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Original Article

Assessment and prognostic analysis of EGFR mutations or/and HER2 overexpression in Uygur's Non-small Cell Lung Cancer

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Abstract: Aim: The epidermal growth factor receptor (EGFR) mutations and human epidermal growth factor receptor HER-2/neu (HER2) have been established roles in the signal transduction pathways leading to cell growth and differentiation. The present study focus on the significance of EGFR mutations combined with HER2 overexpression on survival outcomes in Non-small Cell Lung Cancer patients in Uygur population. Methods: A total of 111 consecutive Uygurods: A total of 111 consecutive Cell Lung Cancer under went lung Cell Lung biopsy or surgery at the Affiliated Tumor Hospital of Xin Jiang Medical University between March 2009 and January 2013 were included in this retrospective study. All the patients included had received gefitinib 250 mg once daily. The HER2 expression were evaluated by immunohistochemical staining with score of membranous staining being 0 = none, 1 = weak, 2 = 10-30% cells, 3≥30% cells stained, and Real-time PCR techniques were conducted to detect mutations of EGFR through 21 kinds of human EGFR gene mutation detection kits. A retrospective review of the medical records was analyzed to determine the correlation between the presence of EGFR mutations combined with HER2 overexpression and clinicopathological factors. Results: The overall rate of EGFR mutation was 10.81% (n = 12), which mainly involved exons 19 (83.33%, n = 10), 21 (16.67%, n = 2). The overall rate of HER2 overexpression was 21.62% (n = 24). EGFR mutation combined with HER2 overexpression analysis was performed in 111 patients, with an overall rate of 5.41% (n = 6). Median progression-free survival and overall survival were significantly longer in the EGFR mutations group than in the wild type group (PFS: 10.0±1.5 versus 3.8±1.4 months, P = 0.000; OS: 27.3±2.9 versus 19.1±4.7 months, P = 0.000). The ORR in patients with HER2 overexpression was 29.17%, and 13.80% in those patients with HER2 negative, but no significant difference (P = 0.121). The median PFS and OS in HER2 positive group showed no significant difference compared with HER2 negative group (PFS: 4.7±1.2 months versus 3.9±1.6 months, P = 0.085; OS: 20.5±2.4 versus 19.2±2.6 months, P = 0.094). As regarding to ORR, PFS and OS, EGFR mutations combined with HER2 overexpression patients showed no superior efficacy to gefitinib treatment compared with EGFR mutations combined with HER2 negative. Conclusion: In Uygur population, progression-free survivals were improved in Non-small Cell Lung Cancer with EGFR mutations. HER2 overexpression provided a poor prognostic factor in Non-small Cell Lung Cancer.

Keywords: Non-small cell lung cancer, epidermal growth factor receptor mutation, gefitinib, HER2 overexpression, Uygur population

Introduction

Nonsmall-cell lung cancer (NSCLC) is the leading cause of cancer death worldwide, with extremely poor prognosis, and the median survival rarely exceeds 10 months irrespective of conventional chemotherapy [1, 2]. The identification of molecular-targeted agent leading to cell differentiation, migration, proliferation or survival could be effective in improving the median overall survival. As the first molecular-targeted agent for NSCLC, gefitinib targets the molecule as an inhibitor or tyrosine kinase of the epidermal growth factor receptor (EGFR-

Table 1. Population Characteristics

Characteristics	N = 111 No (%)	Statistics
Age (years)		
Mean	56.8±11.5	
Range	21-76	
Gender		
Male	78	70.27%
Female	33	29.73%
ECOG performance satus score		
0	8	7.21%
1	62	55.86%
2	41	36.94%
Histologic diagnosis		
Adenocarcinoma	45	40.54%
Squamous cell carcinoma	45	40.54%
Others	21	18.92%
Smoking status		
Previous or current smoker	63	56.76%
Never smoked	48	43.24%
Clinical stage		
IIIB	45	40.54%
IV	66	59.46%
Type of EGFR mutation	12 (Adenocarcinoma)	
EXON 19 deletion	9	75.00%
EXON 21 (L858R)	3	25.00%
Others	0	0

Note: Tumor stage based on tumor-node-metastasis classification advocated by International Union against Cancer. Abbreviations: EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; n, number.

