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Minireview Biomarkers of clinical benefit for anti-epidermal growth factor receptor agents in patients with non-small-cell lung cancer

The title of this article has been corrected since Advance Online Publication.

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Non-small-cell lung cancer (NSCLC) remains by far the major cause of cancer-related death in the Western world in both men and women. The majority of patients will be diagnosed with metastatic disease, and chemotherapy doublets remain the cornerstone of treatment for these patients. However, chemotherapy has a minimal impact on long-term survival and prognosis remains poor for these patients. Further improvement in treatment is likely to require incorporation of novel targeted therapies. Among these agents, inhibitors of the epidermal growth factor receptor (EGFR) have demonstrated significant activity in the first-, second- or third-line treatment of NSCLC. The purpose of current paper is to present the evidence for using several proposed molecular biomarkers as a tool for selection of NSCLC patients for anti-EGFR treatment. According to current data, *EGFR* mutation status appears to be the strongest predictor for the selection of NSCLC patients to first-line treatment with EGFR tyrosine kinase inhibitors vs chemotherapy. Use of other biomarkers remains investigational.

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Chemotherapy has been the backbone of treatment for patients with advanced non-small-cell lung cancer (NSCLC) for the last decades; however, it has clearly reached a plateau of activity, and thus further improvements will require integration of novel therapies. Among the targeted agents, epidermal growth factor receptor (EGFR) inhibitors gefitinib and erlotinib are now established as an option for first-, second- or third-line treatment (Shepherd *et al*, 2005; Kim *et al*, 2008; Mok *et al*, 2009; Maemondo *et al*, 2010; Mitsudomi *et al*, 2010) or as maintenance treatment (Cappuzzo *et al*, 2010a). Furthermore, the addition of cetuximab, a monoclonal antibody, against the extracellular domain of EGFR to the vinorelbine/cisplatin doublet resulted in a statistically significant, but modest, survival prolongation (Pirker *et al*, 2009).

A subset of patients treated with EGFR inhibitors experience a clinical benefit and even these patients eventually develop disease progression. It is clear that we need to identify reliable predictive factors that will allow for the selection of patients who are most likely to benefit from a particular agent, while sparing others from toxicity of ineffective treatments and the health-care systems from the significant costs of these newer agents. The purpose of the present paper is to focus on the current evidence for using several

proposed molecular biomarkers as a tool for selection of NSCLC patients for anti-EGFR treatment.

SEARCH STRATEGY AND SELECTION CRITERIA

A bibliographic search of the Medline database was conducted for papers published from 1 January 2000 to 1 July 2010, with the keywords 'non-small-cell lung cancer', 'epidermal growth factor receptor', 'erlotinib' 'gefitinib' and 'cetuximab'. The search was limited to articles written in English. When considering chemotherapy, targeted therapy or multimodality treatment, only data from phase III trials or randomised phase II trials were incorporated. The Medline search was supplemented by a manual search of meeting abstracts (World Conference on Lung Cancer, European Society of Medical Oncology Annual Congress, American Society of Clinical Oncology Annual Meeting, European Lung Cancer Conference) as well as reference lists of original and review articles. A consensus was reached among all authors for the manuscript.

POSITIVE PREDICTIVE FACTORS

Protein expression by immunohistochemistry

Association of positive EGFR immunostaining, as determined by immunohistochemistry (IHC) in NSCLC specimens, with patient

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sensitivity to EGFR TKI treatment has been studied extensively with both positive (Cappuzzo et al, 2005; Hirsch et al, 2007) and negative (Parra et al, 2004) results reported. Four placebocontrolled phase III trials have evaluated EGFR TKIs as maintenance (Takeda et al, 2010; Cappuzzo et al, 2010a; Sequential Tarceva in Unresectable NSCLC (SATURN) and West Japan Thoracic Oncology Group (WJTOG) 0203 trials), second- or third-line treatment (Shepherd et al, 2005; Thatcher et al, 2005; NCIC Clinical Trials Group BR.21 and Iressa Survival Evaluation in Lung Cancer (ISEL) trials). Another phase III trial, the ATLAS trial (Kabbinavar et al, 2010), was designed to evaluate the addition of erlotinib to bevacizumab maintenance in NSCLC patients who have not progressed after first-line chemotherapy plus bevacizumab. Patients with EGFR-expressing tumours had significantly higher response rate (RR) in the BR.21 (P = 0.03) and ISEL trials (8.2 vs 1.5%; P not reported; Tsao et al, 2005; Hirsch et al, 2006). In three trials (SATURN, BR.21 and ISEL), patients with tumours showing positive EGFR immunostaining had a significantly reduced risk of death or progression with TKI treatment vs placebo (Tsao et al, 2005; Hirsch et al, 2006; Brugger et al, 2009) with hazard ratios (HRs) of 0.68-0.77 in favour of EGFR TKI therapy (Table 1). However, it should be noted that in the ISEL trial, the benefit was of borderline significance (treatment by biomarker interaction test P = 0.049; Hirsch et al, 2006). The WJTOG 0203 was a relatively small and negative trial and the lack of any biomarker published data limits the interpretation and applicability of the findings of this study. In the ATLAS trial, EGFR IHC analysis had no predictive value for progression-free survival (PFS) (Johnson et al, 2009). The cut-off point analyses of two large placebo-controlled trials in the second- and third-line setting revealed that the originally proposed criterion to define EGFR positivity (10% of cells with any staining intensity) had the best predictive discrimination (Hirsch et al, 2008).

Two phase III trials that compared TKIs with chemotherapy either in first-line (Mok *et al*, 2009) or second-line setting (Kim *et al*, 2008) reported biomarker data with tumour EGFR immunostaining. The Iressa Pan-Asian Study (IPASS) study randomly assigned Asian chemo-naive NSCLC patients (neversmokers or former light smokers with adenocarcinoma) to gefitinib or to paclitaxel/carboplatin chemotherapy (Mok *et al*, 2009). This trial met its primary end point of showing noninferiority of gefitinib, but furthermore demonstrated its superiority compared with chemotherapy for PFS (HR 0.74, P < 0.001). The Iressa NSCLC Trial Evaluating Response and Survival *versus* Taxotere (INTEREST) trial was a non-inferiority phase III trial that compared gefitinib with docetaxel as second-line treatment (Kim *et al*, 2008). This study also confirmed that gefitinib was noninferior to docetaxel in terms of overall survival (OS) (HR 1.020). In these trials, using chemotherapy as the comparator, no predictive value of EGFR IHC analysis was observed for response, PFS or survival, and EGFR protein expression status-by-treatment interaction tests were not significant (Fukuoka *et al*, 2009; Douillard *et al*, 2010).

