008;98:154-60.
79. Yoshida K, Yatabe Y, Park JY, et al. Prospective validation for prediction of gefitinib sensitivity by epidermal growth factor receptor gene mutation in patients with non-small cell lung cancer. J Thorac Oncol 2007;2:22-8.
80. Eberhard DA, Giaccone G, Johnson BE. Biomarkers of response to epidermal growth factor receptor inhibitors in Non-Small-Cell Lung Cancer Working Group: standardization for use in the clinical trial setting. J Clin Oncol 2008;26:983-94.
81. Williams C, Ponten F, Moberg C, et al. A high frequency of sequence alterations is due to formalin fixation of archival specimens. Am J Pathol 1999;155:1467-71.
82. Marchetti A, Felicioni L, Buttitta F. Assessing EGFR mutations. N Engl J Med 2006;354:526-8; author reply 526-8.
83. Dahse R, Berndt A, Kosmehl H. PCR-based testing for therapy-related EGFR mutations in patients with non-small cell lung cancer. Anticancer Res 2008;28:2265-70.

84. Sutani A, Nagai Y, Udagawa K, et al. Gefitinib for non-small-cell lung cancer patients with epidermal growth factor receptor gene mutations screened by peptide nucleic acid-locked nucleic acid PCR clamp. Br J Cancer 2006;95:1483-9.
85. Tanaka T, Nagai Y, Miyazawa H, et al. Reliability of the peptide nucleic acid-locked nucleic acid polymerase chain reaction clamp-based test for epidermal growth factor receptor mutations integrated into the clinical practice for non-small cell lung cancers. Cancer Sci 2007;98:246-52.
86. Kimura H, Kasahara K, Kawaishi M, et al. Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. Clin Cancer Res 2006;12:3915-21.
87. Hoshi K, Takakura H, Mitani Y, et al. Rapid detection of epidermal growth factor receptor mutations in lung cancer by the SMart-Amplification Process. Clin Cancer Res 2007;13:4974-83.
88. Yu J, Kane S, Wu J, et al. Mutation-specific antibodies for the detection of EGFR mutations in non-small-cell lung cancer. Clin Cancer Res 2009;15:3023-8.
89. Asahina H, Yamazaki K, Kinoshita I, et al. A phase II trial of gefitinib as first-line therapy for advanced non-small cell lung cancer with epidermal growth factor receptor mutations. Br J Cancer 2006;95:998-1004.
90. Sunaga N, Tomizawa Y, Yanagitani N, et al. Phase II prospective study of the efficacy of gefitinib for the treatment of stage III/IV non-small cell lung cancer with EGFR mutations, irrespective of previous chemotherapy. Lung Cancer 2007;56:383-9.
91. Inoue A, Suzuki T, Fukuhara T, et al. Prospective phase II study of gefitinib for chemotherapy-naive patients with advanced non-small-cell lung cancer with epidermal growth factor receptor gene mutations. J Clin Oncol 2006;24:3340-6.
92. Rosell R, Perez-Roca L, Sanchez JJ, et al. Customized treatment in non-small-cell lung cancer based on EGFR mutations and BRCA1 mRNA expression. PLoS ONE 2009;4:e5133.
93. Tamura K, Okamoto I, Kashii T, et al. Multicentre prospective phase II trial of gefitinib for advanced non-small cell lung cancer with epidermal growth factor receptor mutations: results of the West Japan Thoracic Oncology Group trial (WJTOG0403). Br J Cancer 2008;98:907-14.
94. Costa DB, Kobayashi S, Tenen DG, Huberman MS. Pooled analysis of the prospective trials of gefitinib monotherapy for EGFR-mutant non-small cell lung cancers. Lung Cancer 2007;58:95-103.
95. Hirsch FR, Varella-Garcia M, Dziadziuszko R, et al. Fluorescence in situ hybridization subgroup analysis of TRIBUTE, a phase III trial of erlotinib plus carboplatin and paclitaxel in non-small cell lung cancer. Clin Cancer Res 2008;14:6317-23.