TKI) [3-5]. The phosphorylation of intracellular EGFR tyrosine kinase domain induced by ligand- EGFR complex drives tumor cell survival, proliferation and invasion [6-8]. The HER family includes three other members: EGFR (HER1/ERB1), HER3 (ERBB3) and HER4 (ERBB4), which structure contains three domains: an extracellular domain, homo/heterodimers formation and a transmembrane domain [9, 10]. As the ligand binds to an extracellular domain, the signal, pass through the plasma membrane, then activates two key signaling pathways including the RAS/RAF/MAPK pathway and PI3K/Akt pathway which drives the tumor cell growth [11, 12]. As a relatively new biomarker for NSCLC, the HER2 gene plays its central role in tumor growth, Brabender J et al. [13] highlighted EGFR and HER2-neu mRNA expression is correlated with survival in non-small cell lung cancer, representing an appealing prognostic and predictive factor in NSCLC. In membranous staining, HER2 overexpression was up to 20% of cases, the role of HER2 overexpression in lung cancer remains controversial although HER2 correlating with poor prognosis in breast and ovarian malignant tumors. Jian Ming Xu et al. have demonstrated that HER2 was established as poor prognostic and predictive factor for selecting Chinese patients sensitive to gefitinib treatment [14]. Therefore it appears that no convincing data available on clinical significance of HER2 protein overexpression as a prognosis factor in NSCLC.

In the present study, we performed a retrospective study in Uygur's. NSCLC, aiming at identifying the role of EGFR gene mutations and HER2 overexpression as predictive factors for in selecting patients sensitive to gefitinib treatment.

Patients and methods

111 Uygur NSCLC patients in this study histologically confirmed and treated with gefitinib between March 2009 and January 2013 in the Affiliated Tumor Hospital of Xinjiang Medical University were retrospectively analyzed (**Table 1**). Tumor materials were histologically confirmed. All patients were pretreated with at least one line of platinum-based chemotherapy regimen before receiving gefitinib monotherapy at a daily dose of 250 mg, until an intolerable toxicity event such as grade 3 was observed during the treatment in which gefitinib was administered without any dose reductions until disease progression. The dose of gefitinib will be reduced by changing the everyday schedule to every 2 days schedule when grade 2 toxicity was observed.

Immunohistochemistry

All tumor section staining was performed as the manufacturer's protocol. The HER2 immunohistochemical analysis kit was used (Zymed, USA). The immunostaining was then scored by two independent pathologists who were blind-