Two phase III trials have assessed the role of monoclonal anti-EGFR antibody therapy in addition to first-line chemotherapy in the treatment of NSCLC (Pirker *et al*, 2009; Lynch *et al*, 2010). The FLEX trial investigated the combination of cisplatin/vinorelbine plus or minus cetuximab, and demonstrated a statistically significant although modest survival benefit in favour of cetuximab in patients with tumours positive for EGFR protein expression. A second smaller trial, which compared the combination of a taxane/carboplatin plus or minus cetuximab (BMS-099) in unselected patients, failed to show a PFS or survival benefit in favour of the experimental arm. The biomarker analysis did not reveal any association between EGFR protein expression and response, PFS or survival (Khambata-Ford *et al*, 2010).

According to the above studies, EGFR protein positivity is observed in the vast majority of NSCLC tumour specimens (ranging from approximately 70 to 90% in most studies), which makes this marker unlikely to be used in practice for patient selection. Placebo-controlled phase III trials with EGFR TKIs in the second- or third-line setting were the only studies indicating some predictive value of lack of protein expression in selecting patients who do not benefit from these agents, although its predictive discrimination did not meet the expectations of a clinically useful test (i.e., clinically meaningful difference between patient subsets).

Trial	N	HR	95% CI	<i>P</i> -value	Biomarker by treatment interaction P-value
	1 2005 T 1 200				
BR.21 (Shepherd et d	il, 2005; Tsao et al, 200	(5)			
Positive	184	0.68	0.49-0.95	0.02	NR
Negative	4	0.93	0.63-1.36	0.70	
ISEL (Thatcher et al,	2005; Hirsch et al, 2006	5)			
Positive	264	0.77	0.56-1.08	0.126	0.049
Negative	115	1.57	0.86-2.87	0.140	
SATURN (Brugger et	al, 2009; Cappuzzo et a	ıl, 2010b) ^a			
Positive	NR	0.69	0.58-0.82	< 0.000 I	NR
ATLAS (Johnson et al,	2009) ^a				
Positive	, 191	0.92	0.64-1.32	NR	
Negative	67	1.00	0.55-1.82	NR	NR
INTEREST (Kim et al.	2008: Douillard et al. 2	.010)			
Positive	284	1.00	0.77-1.29	0.98	0.87
Negative	96	1.00	0.65-1.55	0.99	
IPASS (Fukuoka et al,	2009; Mok et al, 2009)	a			
Positive	266	0.73	0.55-0.96	0.0243	0.21
Negative	99	0.97	0.64-1.48	0.8932	
0					

Table I Survival HRs according to EGFR protein expression in phase III trials with EGFR tyrosine kinase inhibitors

Abbreviations: ATLAS = Avastin and Tarceva or Avastin and pLAcebo in patients with NSCLC; CI = confidence interval; EGFR = epidermal growth factor receptor; HR = hazard ratio; ISEL = Iressa Survival Evaluation in Lung Cancer; INTEREST = Iressa NSCLC Trial Evaluating Response and Survival versus Taxotere; IPASS = Iressa Pan-Asian Study; NR = not reported; SATURN = Sequential Tarceva in Unresectable NSCLC. ^aHR for progression-free survival.

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Table 2 Survival HRs according to EGFR gene copy number as assessed by FISH in phase III trials with EGFR tyrosine kinase inhibitors

Trial	N	HR	95% CI	P-value	Biomarker by treatment interaction P-value
BR 21 (Shepherd et al. 20)05: Tsao et al. 2005: 7	7bu et al 2008)			
FISH positive	61	0.43	023-078	0.0042	012
FISH negative	98	0.80	0.49 - 1.29	0.3525	0.12
ISEL (Thatcher et al. 2005	5; Hirsch et al, 2006)				
FISH positive	4	0.61	0.36-1.04	0.067	0.045
FISH negative	256	1.16	0.81-1.64	0.417	
SATURN (Brugger et al. 2	009: Cappuzzo et al. 2	010b) ^a			
FISH positive	· · · , · · · · · · · ,	0.69	NR	0.0001	NR
ATLAS (Johnson et al, 200)9) ^a				
FISH positive	87	0.66	0.39-1.13	NR	NR
FISH negative	109	1.40	0.86-2.28	NR	
INTEREST (Kim et al, 200	8; Douillard et al, 2010))			
FISH positive	174	1.09	0.78-1.51	0.62	0.52
FISH negative	200	0.93	0.68-1.26	0.64	
IPASS (Fukuoka et al, 200	9; Mok et al, 2009)ª				
FISH positive	249	0.66	0.50-0.88	0.0050	0.0437
FISH negative	157	1.24	0.87-1.76	0.2368	

Abbreviations: ATLAS = Avastin and Tarceva or Avastin and pLAcebo in patients with NSCLC; CI = confidence interval; EGFR = epidermal growth factor receptor; FISH = fluorescence *in situ* hybridisation; HR = hazard ratio; ISEL = Iressa Survival Evaluation in Lung Cancer; INTEREST = Iressa NSCLC Trial Evaluating Response and Survival versus Taxotere; IPASS = Iressa Pan-Asian Study; NR = not reported; SATURN = Sequential Tarceva in Unresectable NSCLC. ^aHR for progression-free survival.

