

'Classical' but not 'other' mutations of EGFR kinase domain are associated with clinical outcome in gefitinib-treated patients with non-small cell lung cancer

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'Classical' mutations in the EGFR tyrosine kinase domain (exons 18, 19 and 21) have been associated with sensitivity to tyrosine kinase inhibitors (TKIs) in patients with NSCLC. The aim of the current study was to evaluate whether other than the classical G719X, DEL19 and L858R mutations of EGFR confer sensitivity to TKIs. Genomic DNA was extracted from microdissected formalin-fixed paraffin-embedded tumour tissue from 86 patients treated with gefitinib. Exons 18, 19 and 21 were amplified and subjected to direct sequencing. Eleven (13%) patients harboured the classical exon's 18, 19 and 21 mutations, while 14 (16%) had 'other' variants. There was a significantly higher percentage of 'never-smoker' patients with 'classical' EGFR mutations ($P=0.002$). Among patients with 'classical' mutations 3 patients achieved PR and 7 SD, while in the 'other' mutations group 10 patients had SD as best response. In the wild-type group, there were 3 patients with PR and 25 with SD. Median TTP was 16, 64 ($P=0.002$) and 21 weeks and median survival was 36, 78 and 67 weeks for patients with wild-type, 'classical' and 'other' EGFR mutations, respectively. The clinical relevance of 'other' EGFR mutation variants remains uncertain and requires further assessment in a prospective study.

British Journal of Cancer (2007) **97**, 1560–1566. doi:10.1038/sj.bjc.6604068 www.bjcancer.com

Published online 13 November 2007

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Keywords: NSCLC; EGFR; gefitinib; EGFR mutations

Despite the use of newer chemotherapeutic agents, survival of patients with advanced/metastatic NSCLC after first- or second-line chemotherapy seems to have reached a plateau (Carney, 2002). Further improvement in treatment is likely to require integration of novel, molecular agents such as EGFR inhibitors. The BR.21 placebo-controlled trial of erlotinib demonstrated that therapy with this EGFR tyrosine kinase inhibitor (TKI) was associated with statistically significant and clinically relevant differences in terms of overall and progression-free survival compared to the placebo (Shepherd *et al*, 2005). Conversely, in a similar study with gefitinib, (ISEL trial), a survival benefit was not demonstrated (Thatcher *et al*, 2005). Several clinical factors have been correlated with response to TKIs, including never-smoking status, female sex, Asian ethnicity and adenocarcinoma histology (Fukuoka *et al*, 2003; Kris *et al*, 2003; Miller *et al*, 2004; Shepherd *et al*, 2005). In addition, several groups have shown that somatic mutations in the

tyrosine kinase domain of the EGFR gene, in the exons 18 (G719A/C/S), 19 (DEL19, in-frame deletions which eliminate four to six amino acids just downstream of a critical lysine residue at position 745) and 21 (L858R) (commonly reported as 'classical' mutations), were significantly correlated with clinical response to gefitinib therapy (Lynch *et al*, 2004; Paez *et al*, 2004; Eberhard *et al*, 2005; Tsao *et al*, 2005). Patients with 'classical' EGFR mutations exhibit objective responses ranging from 75 to 95% (Huang *et al*, 2004; Han *et al*, 2005; Mitsudomi *et al*, 2005; Taron *et al*, 2005; Tokumo *et al*, 2005; Inoue *et al*, 2006; Yoshida *et al*, 2007). Furthermore, recently it was reported that polysomy or amplification of the EGFR gene but not EGFR mutational status was associated with clinical outcome of patients treated with erlotinib (Hirsch *et al*, 2005; Tsao *et al*, 2005; Dziadziuszko *et al*, 2006).