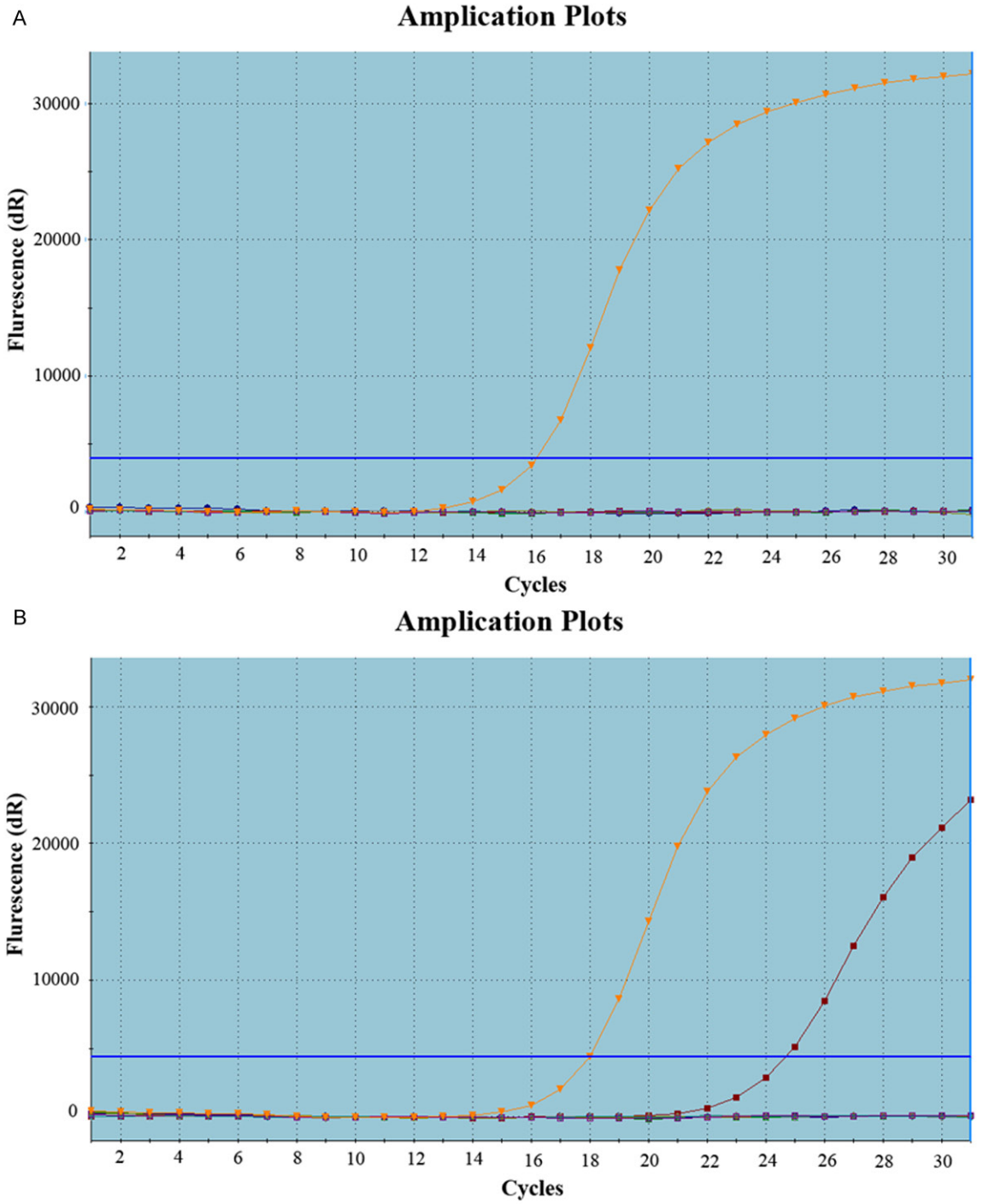

Figure 1. Representative image showing EGFR mutation in NSCLC, Real-time polymerase chain reaction: (A) negative, (B) positive respectively.

ed to the clinical information. Tumor membranous staining intensity was scored using a four-grade scale: (0, 1+, 2+, or 3+), 0 = no staining, +1 = if less than 10% tumor cells had weak staining, +2 = if at least 10%tumor cells had moderate staining, and +3 = if at least 10% tumor cells had strong staining. Cases Grade of 0 or +1 was considered as negative, and +2 or +3 was considered as positive.

Real-time fluorescence quantitative PCR

ARMS (Amplification Refractory Mutation System) was used to detect EGFR mutations:

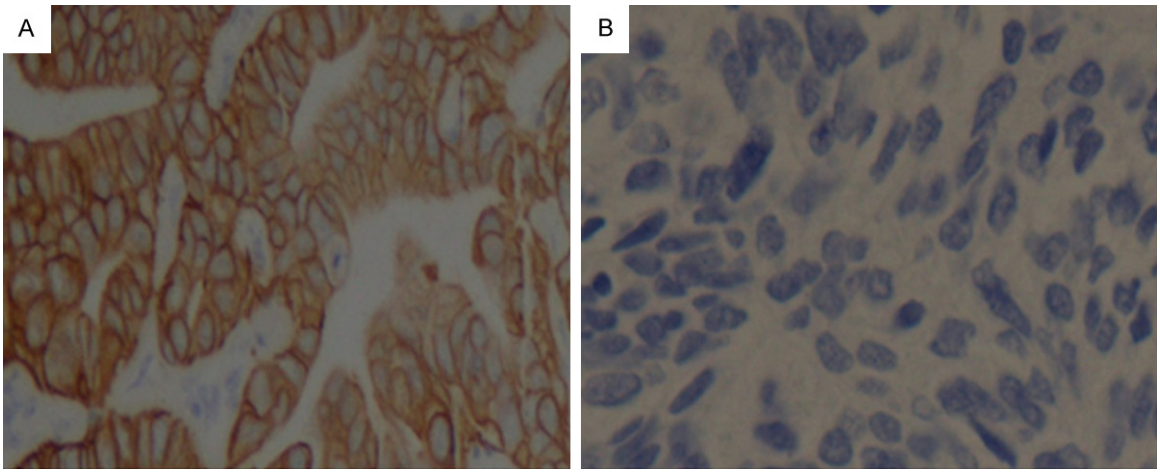

Figure 2. Representative image showing HER2 overexpression in NSCLC. Notes: (A) positive, (B) negative, respectively (original magnification ×400).