EGFR gene copy number

EGFR gene copy number, assessed by fluorescence in situ hybridisation (FISH), has been tested extensively as a predictive factor for response and survival benefit from TKI treatment. The original classification of FISH positivity includes both gene amplification (rare in NSCLC) and high polysomy (≥4 copies of the EGFR gene in >40% of tumour cell nuclei; Cappuzzo et al, 2005). In placebo-controlled studies (BR.21 and ISEL studies; Shepherd et al, 2005; Thatcher et al, 2005), high EGFR copy number was associated with higher response rate and significantly prolonged OS from EGFR TKI treatment (Tsao et al, 2005; Zhu et al, 2008; Table 2). Moreover, in the BR.21 study, high EGFR copy number by FISH was both prognostic for worse survival in untreated patients (P = 0.025) and predictive of greater survival benefit in erlotinib-treated patients (P = 0.005). In the ISEL trial, high EGFR copy was associated with a survival benefit in patients receiving gefitinib compared with placebo (HR 0.61; P = 0.067), whereas no benefit was observed in patients with FISH-negative tumours (HR 1.16; P = 0.417; comparison of HRs high vs low copy number; P = 0.045; Hirsch et al, 2006). In patients treated with placebo, high EGFR copy was associated with a numerically shorter survival, indicating that copy number might also be prognostic. In the biomarker analysis of the SATURN trial, patients derived a PFS benefit with erlotinib irrespective of EGFR FISH status in their tumours (Brugger et al, 2009). Similarly, in the biomarker analysis of the ATLAS trial, EGFR FISH status had no statistically significant predictive value for PFS, although HRs for PFS were numerically different within patient subsets (Table 2; Johnson et al, 2009)

The FISH EGFR assay had no predictive value for survival in randomised trials comparing TKI treatment with chemotherapy (Kim *et al*, 2008; Mok *et al*, 2009). In the INTEREST trial, RR was higher in EGFR FISH-positive patients treated with gefitinib compared with docetaxel (13.0 vs 7.4%; P = 0.04; Douillard *et al*, 2010). Overall survival and PFS were similar between the two treatment arms, irrespectively of *EGFR* copy number (OS treatment effect between high and low copy number: HR 1.09 and 0.93, respectively; *EGFR* copy number status-by-treatment interaction test; P = 0.52). In the IPASS study, EGFR FISH positivity was associated with higher response rate and a borderline PFS benefit from gefitinib when compared with platinum-based chemotherapy (P = 0.044; Fukuoka *et al*, 2009). Placebo-controlled phase III trials of cetuximab in combination with chemotherapy (FLEX and BMS-099) failed to show an association between *EGFR* gene copy number status and clinical end points, including PFS, OS and RR (O'Byrne *et al*, 2009; Khambata-Ford *et al*, 2010).

A phase II trial was performed with prospective EGFR gene copy number assessment (Cappuzzo *et al*, 2007). The trial was not limited exclusively to patients with EGFR FISH positive tumours. The biomarker results indicate that PFS and OS benefit in patients with high *EGFR* gene copy number in their tumours appears to be derived from overlapping *EGFR* mutation positivity.

In summary, *EGFR* copy number is predictive of survival benefit from erlotinib or gefitinib in placebo-controlled trials in patients who failed previous chemotherapy (Tsao *et al*, 2005; Hirsch *et al*, 2006). These observations were not confirmed in clinical trials comparing EGFR TKI treatment with chemotherapy (Kim *et al*, 2008; Mok *et al*, 2009), suggesting that the predictive value of *EGFR* gene copy number assessment is confined to second/third line trials with placebo arm as a comparator. At present, *EGFR* gene copy number testing is not recommended in the selection of first- or second-line treatment of advanced NSCLC patients. Data from phase III trials do not suggest a role for *EGFR* gene copy number in predicting benefit from anti-EGFR monoclonal antibodies in NSCLC.

Somatic EGFR mutations

Most somatic mutations of the *EGFR* gene observed in NSCLC involve the tyrosine kinase coding domain (exons 18–21). Discovery of these mutations in tumours from NSCLC patients was immediately linked with response to gefitinib (Lynch *et al*, 2004; Paez *et al*, 2004). In placebo-controlled phase III studies of

gefitinib (Thatcher *et al*, 2005) and erlotinib (Shepherd *et al*, 2005; Cappuzzo *et al*, 2010a), patients with *EGFR*-mutated tumours had significantly higher RR compared with patients with wild-type tumours. In the BR.21 study, both groups derived a survival benefit (Zhu *et al*, 2008). In the ISEL study, there were too few patients with mutations for survival subset analysis (Hirsch *et al*, 2006), whereas in the SATURN trial, a remarkable PFS benefit was observed in patients with tumours with *EGFR* mutations in the erlotinib arm (HR 0.10; P < 0.0001; Brugger *et al*, 2009). Similarly, the biomarker analysis of the ATLAS trial reported a significant benefit in terms of PFS in patients with tumours bearing *EGFR* mutations in the erlotinib arm (HR 0.44; Johnson *et al*, 2009).

In the INTEREST trial, EGFR mutation-positive patients had significantly longer PFS (HR 0.16; P = 0.001) and higher RR when treated with gefitinib when compared with docetaxel (ORR 42.1 vs 21.1%; P = 0.04; Douillard et al, 2010). Patients harbouring EGFR mutation-positive tumours had longer survival in both gefitinib and docetaxel groups (median survival 14.2 and 16.6 months, respectively) than in the overall population (7.6 and 8.0 months, respectively), and in the population with wild-type EGFR (6.4 and 6.0 months, respectively), indicating that EGFR mutations have a positive prognostic role. There was no OS difference between treatment groups according to EGFR mutation status (subset of patients with mutated tumours, HR = 0.83 vs those with wild-type EGFR, HR = 1.02, interaction test; P = 0.59; Douillard *et al*, 2010). In the IPASS study, patients with EGFR-mutated tumours had significantly higher RR with gefitinib compared with chemotherapy (71.2 vs 47.3%; P = 0.0001; Fukuoka et al, 2009). There was also a striking difference in PFS in patients with EGFR-mutated tumours treated with gefitinib compared with those treated with chemotherapy (9.5 vs 6.3 months; HR = 0.48; P < 0.001). The predictive role of EGFR mutation was also demonstrated by the noteworthy differences in PFS observed in patients with *EGFR* mutation-positive or -negative tumours when treated with gefitinib (9.5 vs 1.5 months). In patients without EGFR TK mutations, PFS was significantly superior in the group treated with chemotherapy compared with gefitinib (HR = 2.85; P < 0.001; Table 3). The results of two phase III Japanese trials comparing gefitinib and chemotherapy as first-line treatment in NSCLC patients exclusively with tumours harbouring *EGFR* mutations confirmed improved outcomes with EGFR TKIs (Maemondo *et al*, 2010; Mitsudomi *et al*, 2010). Similar results were also observed in a Chinese trial with erlotinib (Zhou *et al*, 2010).