Although classical EGFR mutations are present in most cases of NSCLC responding to TKIs therapy, approximately 10–20% of patients who do show a clinical response to gefitinib do not have these EGFR mutations, indicating that other, than these classical mutations, may confer sensitivity to TKIs, or that EGFR mutations are not the sole determinants of TKI response (Sharma *et al*, 2007). Since the original reports by Paez *et al* (2004) and Lynch *et al* (2004) several 'other' mutations of EGFR gene have been reported (Riely *et al*, 2006b). During sequence analysis performed by our group, several mutations of EGFR's exons 18, 19 and 21, other than

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⁶Drs AG Pallis and A Voutsina have contributed equally to this work. Received 10 July 2007; revised 8 October 2007; accepted 9 October 2007; published online 13 November 2007

the classical ones, which will be collectively reported in the text as ‘other’, have been identified; however, their biological as well as their clinical relevance is still unclear (Marchetti *et al*, 2005). The aim of the present study was to determine whether the presence of these ‘other’ variants is correlated with the clinical outcome of patients treated with gefitinib.

In the present study, the clinical outcome of NSCLC patients treated with gefitinib in the context of an expanded access program (EAP) was analysed according to the presence of classical or ‘other’ variants of EGFR mutations.

PATIENTS AND METHODS

Patient selection

Patients with histologically documented NSCLCs were included in this retrospective analysis. Additional inclusion criteria were as follows: a gefitinib treatment of at least 4 weeks (to evaluate efficacy after adequate exposure to the drug); complete information regarding tumour size, tumour location, extent of disease and prior treatments for NSCLC; bi-dimensionally measurable disease with imaging assessment performed at least 3 weeks before starting gefitinib therapy and at least one subsequent imaging assessment; tumour tissue was required for the assessment of EGFR mutations.

Expanded access program

Gefitinib EAP, was a non-randomised, open-label compassionate use program, which enrolled patients with advanced/metastatic NSCLC. The EAP protocol and informed consent forms were approved by the Scientific and Ethics Committees of the participating Institutions. Patient enrollment began on July 2001 and was closed on April 2006. Eligible patients with NSCLCs (aged ≥ 18 years) were those who either (i) had disease progression with standard systemic chemotherapy or radiation therapy, or (ii) were ineligible for chemotherapy or radiation therapy, finally.

Treatment

Gefitinib was administered orally, as a once daily dose of 250 mg. Treatment was continued until disease progression, the appearance of unacceptable toxicity or patient’s withdrawal of consent. Objective tumour responses were evaluated according to WHO response criteria (Miller *et al*, 1981).

Immunohistochemistry

Representative, tumour sections from formalin-fixed paraffin-embedded (FFPE) tumour samples were stained immunohistochemically, as described previously (Koutsopoulos *et al*, 2007), using the mouse monoclonal anti-EGFR antibody (clone H11, code M3563, DakoCytomation, Glostrup, Denmark), in dilution 1/50 and incubation time 1 h at room temperature. For the detection of antigen-antibody reaction the Ultra-Vision detection system AP Polymer kit (catalogue no. TL-125-AL, Lab Vision, Cheshire, UK), was used according to the manufacturer’s instructions. Immunoreaction was considered as weakly positive (2+) when more than 10% of the tumour cells showed weak to moderate complete membrane staining or as strongly positive (3+) when a strong complete membrane staining was observed in more than 10% of the tumour cells. All other staining patterns were interpreted as negative (0 or 1+) (Koutsopoulos *et al*, 2007).

DNA extraction and mutation analysis

All tumour samples were FFPE tissues. Representative sections from tissue used for DNA extraction were stained with H&E and subjected to histopathologic examination. Subsequently, tissue

samples were macrodissected or microdissected (piezo power Eppendorf Microdissector, Germany) to ensure that specimens contained at least 80% tumour cells. Approximately 5–10 μm sections or 30–100 000 cells were collected from normal (when available) and tumour samples and placed in 2% SDS/proteinase K (10 mg ml⁻¹) at 56°C overnight. DNA was extracted from the FFPE tissue using the MasterPure Complete DNA/RNA Purification kit (EPICENTRE; Biotechnologies, Madison, USA) according to the manufacturer’s instructions. Exons 18, 19 and 21 were amplified and subjected to direct sequencing. The PCR primers were as follows:

155273L23(18ex) 5’-TCCCAAACACTCAGTGAAACAAA-3’, 155348L22(18ex) 5’-TGGTCTCACAGGACCAGTATT-3’, 154838U22(18) 5’-TCAGAGCCTGTGTTTCTACCAA-3’, 154899U20(18) 5’-TCCAAATGAGCTGGCAAGTG-3’, 55634U24(19ex) 5’-AAATAATCAGTGTGATTCGTGGAG-3’, 156027L20(19) 5’-TGTGGAGATGAGCAGGGTCT-3’, 156107L22(19ex) 5’-GAGGCCAGTGCTGTCTCTAAGG-3’, 155750U20(19) 5’-GTGCATCGCTGGTAACATCC-3’, 173160L22(21Ex) 5’-CAGTCTGGCTCACACTACCAG-3’, 173076L19(21) 5’-CATCCTCCCCTGCATGTGT-3’, 172656U22(21Ex) 5’-GCAGCGGGTTACATCTTCTTTC-3’, 172747U19(21) 5’-GCTCAGAGCCTGGCATGAA-3’. The first PCR was carried out in total volume of 10 μl containing 1/10 of the extracted genomic DNA using 1 U of Platinum Taq DNA polymerase (Invitrogen Corporation, Carlsbad, CA, USA). The initial denaturing step was 94°C for 15 min, followed by 35 cycles of denaturing step at 94°C for 20 s, annealing step at 60°C for 30 s and extension step at 72°C for 1 min, ending with a final extension step at 72°C for 7 min. Nested PCR was carried in a total volume of 20 μl and the conditions were identical to the first PCR. Cycle sequencing reactions were performed using the nested PCR primers and the ABI BigDye Terminator kit (v3.1, Applied Biosystems, Foster City, CA, USA) and electrophoresed on an ABI3100 genetic analyzer (Applied Biosystems). Sequence variants were determined using the Seqscape software (Applied Biosystems) and confirmed by an independent PCR amplification and sequencing in both directions.

Statistical analysis

Descriptive statistics for the patient group are reported as median and range. Statistical comparisons between group rates (proportions) were assessed by Pearson’s χ^2 -test or Fisher’s test where appropriate (Altman, 1991). As this was a compassionate use program, the primary outcome variables were safety, survival and assessment of response. Overall survival (OS) was measured from entry into the study until death; 1-year survival was estimated using the Kaplan–Meier method (Collet, 1994).

RESULTS

Patients’ demographics

Eighty-six patients with a median age of 61 years (range = 35–82) were enrolled in the present analysis. Most of the patients (75%) were male, 53 (61.6%) had an adenocarcinoma histology and 61 (79%) had stage IV disease. A total of 28 (33%) patients were never-smokers. All patients had received a platinum-based chemotherapy, except two patients with severe heart failure, which precluded cisplatin administration. A total of 66 patients had gefitinib as third-line treatment; 14 patients (21%) received taxane-based second-line therapy, 21 (32%) a platinum-based therapy while the remaining 31 (47%) received a gemcitabine or irinotecan-based regimen as second-line treatment. Patients’ characteristics are presented in Table 1. EGFR membrane expression was assessed by immunohistochemistry in all tumour specimens; A total of 42 (49%) of these specimens were considered as positive (2+ or 3+) for EGFR expression.

Table 1 Patients' characteristics

	n	%
Age		
Median	61	
(minimum–maximum)	35–82	
Sex		
Male	64	75
Female	22	26
Histology		
Adenocarcinoma	47 (53)	55 (61.6)
Squamous cell carcinoma	27 (29)	31 (33.7)
Large cell carcinoma	1	1 (1.1)
Bronchoalveolar	4	5 (4.7)
Other	7	8 (9.3)
PS (WHO)		
0–1	53	62
≥2	33	38
Stage		
IIIB	25	21
IV	61	79
EGFR expression (IHC)		
0–1+	44	51
2–3+	42	49
Line of therapy		
2nd	20	24
≥3rd	66	76
Smoking status		
Smoker	39	45
Ex-smoker	19	22
Never-smoker	28	33
No. of organs involved		
1	19	22
2	48	56
≥3	19	22
Median (range)	2 (1–4)	

Mutational analysis

EGFR mutation detection was performed by sequencing exons 18, 19 and 21 in tumours of all patients ($n=86$) and matched-normal tissue ($n=22$) or blood ($n=3$) of patients carrying EGFR mutations.