genomic DNA extracted from five parafin sections or frozen tumor tissues was used for Real-time fluorescence quantitative PCR to detect EGFR mutations. Each PCR reaction contained 10-15 ng DNA, 5-10 pM forward and reverse primers in 25 uL reaction volume with cycling parameters: first cycle of 95°C for 2 min and 30 s, 15 cycles of 95°C for 25 s, 64°C for 20 s and 72°C for 20 s, 31 cycles of 93°C for 25 s, 60°C for 35 s and 72°C for 20 s, and one cycle of 60°C for 7 min followed by analysis of FEM and HEX signals determing EGFR mutations.

Treatment with gefitinib

According to the RECIST criteria, the response to gefitinib was analyzed at 4 weeks [15]. The treatment response: complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD) were confirmed no less than 4 weeks apart. Objective response rate (ORR) and median progression free time (PFS) were assessed.

Statistical analysis

The χ^2 test was used to analyze EGFR mutations or HER2 overexpression and patient characteristics. The logistic regression model was used in multivariate analysis. Kaplan-Meier product-limit method was used to calculate median overall survival and PFS (Progression Free Survival). A multivariate analysis using the stepwise Cox regression model was carried out.

Results

EGFR mutations

EGFR mutations were examined in 111 Uygurlculate median PFS. s use adenocarcinoma cases 45 squamous cell carcinoma and 20 other cases, with an overall mutation rate of 10.81% (12/111), in which 9/111 (8.11%) mutions in exon 19, 3/111 (2.70%) mutations in exon 21 (**Figure 2** and **Table 1**). No significant difference was observed in the analysis of the presence of EGFR mutations between different gender patients. EGFR mutations positivity was statistically associated (P = 0.000) with adenocarcinoma histology, while not influenced by mean age, smoking status, clinical stage and ECOG performance satus score (**Table 2**).

EGFR-mutation group showed superior efficacy (response rate) to gefitinib treatment compared with wild-type group (**Table 3**). Further survival analysis showed that PFS for EGFR-mutation group (10.0±1.5 months, 95% CI = 6.5-12.2 months, P<0.001) tended to be superior to that of wild type group (3.8±1.4 months, 95% CI = 3.1-4.7 months, **Table 3** and **Figure 3**). Mean overall survival (27.3±2.9 months, 95% CI = 13.8-30.2 months, P<0.001) in EGFR mutant group was also significantly different from wild type (19.1±4.7 months, 95% CI = 8.8-21.9 months, **Table 3** and **Figure 3**).

HER2 overexpression

The HER2 overexpression status was determined in 111 cases, in which 21.62% (n = 24),

Table 2. EGFR Mutation/HER2 Overexpression Status According to Demographics

Variable/ Categories	EGFR		HER2	
	Mutation N%	Wide-type N%	Positive N%	Negative N%
Gender				
Male	9/11.84	69/88.16	15/19.23	63/80.77
Female	3/9.09	30/90.9	9/27.27	24/72.72
P	0.497		0.243	
Age				
≤65	6/7.41	75/92.59	15/19.23	63/80.77
≥65	6/20	24/80	9/27.27	24/72.72
P	0.065		0.243	
Ecog				
0-1	5/7.14	65/92.86	9/15.79	48/84.21
2-3	7/17.07	34/82.93	15/27.78	39/72.22
P	0.065		0.065	
Histology				
Ade	12/26.67	33/73.33	18/40	27/60
No-Ade	0/0	66/100	6/9.09	60/90.9
P	0.000		0.000	
Smoking History				
Never Smoker	6/15.38	33/84.62	15/23.81	48/76.19
Smoker	6/8.33	66/91.67	9/18.75	39/81.25
P	0.204		0.344	
Stage				
IIIB	4/8.88	41/91.11	9/15.79	48/84.21
IV	8/12.12	58/87.87	15/27.78	39/72.22
P	0.417		0.096	

Note: ECOG Eastern Cooperative Oncology Group, *Ade* adenocarcinoma.

were found to be positive (**Figure 1** and **Table 1**). The HER2 overexpression was significantly associated (P = 0.000) with adenocarcinoma histology (40% in adenocarcinoma histology versus 9.09% in non-adenocarcinoma histology), while no association with gender, smoking status, clinical stage and ECOG performance satus score (**Table 2**).