The NSCLC cell lines harbouring *EGFR* gene mutations are less sensitive to monoclonal antibodies than to EGFR tyrosine kinase inhibitors (Mukohara *et al*, 2005). In the BMS-099 trial, *EGFR* mutation status did not predict benefit from concurrent treatment with cetuximab and chemotherapy. Survival tended to be longer in patients with mutated *EGFR* compared with those with wild-type *EGFR* (HR 0.61; P = 0.09). This trend was more apparent in the chemotherapy group (HR 0.46; P = 0.06) than in the cetuximab group (HR 0.84; P = 0.66), confirming the prognostic role of *EGFR* mutations (Khambata-Ford *et al*, 2010).

Based on the above mentioned trials, *EGFR* mutation testing is now recommended as part of routine care of NSCLC patients to guide decisions about first-line treatment.

Germline EGFR polymorphisms

Regulatory sequences of the *EGFR* gene are located within the 5' flanking region, and a highly polymorphic $(CA)_n$ repeat is situated in intron 1 of the gene. *In vitro* as well as *in vivo* data indicate that *EGFR* transcriptional activity may be influenced by the number of CA repeats (Gebhardt *et al*, 2000). Given the association between gene expression and the number of CA repeats, the efficacy of anti-

Table 3 Survival HRs according to EGFR mutation status in phase III clinical trials with EGFR tyrosine kinase inhibitors

Trial	N	HR	95% CI	P-value	Biomarker by treatment interaction P-value
BR.21 (Shepherd et al, 2005; Tsao et al, 2005; Z	Zhu et al, 2008)				
EGFR mutated	30	0.55	0.25-1.19	0.1217	0.47
EGFR wild-type	176	0.74	0.52-1.05	0.0924	
ISEL (Thatcher et al. 2005: Hirsch et al. 2006)					
EGFR mutated	26	NR	NR	NR	NR
EGFR wild-type	189				
SATURN (Brugger et al, 2009; Cappuzzo et al, 2	010b) ^a				
EGFR mutated	22	0.10	0.04-0.25	< 0.000	
EGFR wild-type	199	0.78	0.63-0.96	0.0195	
ATLAS (Johnson et al. 2009) ^a					
EGFR mutated	52	0.44	0.22-0.86	NR	NR
EGFR wild-type	295	0.85	0.64-1.13	NR	
INTEREST (Kim et al, 2008; Douillard et al, 2010))				
EGFR mutated	,	0.83	0.41-1.67	0.60	0.59
EGFR wild-type	NR	1.02	0.78-1.33	0.91	
IPASS (Fukuoka et al. 2009: Mok et al. 2009)ª					
EGFR mutated	261	0.48	0.36-0.64	< 0.00	< 0.000
EGFR wild-type	176	2.85	2.05-3.98	< 0.00	
WITOG3405 (Mitsudomi et al, 2010) ^{a,b}	177	0.489	0.336-0.710	< 0.0001	NA
NEJ002 (Maemondo et al, 2010) ^{a,c}	230	0.36	0.25-0.51	< 0.001	NA
CTONG 0802 (Zhou et al, 2010)	154	0.16	0.10-0.26	< 0.000 I	NA

Abbreviations: ATLAS = Avastin and Tarceva or Avastin and pLAcebo in patients with NSCLC; CI = confidence interval; EGFR = epidermal growth factor receptor; HR = hazard ratio; ISEL = Iressa Survival Evaluation in Lung Cancer; INTEREST = Iressa NSCLC Trial Evaluating Response and Survival*versus*Taxotere; IPASS = Iressa Pan-Asian Study; NR = not reported; NA = not applicable; SATURN = Sequential Tarceva in Unresectable NSCLC; TKI = tyrosine kinase inhibitor. ^aHR for progression-free survival. ^bGefitinib vs cisplatin/docetaxel. ^cGefitinib vs paclitaxel/carboplatin.

EGFR treatment could vary according to a patient's genotypic differences. Two clinical single cohort studies in Asian patients (Han *et al*, 2007; Nie *et al*, 2007) have reported higher response rates in patients with low CA repeats, and longer time to progression (HR 0.54, P = 0.014; Han *et al*, 2007) and OS (20 vs 11 months, RR: 1.89; P = 0.039; Nie *et al*, 2007). Similarly, an American study (Liu *et al*, 2008) reported improved PFS in patients homozygous for the shorter lengths of CA repeats. This observation was not confirmed by other studies (Gregorc *et al*, 2008), and one study reported an association between shorter CA repeats and poorer survival in the absence of anti-EGFR treatment (Dubey *et al*, 2006). Molecular analysis of the SATURN trial did not confirm predictive value of the number of intron 1 CA repeats (Brugger *et al*, 2009).

In addition to EGFR polymorphisms, much interest is focussed on polymorphisms of the *ABCG2* gene, which codes for a multidrug transporter that has been shown to effectively remove gefitinib and erlotinib from cells (Li *et al*, 2007). The ABCG2 421C>A (Q141K) polymorphism results in a glutamine to lysine substitution in codon 141 and has been associated with increased toxicity in patients treated with gefitinib (Cusatis *et al*, 2006) or with increased concentrations of both gefitinib and erlotinib (Li *et al*, 2007; Rudin *et al*, 2008).

It should be noted that all these data are based on retrospective review of small, single cohort studies, using different definitions of key variables such as 'short' or 'long' intron 1 CA repeats. Therefore, these studies are unable to properly define the predictive or prognostic role of these polymorphisms in NSCLC patients treated with EGFR TKIS.

No data exist about the role of EGFR polymorphisms as predictors for treatment outcome with anti-EGFR monoclonal antibodies.

NEGATIVE PREDICTIVE FACTORS

EGFR mutations and resistance to anti-EGFR treatment

One of the mechanisms of primary and acquired resistance in patients who receive TKI treatment is insertion point mutations in exon 20 of the *EGFR* gene. The spectrum of resistant mutations includes the exon 20 insertion mutants D770_N771 (ins NPG), D770_(ins SVQ) and D770_(ins G) N771T (Gazdar, 2009). Nevertheless, it should be noted that these mutations are relatively rare, suggesting that other mechanisms also contribute to primary resistance to EGFR TKI treatment.