According to the mutational status, three groups of patients were identified as follows: (i) the 'wild-type' group ($n=61$ patients; 71%) with no detectable mutations; (ii) 'classical' mutations group ($n=11$ patients, 13%; 6 of these patients harboured the classical exon 19 deletion, one the G719D, one the E746V and three the exon 21 L858R point mutation) and (iii) the 'other' mutations group ($n=14$ patients, 16%). EGFR mutational status is presented in Table 2. None of the reported EGFR mutations was found in matched-normal tissues suggesting their somatic origin and eliminating the possibility to be single-nucleotide polymorphisms. A total of 8 (57%) cases had 'other' mutations that have been previously reported and 6 cases had novel EGFR mutations.

A total of 1 (1.1%) patient (no. 13) had two 'other' mutations, while 3 (3.4%) patients (nos. 9, 11 and 18), who were included in the 'classical mutations' group, had both the exon 21 L858R mutation and an 'other' mutation (Table 3). The incidence of 'classical' mutations was gender – (9.4% for males vs 22.7% for

females; $P=0.139$) and histology – (11.8% in adenocarcinomas vs 14.3% in non-adenocarcinomas; $P=0.752$) independent, while it was significantly higher in 'never-smoker' patients compared to 'smokers' (32 vs 2.1%, respectively; $P=0.001$). Similarly, regarding the 'other' variants, there was no difference in their incidence according to sex (male vs female: 18.8 vs 13.6%, $P=0.750$), histology (adenocarcinoma vs other: 15.7 vs 20.0%, $P=0.773$) and smoking habits (smoker vs never-smoker: 20.8 vs 8.0%, $P=0.200$). Finally, no correlation was observed between EGFR expression as assessed by IHC and presence of 'classical' mutations ($P=0.732$); conversely, the detection of 'other' variants was more frequent in patients with tumours, which did not express EGFR by IHC (28.9 vs 8.3%, $P=0.036$).

Treatment

The median duration of gefitinib administration was 17 weeks (range = 4–140). The reason for treatment discontinuation was disease progression in all but six (6.9%) patients (drug-related toxicity ($n=3$ patients) and personal reasons non-related to treatment or the disease ($n=3$ patients)). There was no clear association between treatment duration and any of the following: PS ($P=0.262$), histology ($P=0.751$), disease stage ($P=0.103$), smoking status ($P=0.950$), sex ($P=0.663$) and skin rash ($P=0.357$) and EGFR expression by IHC ($P=0.254$). However, patients belonging to the group with 'classical' mutations had significantly longer treatment duration when compared with patients of the 'wild-type' group (67 weeks vs 17 weeks, respectively; $P=0.018$), while there was a trend towards longer treatment duration when compared with patients belonging to group of 'other' mutations (67 vs 21 weeks, respectively; $P=0.069$). On the contrary, there was no significantly different treatment duration between patients belonging to the 'other' mutations and those of the 'wild-type' group (21 and 17 weeks, respectively; $P=0.141$).

Response to treatment

No patient achieved a complete response, while six (7.0%) experienced a partial response (PR) (overall response rate = 7.0%; 95% CI = 1.59–12.36) and 40 (46.5%) stable disease (SD); the disease control rate (DCR; PR + SD) was 53.5% (95% CI = 42.95–64.03%). Additionally, progressive disease (PD) was observed in 40 (46.5%) patients. Disease control rate was significantly higher for the following: (i) patients with PS 0–1 as compared with those with PS ≥2 (67 and 22%, respectively; $P<0.001$); (ii) women as compared to men (77.3 and 42.9%, respectively; $P=0.007$); (iii) never-smokers vs smokers (84 and 34%, respectively; $P<0.001$) and (iv) patients who developed skin rash as compared with those who did not (85.2 and 35.7%, respectively; $P=0.003$). In addition, there was a trend towards a higher DCR in patients with adenocarcinoma compared with those bearing other histologic types (54.9 and 47.1%, respectively; $P=0.057$). Regarding patients achieving SD, significant association was observed for those with a PS of 0–1 ($P=0.003$), never-smoker status ($P=0.001$) and development of skin rash ($P=0.003$); conversely, there was no association between DCR and sex ($P=0.053$) or histology ($P=0.355$) (Table 4).

The DCR was significantly higher in patients of the 'classical' mutations than in patients of the 'wild-type' (90.9 and 43.3%, respectively; $P=0.006$) group; conversely, there was no significant difference between the DCR observed in patients of the 'classical' mutations group and that of patients of the 'other mutations' group (90.9 and 57.1%, respectively; $P=0.090$) or of patients of the 'other mutations' group and those of the 'wild-type' group (57.1 and 43.3%, respectively; $P=0.386$). All patients (six out of six) with exon 19 deletion achieved disease control (two patients

Table 2 Results of EGFR mutational analysis

Patients no.	'Classical mutations' group	'Other mutations' group	Histology	Gender	Smoking status	Response
1	DEL 19		Squamous	Male	Never-smoker	SD
2		Y727H	Squamous	Male	Smoker	SD
3	DEL 19		Adenocarcinoma	Male	Never-smoker	SD
4		V843I	Squamous	Male	Ex-smoker	SD
5		L747S	Adenocarcinoma	Female	Never-smoker	SD
6	G719D		Adenocarcinoma	Male	Never-smoker	SD
7	DEL 19		Adenocarcinoma	Female	Never-smoker	PR
8		P691S	Adenocarcinoma	Male	Ex-smoker	SD
9	L858R, L861P		Undifferentiated	Male	Smoker	PD
10		K860E	Squamous	Male	Smoker	PD
11	L858R, V843I		Adenocarcinoma	Female	Never-smoker	SD
12		G863S	Adenocarcinoma	Female	Never-smoker	SD
13		T847A, G863S	Adenocarcinoma	Male	Smoker	PD
14		L692P	Squamous	Male	Smoker	PD
15		L703F	Adenocarcinoma	Female	Smoker	PD
16	E746V		Adenocarcinoma	Female	Never-smoker	SD
17		G729R	Undifferentiated	Male	Smoker	PD
18	L858R, E709K		Squamous	Female	Never-smoker	PR
19		V726M	Adenocarcinoma	Male	Smoker	SD
20	DEL19		Adenocarcinoma	Male	Never-smoker	PR
21	DEL19		Squamous	Male	Never-smoker	SD
22		G857E	Adenocarcinoma	Male	Smoker	SD
23		E711K	Squamous	Male	Smoker	PD
24	DEL19		BAC	Female	Never-smoker	SD
25		G874S	Adenocarcinoma	Female	Smoker	SD
Total	11	14				

Table 3 Clinical characteristics of patients bearing both a classical and an 'other' mutation

Patients no.	Sex	Histology	Smoking status	Response to gefitinib	Duration
9	Male	Undifferentiated	Smoker	PD	—
11	Female	Adenocarcinoma	Never-smoker	SD	64 weeks
18	Female	Squamous	Never-smoker	PR	12 weeks

Table 4 Clinical and molecular characteristics of patients achieving disease control

	n	Female sex n (%)	Adenocarcinoma histology ^a n (%)	Never smoker n (%)	Skin rash development n (%)	EGFR mutations
PR	6	4 (67%)	2 (33%)	5 (83)	4 (67%)	DEL 19: n = 2 (33%) L858R: n = 1 (17%) Wild type: n = 3 (50%)
SD	40	14 (35%)	28 (70%)	18 (45%)	19 (47.5%)	DEL 19: n = 4 (10%) L858R: n = 1 (2.5%) Other variant: n = 10 (25%) Wild type: n = 25 (62.5%)

PR = partial response, SD = stable disease. ^aBronchoalveolar included.

achieved PR and four SD); in addition, two out of three patients with exon 21 L858R mutation experienced disease control (one patient with PR and one with SD) while both patients with the G719D and E746V point mutations achieved SD. In the multivariate analysis, the presence of mutations (either 'classical', or 'other', as well as all patients with mutations) did not emerge as a significant factor associated with DCR or SD.