Virtually all patients responding to TKI treatment will inevitably develop resistance to these agents. A point mutation in the tyrosine kinase domain (T790M) is found in approximately half of patients at the time of acquired resistance to EGFR TKI therapy (Gazdar, 2009). This mutation has been observed in a small fraction of cells in tumours from pretreated patients, believed to be gained through selective pressure during treatment (Gazdar, 2009). At present, there are insufficient data to treat patients with tumours having classical activating exon 19 or 21 mutations that coexist with exon 20 T790M mutations differently than patients without exon 20 mutations. Physicians should be aware that the detection of resistance mutation may herald the development of clinical resistance to gefitinib or erlotinib.

K-RAS

Ras plays an important role in the EGFR downstream signalling pathway, by activating Raf-kinase, MAPK and promoting cell proliferation (Hynes and Lane, 2005). The K-RAS mutations result in EGFR-independent activation of MAPK and are mutually exclusive with EGFR mutations (Pao *et al*, 2005). These mutations have been proposed as a mechanism of primary resistance to TKIs



in NSCLC and are observed in ~15-30% of NSCLC patients. Several studies suggest that K-RAS mutations are negative predictive factors of response to single-agent TKI treatment in advanced/metastatic NSCLC (Zhu *et al*, 2008). However, the molecular analysis of the SATURN trial showed that the benefit from maintenance erlotinib is similar in patients with and without K-RAS mutations in their tumours (HR for PFS 0.77 and 0.70, respectively; Brugger *et al*, 2009). Although several studies support that anti-EGFR monoclonal antibodies are not active in colorectal cancer patients with K-RAS gene mutations, it seems that K-RAS mutations have no predictive role in NSCLC patients treated with these agents (O'Byrne *et al*, 2009; Khambata-Ford *et al*, 2010), although limited data are available. At present, there are insufficient data to use K-RAS mutation status for lung cancer patient selection to EGFR inhibitor therapy.

Serum proteomic determination of predictive biomarkers for TKIs

Matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry is a potentially powerful and inexpensive tool for identifying protein signatures in serum. Using this approach, a TKI prediction algorithm was identified using a training set of 139 samples of serum or plasma (Taguchi et al, 2007). Based on eight discriminating features and validated in two independent cohorts, it selectively predicted survival in patients who had received an EGFR TKI. In cohort 1, there were 67 patients treated with gefitinib. Survival in the high-risk group was 92 vs 207 days in the low-risk group with HR of 0.50 and 95% CIs of 0.24-0.78. In cohort 2, survival was 107 vs 306 days with HR of 0.41 and 95% CIs 0.17-0.63. This serum proteomic classifier has been commercially developed (Veristrat) and was shown to associate with outcome in a clinical trial of erlotinib and bevacizumab (Carbone et al, 2010a). An 11 proteomic feature-based classifier has been developed that associated with OS in a Cox proportional hazards model in the training set (P = 0.0006) and also when applied in a blinded test to patients treated with erlotinib alone in the phase II first-line monotherapy trial, ECOG 3503 (n = 82, P < 0.0001; Salmon et al, 2009). Analysis of the proteomic classifier in the sera from patients included in the BR.21 trial was recently reported and showed that this marker had mainly prognostic role (Carbone et al, 2010b).

DISCUSSION

Identification of predictive markers is important for selection of patients with advanced/metastatic NSCLC who are likely to obtain a clinical benefit from anti-EGFR treatment. A panel of such biomarkers has been extensively evaluated in NSCLC patients treated in clinical trials with these agents. The EGFR expression as determined by IHC should not be considered as a valid predictive marker given that published results are conflicting with some studies showing weak predictive value (mainly placebo-controlled second/third-line trials), not confirmed in other studies. High EGFR gene copy number, as assessed by FISH, has been associated with a survival benefit in the placebo-controlled phase III TKI trials (Hirsch et al, 2006; Zhu et al, 2008), but had no predictive value in randomised trials comparing TKI treatment with chemotherapy (Fukuoka et al, 2009; Douillard et al, 2010). On the contrary, EGFR mutations were associated with a dramatic benefit in terms of PFS in both placebo-controlled (Brugger et al, 2009) and chemotherapy-controlled trials (Fukuoka et al, 2009; Douillard et al, 2010). Furthermore, a recent meta-analysis by Dahabreh et al (2010) reported that EGFR mutations are predictive of response to TKIs with a higher sensitivity and specificity compared with EGFR gene gain, although survival improvement may not be confined exclusively to patients with tumour shrinkage. On the basis of the data from clinical trials comparing

EGFR TKIs with chemotherapy, *EGFR*-activating mutation status appears to be the most valid marker for the selection of patients who would derive the most benefit from TKI treatment.

It is not clear why conflicting results are reported between trials. The major strength of the above presented conclusions is that they are based (with the exception of germline EGFR polymorphisms) on data derived from large randomised phase III trials. On the other hand, it should be noted that molecular analyses derived from placebo-controlled studies (BR.21 and ISEL) were retrospective, not preplanned and restricted to patient subsets with available samples and thus likely to be biased (McShane et al, 2005). Therefore, all results based on these trials should be considered exploratory (Zhu et al, 2008). On the contrary, chemotherapy-controlled trials (INTEREST, IPASS) had a prospective preplanned biomarker analysis. Furthermore, conflicting results about the predictive role of EGFR gene copy number could be explained by possible biological differences between early (first line or maintenance) vs late (second and third line) settings. The role of EGFR mutations was confirmed in three phase III trials specially designed for the population of patients treated in the first-line setting (Maemondo et al, 2010; Mitsudomi et al, 2010; Zhou et al, 2010).

An important issue is when to use EGFR TKI in patients who have EGFR mutations in their tumours – should these agents be administered as first-line, maintenance or as second/third-line treatment? Only comparative data exist to answer this question; no prospective study has been specifically designed to address this issue and cross-study comparisons are not reliable. It is unlikely that a clinical trial will be designed to answer this question, given the large number of patients who will be needed. Given that there is unquestionable benefit in terms of PFS, RR and quality of life in the first-line setting, and that only a subgroup of patients will be suitable for second-line treatment, EGFR TKIs should be recommended in NSCLC patients harbouring EGFR mutations for first-line treatment (D'Addario *et al*, 2010).