Time to tumour progression

The median follow-up period was 109 weeks and the median time to tumour progression (TTP) 20 weeks (range = 4–140). A total of 23 (36%) patients had a TTP > 24 weeks and 7 (10.9%) > 52 weeks (Table 5). There was no difference in TTP according to sex ($P=0.468$), histology ($P=0.676$), EGFR positivity (by IHC)

Table 5 EGFR mutation status for patients with ≥ 24 and ≥ 52 weeks TTP

TTP	n	Wild type	Exon 19 deletion	L858R	Other variant
≥ 24 weeks	23	11 (48%)	5 (22%)	1 (4%)	6 (26%)
≥ 52 weeks	7	1 (14%)	2 (29%)	1 (14%)	3 (43%)

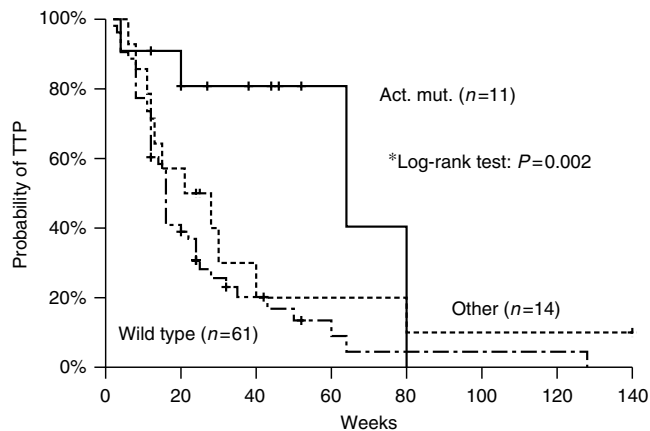


Figure 1 Kaplan–Meier curve of time to tumour progression (TTP) of wild-type EGFR patients group, 'classical' and 'other' mutations group. *P-value between 'classical' and wild type.

($P = 0.267$) and PS ($P = 0.437$); conversely, patients who had never-smoked and patients who developed skin rash had significantly higher TTP ($P = 0.003$ and $P = 0.006$, respectively). Time to tumour progression in the 'classical' mutations group was significantly longer than in the 'wild-type' group (64 vs 16 weeks; $P = 0.002$, Figure 1). On the contrary, there was no difference in TTP between 'other' mutations group and 'wild-type' group (21 vs 16 weeks; $P = 0.363$) while there was a trend towards a significant difference in TTP between 'other' mutation group and 'classical' mutations group (21 vs 64 weeks; $P = 0.069$). Although patients with DEL19 mutation had numerically higher TTP, when compared with L858R mutation patients, this difference failed to reach statistical significance (80 vs 64 weeks, $P = 0.786$).

Survival

Median OS was 48 weeks (range = 4–140). None of the following factors had a significant impact on OS: PS ($P = 0.403$), histology ($P = 0.198$), smoking ($P = 0.242$), sex ($P = 0.475$), skin rash ($P = 0.182$) and EGFR IHC expression ($P = 0.637$). Median OS was significantly longer in patients with the DEL19 mutation (not reached) compared to 'wild-type' patients (36 weeks; $P = 0.043$, Figure 2); however, when all patients with 'classical' mutations were included in the analysis, this difference was lost (78 vs 36 weeks; $P = 0.052$, Figure 3); there was no statistically significant difference regarding OS between patients with DEL19 and L858R mutation (not reached vs 78 weeks, $P = 0.896$). Similarly, patients of the 'other' mutation group had a median survival of 67 weeks, which was not different when compared with 'wild-type' group ($P = 0.094$) or 'classical' mutations group ($P = 0.491$). Efficacy results according to mutational status are presented in Table 6.

DISCUSSION

The incidence of the classical somatic mutations has been reported to range between 3 and 13% in Caucasian populations and between 30 and 40% in populations of Asian descent (Shigematsu et al,

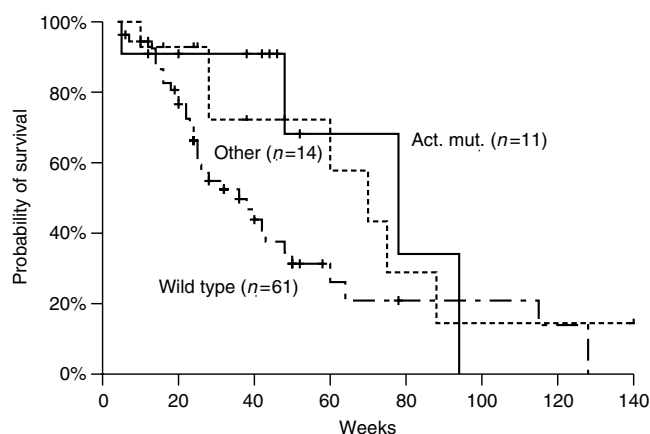


Figure 2 Kaplan–Meier survival curve of wild-type EGFR patients group, 'classical' and 'other' mutations group.