REFERENCES

- Brugger W, Triller N, Blasinska-Morawiec M, Curescu S, Sakalauskas R, Manikhas G, Mazieres J, Whittom R, Rohr K, Cappuzzo F (2009) Biomarker analyses from the phase III placebo-controlled SATURN study of maintenance erlotinib following first-line chemotherapy for advanced NSCLC. J Clin Oncol 27(15s): abstract 8020
- Cappuzzo F, Ciuleanu T, Stelmakh L, Cicenas S, Szczesna A, Juhasz E, Esteban E, Molinier O, Brugger W, Melezinek I, Klingelschmitt G, Klughammer B, Giaccone G (2010a) Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. *Lancet Oncol* 11(6): 521-529
- Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn Jr PA, Varella-Garcia M (2005) Epidermal growth factor receptor gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst 97(9): 643-655
- Cappuzzo F, Ligorio C, Janne PA, Toschi L, Rossi E, Trisolini R, Paioli D, Holmes AJ, Magrini E, Finocchiaro G, Bartolini S, Cancellieri A, Ciardiello F, Patelli M, Crino L, Varella-Garcia M (2007) 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 25(16): 2248-2255
- Cappuzzo F, Tallini G, Finocchiaro G, Wilson RS, Ligorio C, Giordano L, Toschi L, Incarbone M, Cavina R, Terracciano L, Roncalli M, Alloisio M, Varella-Garcia M, Franklin WA, Santoro A (2010b) Insulin-like growth factor receptor 1 (IGF1R) expression and survival in surgically resected non-small-cell lung cancer (NSCLC) patients. *Ann Oncol* 21(3): 562-567
- Carbone DP, Salmon JS, Billheimer D, Chen H, Sandler A, Roder H, Roder J, Tsypin M, Herbst RS, Tsao AS, Tran HT, Dang TP (2010a)

Another important issue is whether treatment with EGFR TKIs should be denied to patients with tumours showing wild-type EGFR gene. The BR.21 study reported that both groups (tumours having EGFR mutations or wild-type EGFR) derive a survival benefit from treatment with erlotinib compared with placebo, although the effect of erlotinib was much greater in patients with EGFR-mutated tumours (Shepherd et al, 2005). A similar observation was reported in the SATURN trial (Cappuzzo et al, 2010a). Based on the results of this and other trials, EGFR TKI treatment should not be confined to patients harbouring EGFR mutations, although the smaller benefit in patients with EGFR wild-type tumours should be taken into account in pharmacoeconomic analyses to guide reimbursement decisions. It is likely that a combination of markers, such as K-RAS mutations or other as yet unidentified markers, will be used in future to identify patients who will not benefit from EGFR TKI therapy.

An important concern is the feasibility of large-scale screening of NSCLC patients for *EGFR* mutations. A study from the Spanish Lung Cancer Group reported the screening of >2000 NSCLC patients and found mutations in 350 patients (16.6%; Rosell *et al*, 2009). Median PFS and OS for 217 patients who received erlotinib were 14 and 27 months, respectively. New techniques of *EGFR* mutation testing, in particular IHC with antibodies constructed against abnormal EGFR proteins as a result of gene mutations, should facilitate large-scale testing (Kitamura *et al*, 2010).

The evolution of our knowledge of biomarkers to guide treatment in NSCLC patients treated with *EGFR* inhibitors came in parallel to the clinical trials testing these agents. This knowledge should serve as a lesson in the current development of other agents in NSCLC, where many trials do not meet their end points because of a questionable clinical benefit in unselected populations. Rational drug development, based on profound understanding of tumour biology, a drug's mechanism of action and clinical implications of patient selection, is hoped to impact on the current poor treatment outcomes of NSCLC patients.

VeriStrat((R)) classifier for survival and time to progression in nonsmall cell lung cancer (NSCLC) patients treated with erlotinib and bevacizumab. *Lung Cancer* **69**(3): 337–340

- Carbone DP, Seymour L, Ding K, Roder H, Tsao M, Shepherd FA (2010b) Serum proteomic prediction of outcomes in advanced NSCLC patients treated with erlotinib/placebo in the NCIC clinical trials group BR.21 trial. J Thorac Oncol 5(5 (Suppl 1): abstract 2030
- Cusatis G, Gregorc V, Li J, Spreafico A, Ingersoll RG, Verweij J, Ludovini V, Villa E, Hidalgo M, Sparreboom A, Baker SD (2006) Pharmacogenetics of ABCG2 and adverse reactions to gefitinib. J Natl Cancer Inst 98(23): 1739-1742
- D'Addario G, Fruh M, Reck M, Baumann P, Klepetko W, Felip E (2010) Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **21**(Suppl 5): v116-v119
- Dahabreh IJ, Linardou H, Siannis F, Kosmidis P, Bafaloukos D, Murray S (2010) Somatic EGFR mutation and gene copy gain as predictive biomarkers for response to tyrosine kinase inhibitors in non-small cell lung cancer. *Clin Cancer Res* **16**(1): 291-303
- Douillard JY, Shepherd FA, Hirsh V, Mok T, Socinski MA, Gervais R, Liao ML, Bischoff H, Reck M, Sellers MV, Watkins CL, Speake G, Armour AA, Kim ES (2010) 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* **28**(5): 744-752
- Dubey S, Stephenson P, Levy DE, Miller JA, Keller SM, Schiller JH, Johnson DH, Kolesar JM (2006) EGFR dinucleotide repeat polymorphism as a prognostic indicator in non-small cell lung cancer. *J Thorac Oncol* 1(5): 406-412
- Fukuoka M, Wu Y, Thongprasert S, Yang C, Chu D, Saijo N, Watkins C, Duffield EL, Armour AA, Mok T (2009) Biomarker analyses from a phase III, randomized, open-label, first-line study of gefitinib (G) vs

carboplatin/paclitaxel (C/P) in clinically selected patients (pts) with advanced non-small cell lung cancer (NSCLC) in Asia (IPASS). J Clin Oncol 27(15s): abstract 8006

- Gazdar AF (2009) Activating and resistance mutations of EGFR in nonsmall-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene 28(Suppl 1): S24-S31
- Gebhardt F, Burger H, Brandt B (2000) Modulation of EGFR gene transcription by secondary structures, a polymorphic repetitive sequence and mutations-a link between genetics and epigenetics. *Histol Histopathol* 15(3): 929-936
- Gregorc V, Hidalgo M, Spreafico A, Cusatis G, Ludovini V, Ingersoll RG, Marsh S, Steinberg SM, Vigano MG, Ghio D, Villa E, Sparreboom A, Baker SD (2008) Germline polymorphisms in EGFR and survival in patients with lung cancer receiving gefitinib. *Clin Pharmacol Ther* 83(3): 477-484
- Han SW, Jeon YK, Lee KH, Keam B, Hwang PG, Oh DY, Lee SH, Kim DW, Im SA, Chung DH, Heo DS, Bang YJ, Kim TY (2007) Intron 1 CA dinucleotide repeat polymorphism and mutations of epidermal growth factor receptor and gefitinib responsiveness in non-small-cell lung cancer. *Pharmacogenet Genomics* 17(5): 313-319
- Hirsch FR, Dziadziuszko R, Thatcher N, Mann H, Watkins C, Parums DV, Speake G, Holloway B, Bunn Jr PA, Franklin WA (2008) Epidermal growth factor receptor immunohistochemistry: comparison of antibodies and cutoff points to predict benefit from gefitinib in a phase 3 placebocontrolled study in advanced nonsmall-cell lung cancer. *Cancer* **112**(5): 1114-1121
- Hirsch FR, Varella-Garcia M, Bunn Jr PA, Franklin WA, Dziadziuszko R, Thatcher N, Chang A, Parikh P, Pereira JR, Ciuleanu T, von Pawel J, Watkins C, Flannery A, Ellison G, Donald E, Knight L, Parums D, Botwood N, Holloway B (2006) Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-smallcell lung cancer. J Clin Oncol 24(31): 5034–5042
- Hirsch FR, Varella-Garcia M, Cappuzzo F, McCoy J, Bemis L, Xavier AC, Dziadziuszko R, Gumerlock P, Chansky K, West H, Gazdar AF, Crino L, Gandara DR, Franklin WA, Bunn Jr PA (2007) Combination of EGFR gene copy number and protein expression predicts outcome for advanced non-small-cell lung cancer patients treated with gefitinib. Ann Oncol 18(4): 752-760
- Hynes NE, Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5(5): 341-354
- Johnson B, Miller V, Amler LC, Stern H, Soh C, O'Connor P, Kabbinavar F (2009) Biomarker evaluation in the randomized, double-blind, placebocontrolled, phase IIIb ATLAS trial, comparing bevacizumab (B) therapy with or without erlotinib (E), after completion of chemotherapy with B for the treatment of locally advanced, recurrent, or metastatic non-small cell lung cancer (NSCLC). *Eur J Cancer* 7(3): 5
- Kabbinavar F, Miller VA, Johnson BE, O'Connor P, Soh C, for the ATLAS investigators (2010) Overall survival (OS) in ATLAS, a phase IIIb trial comparing bevacizumab (B) therapy with or without erlotinib (E) after completion of chemotherapy (chemo) with B for first-line treatment of locally advanced, recurrent, or metastatic non-small cell lung cancer (NSCLC). J Clin Oncol 28(15s): abstract 7526
- Khambata-Ford S, Harbison CT, Hart LL, Awad M, Xu LA, Horak CE, Dakhil S, Hermann RC, Lynch TJ, Weber MR (2010) Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non-small-cell lung cancer. J Clin Oncol 28(6): 918–927
- Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, Li LY, Watkins CL, Sellers MV, Lowe ES, Sun Y, Liao ML, Osterlind K, Reck M, Armour AA, Shepherd FA, Lippman SM, Douillard JY (2008) Gefitinib versus docetaxel in previously treated non-small-cell lung cancer (INTEREST): a randomised phase III trial. *Lancet* 372(9652): 1809–1818
- Kitamura A, Hosoda W, Sasaki E, Mitsudomi T, Yatabe Y (2010) Immunohistochemical detection of EGFR mutation using mutationspecific antibodies in lung cancer. *Clin Cancer Res* 16(13): 3349–3355
- Li J, Cusatis G, Brahmer J, Sparreboom A, Robey RW, Bates SE, Hidalgo M, Baker SD (2007) Association of variant ABCG2 and the pharmacokinetics of epidermal growth factor receptor tyrosine kinase inhibitors in cancer patients. *Cancer Biol Ther* **6**(3): 432–438
- Liu G, Gurubhagavatula S, Zhou W, Wang Z, Yeap BY, Asomaning K, Su L, Heist R, Lynch TJ, Christiani DC (2008) Epidermal growth factor receptor polymorphisms and clinical outcomes in non-small-cell lung cancer patients treated with gefitinib. *Pharmacogenomics J* 8(2): 129–138

Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani

DC, Settleman J, Haber DA (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* **350**(21): 2129–2139