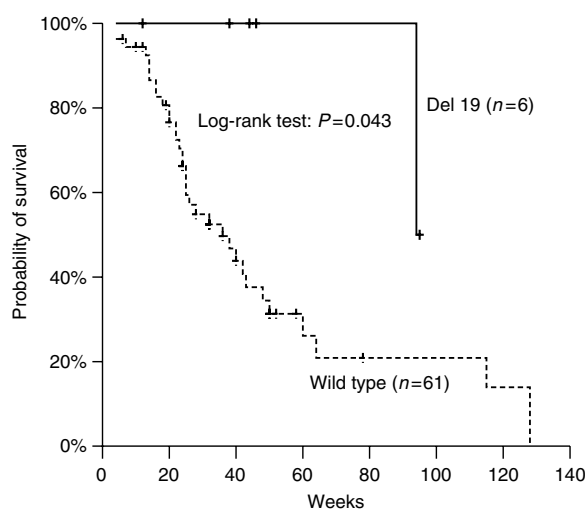


Figure 3 Kaplan–Meier survival curve of patients with the 'classical' DEL19 mutation and wild-type EGFR.

Table 6 Efficacy results according to mutational status

	'Classical' mutations	'Other' mutations	Wild type
DCR	90.9%*	57.1%	43.3
TTP	64 weeks*	21 weeks	16 weeks
OS	78 weeks**	67 weeks	36 weeks

DCR = disease control rate, OS = overall survival; TTP = time to tumour progression. * $P < 0.05$ (vs wild type). ** $P = 0.052$ (vs wild type).

2005; Calvo and Baselga, 2006). In a Greek study (Murray et al, 2006), somatic mutations of EGFR were reported in 15% of a group of 60 patients with NSCLC, while Dova et al (2007) reported two mutations in 50 Greek patients with cancer of unknown primary. In our series, mutational analysis revealed the presence of 'classical' mutations in 11 (13%) patients which is in agreement with other reports (Murray et al, 2006; Dova et al, 2007), while 14 (16%) patients had 'other' mutations. A similar high incidence of 'other' EGFR mutations has been reported by Tsao et al (2005) who have also used microdissected FFPE tumour samples. Microdissection of tumour samples with a low percentage and/or uneven distribution of cancer cells allows the detection of mutations with higher sensitivity; this could be the reason for the high incidence of

'other' mutations observed in our study. On the other hand, the low-DNA template input in PCR could generate false mutations. However, in our case the latter is unlikely, given that the sequence analysis of FFPE normal tissue specimens of 22 patients with EGFR mutations revealed no mutations (data not shown).

The presence of 'other' mutations in our study was not correlated with sensitivity to gefitinib. Indeed, patients whose tumours harbour 'other' variants of EGFR mutations had a higher, but not statistically significant, DCR when compared with patients bearing wild-type EGFR. However, this observation should be considered with caution given the small number of patients analysed. On the other hand, tumour growth control was significantly higher ($P=0.013$) in patients who presented the classical EGFR mutations compared to that of patients with wild-type EGFR, as already has been reported (Argiris *et al*, 2003; Miller *et al*, 2004; Han *et al*, 2005; Kim *et al*, 2005; Thatcher *et al*, 2005; Tsao *et al*, 2005). Similarly, although patients with 'other' EGFR mutations had a numerically longer survival as compared with patients of the 'wild-type' group, this difference failed to reach statistical significance. This observation may suggest that, collectively, 'other' EGFR mutation variants *per se* could not be considered as predictors of clinical outcome.

Furthermore, there was a trend towards higher survival for patients with classical mutations, when compared with 'wild-type' patients, but this difference failed to reach statistical significance. A possible reason for this observation is the small number of patients studied. This observation is similar with that reported in the BR.21 study (Tsao *et al*, 2005) but it is in conflict with that reported by other studies (Hirsch *et al*, 2005; Takano *et al*, 2005).

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