- Lynch TJ, Patel T, Dreisbach L, McCleod M, Heim WJ, Hermann RC, Paschold E, Iannotti NO, Dakhil S, Gorton S, Pautret V, Weber MR, Woytowitz D (2010) Cetuximab and first-line taxane/carboplatin chemotherapy in advanced non-small-cell lung cancer: results of the randomized multicenter phase III trial BMS099. *J Clin Oncol* 28(6): 911–917
- Maemondo M, Inoue A, Kobayashi K, Sugawara S, Oizumi S, Isobe H, Gemma A, Harada M, Yoshizawa H, Kinoshita I, Fujita Y, Okinaga S, Hirano H, Yoshimori K, Harada T, Ogura T, Ando M, Miyazawa H, Tanaka T, Saijo Y, Hagiwara K, Morita S, Nukiwa T (2010) Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N Engl J Med* **362**(25): 2380–2388
- McShane LM, Altman DG, Sauerbrei W, Taube SE, Gion M, Clark GM (2005) REporting recommendations for tumour MARKer prognostic studies (REMARK). Br J Cancer 93(4): 387-391
- Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K, Fukuoka M (2010) 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* 11(2): 121–128
- Mok TS, Wu YL, Thongprasert S, Yang CH, Chu DT, Saijo N, Sunpaweravong P, Han B, Margono B, Ichinose Y, Nishiwaki Y, Ohe Y, Yang JJ, Chewaskulyong B, Jiang H, Duffield EL, Watkins CL, Armour AA, Fukuoka M (2009) Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med 361(10): 947-957
- Mukohara T, Engelman JA, Hanna NH, Yeap BY, Kobayashi S, Lindeman N, Halmos B, Pearlberg J, Tsuchihashi Z, Cantley LC, Tenen DG, Johnson BE, Janne PA (2005) Differential effects of gefitinib and cetuximab on non-small-cell lung cancers bearing epidermal growth factor receptor mutations. J Natl Cancer Inst **97**(16): 1185–1194
- Nie Q, Wang Z, Zhang GC, An SJ, Lin JY, Guo AL, Li R, Gan B, Huang Y, Mok TS, Wu YL (2007) The epidermal growth factor receptor intron1 (CA) n microsatellite polymorphism is a potential predictor of treatment outcome in patients with advanced lung cancer treated with Gefitinib. *Eur J Pharmacol* 570(1-3): 175-181
- O'Byrne K, Bondarenko I, Barrios CH, Eschbach C, Martens U, Hotko Y, Kortsik C, Celik I, Stroh C, Pirker R (2009) Molecular and clinical predictors of outcome for cetuximab in non-small cell lung cancer (NSCLC): data from the FLEX study. J Clin Oncol 27(15s): abstract 8007
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**(5676): 1497-1500
- Pao W, Wang TY, Riely GJ, Miller VA, Pan Q, Ladanyi M, Zakowski MF, Heelan RT, Kris MG, Varmus HE (2005) KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med* 2(1): e17
- Parra HS, Cavina R, Latteri F, Zucali PA, Campagnoli E, Morenghi E, Grimaldi GC, Roncalli M, Santoro A (2004) Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib ('Iressa', ZD1839) in non-small-cell lung cancer. Br J Cancer 91(2): 208-212
- Pirker R, Pereira JR, Szczesna A, von PJ, Krzakowski M, Ramlau R, Vynnychenko I, Park K, Yu CT, Ganul V, Roh JK, Bajetta E, O'Byrne K, de MF, Eberhardt W, Goddemeier T, Emig M, Gatzemeier U (2009) Cetuximab plus chemotherapy in patients with advanced non-small-cell lung cancer (FLEX): an open-label randomised phase III trial. *Lancet* 373(9674): 1525-1531
- Rosell R, Moran T, Queralt C, Porta R, Cardenal F, Camps C, Majem M, Lopez-Vivanco G, Isla D, Provencio M, Insa A, Massuti B, Gonzalez-Larriba JL, Paz-Ares L, Bover I, Garcia-Campelo R, Moreno MA, Catot S, Rolfo C, Reguart N, Palmero R, Sanchez JM, Bastus R, Mayo C, Bertran-Alamillo J, Molina MA, Sanchez JJ, Taron M (2009) Screening for epidermal growth factor receptor mutations in lung cancer. N Engl J Med 361(10): 958-967
- Rudin CM, Liu W, Desai A, Karrison T, Jiang X, Janisch L, Das S, Ramirez J, Poonkuzhali B, Schuetz E, Fackenthal DL, Chen P, Armstrong DK, Brahmer JR, Fleming GF, Vokes EE, Carducci MA, Ratain MJ (2008) Pharmacogenomic and pharmacokinetic determinants of erlotinib toxicity. J Clin Oncol 26(7): 1119-1127

- Salmon S, Chen H, Chen S, Herbst R, Tsao A, Tran H, Sandler A, Billheimer D, Shyr Y, Lee JW, Massion P, Brahmer J, Schiller J, Carbone D, Dang TP (2009) Classification by mass spectrometry can accurately and reliably predict outcome in patients with non-small cell lung cancer treated with erlotinib-containing regimen. J Thorac Oncol 4(6): 689–696
- Shepherd FA, Rodrigues PJ, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van KM, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabarbara P, Seymour L (2005) Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 353(2): 123-132
- Taguchi F, Solomon B, Gregorc V, Roder H, Gray R, Kasahara K, Nishio M, Brahmer J, Spreafico A, Ludovini V, Massion PP, Dziadziuszko R, Schiller J, Grigorieva J, Tsypin M, Hunsucker SW, Caprioli R, Duncan MW, Hirsch FR, Bunn Jr PA, Carbone DP (2007) Mass spectrometry to classify non-small-cell lung cancer patients for clinical outcome after treatment with epidermal growth factor receptor tyrosine kinase inhibitors: a multicohort cross-institutional study. J Natl Cancer Inst 99(11): 838-846
- Takeda K, Hida T, Sato T, Ando M, Seto T, Satouchi M, Ichinose Y, Katakami N, Yamamoto N, Kudoh S, Sasaki J, Matsui K, Takayama K, Kashii T, Iwamoto Y, Sawa T, Okamoto I, Kurata T, Nakagawa K, Fukuoka M (2010) Randomized phase III trial of platinum-doublet chemotherapy followed by gefitinib compared with continued platinumdoublet chemotherapy in Japanese patients with advanced non-small-cell

lung cancer: results of a west Japan thoracic oncology group trial (WJTOG0203). J Clin Oncol 28(5): 753-760

- Thatcher N, Chang A, Parikh P, Rodrigues PJ, Ciuleanu T, von PJ, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K (2005) 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 366(9496): 1527-1537
- Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, Lorimer I, Zhang T, Liu N, Daneshmand M, Marrano P, da Cunha SG, Lagarde A, Richardson F, Seymour L, Whitehead M, Ding K, Pater J, Shepherd FA (2005) Erlotinib in lung cancer – molecular and clinical predictors of outcome. N Engl J Med **353**(2): 133–144
- Zhou C, Wu YL, Chen G, Feng J, Liu X, Wang C, Zhang S, Wang J, Zhou S, Ren S (2010) Efficacy results from the randomised phase III OPTIMAL (CTONG 0802) study comparing first-line erlotinib versus carboplatin (CBDCA) plus gemcitabine (GEM), in Chinese advanced non-small-cell lung cancer (NSCLC) patients (Pts) with EGFR activating mutations. *Ann Oncol* 21(Suppl 8): abstract LBA 13
- Zhu CQ, da Cunha SG, Ding K, Sakurada A, Cutz JC, Liu N, Zhang T, Marrano P, Whitehead M, Squire JA, Kamel-Reid S, Seymour L, Shepherd FA, Tsao MS (2008) 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 26(26): 4268-4275