Objective response, median PFS and median OS in HER2 overexpression positivity group tended to be superior to that of wild type group (OR: 29.17% versus 13.80%, P = 0.121; PFS: 4.7±1.2 versus 3.9±1.6 months, P = 0.085; OS: 20.5±2.4 versus 19.2±2.6 months, P = 0.094, **Table 3** and **Figure 3**).

EGFR mutations combined with HER2 overexpression

EGFR mutations combined with HER2 overexpression patients showed no superior efficacy (objective response rate) to gefitinib treatment compared with EGFR mutations combined with HER2 negative, (the response rate was 83.33%, 83.33% respectively, P = 1.00, **Table 3**). For median PFS and OS of EGFR mutations combined with HER2 overexpression patients, no significant difference was found compared with EGFR mutations combined with HER2 negative (PFS: 10.3±1.3 versus 9.4±1.5 months, P = 0.991; OS: 28.1±3.1 versus 26.5±2.7, P = 0.893 **Table 3** and **Figure 3**).

Multivariate analysis of clinical outcome

Univariate analysis was performed to identify which variables were significantly associated with clinical outcome. Those variables statistically significant in the univariate analysis including presence of EGFR mutations and adenocarcinoma histology were added to a multivariate model exploring the predictive factors. A step-wise logistic regression analysis revealed that presence of EGFR mutations (RR 15.7, 95% CI = 6.2-47.8) was the strongest prognostic factors for OR to gefitinib. According to the multivariate Cox regression model, presence of EGFR mutations (RR 5.1, P<0.0001) and adenocarcinoma histology (RR 4.8, P<0.001) were significant predictors of long PFS. Also, presence of EGFR mutations (RR 5.3, P<0.0001) and adenocarcinoma histology (RR 3.9, P< 0.001) predicted long OS.

Discussion

In East Asian, North American, West European and East European populations, the frequency of EGFR gene mutations in NSCLC has been shown different [16-20]. In this study, it is the first time, to our knowledge, for assessment of the prevalence of EGFR gene mutations and HER2 overexpression in Uygur population.

In concordance with previous studies [21-24], the main factor affecting frequency of EGFR

Table 3. Tumor response and prognosis of EGFR mutation and HER2 overexpression to gefitinib

Marker	N/%	OR (%)	Median PFS (months)	Median OS (months)
EGFR+	12/10.81	10/83.33	10.0±1.5	27.3±2.9
EGFR-	99/89.19	9/9.09	3.8±1.4	19.1±4.7
P		0.000	0.000	0.000
HER2+	24/21.62	7/29.17	4.7±1.2	20.5±2.4
HER2-	87/78.38	12/13.80	3.9±1.6	19.2±2.6
P		0.121	0.085	0.094
EGFR+/HER2+	6/5.41	5/83.33	10.3±1.3	28.1±3.1
EGFR+/HER2-	6/5.41	5/83.33	9.4±1.5	26.5±2.7
P		1.000	0.991	0.893
EGFR-/HER2+	18/16.22	2/11.11	4.5±1.3	20.2±5.3
EGFR-/HER2-	81/72.97	7/8.64	3.8±1.1	18.9±3.7
P		0.667	0.732	0.000

Note: OR Objective response; *PFS* Median progression free time; OS Overall survival.

gene mutations in NSCLC is histological subtype (P = 0.000), but no smoking status (P = 0.204) and gender (P = 0.497).

In the Chinese population, EGFR gene mutations were detected in 30.2% of the samples [14], which mainly involved exons 19 (53.1%), 21 (21.9%), and 18 (18.8%). In our study, EGFR gene mutations involve exon 19 (83.33%) and exon 21 (16.67%). In conformity with previous studies in different ethnics, EGFR gene mutations in Uygur population occur more frequently in ADCs (26.67%).

The never smokers was found to have higher gene mutations frequency than former and current smokers [25, 26]. In this study, we compared it in Uygur population; however, no significant difference was found which should be confirmed with more consistent number of patients.

In concordance with previously reported studies [4, 26-30], we observed that EGFR-mutation group showed superior efficacy (response rate, 83.33%), long median PFS and (PFS: 10.0±1.5 months, OS: 27.3±2.9 months) to gefitinib treatment. Overall, our study confirmed that the presence of EGFR mutations is a valuable prognosis factor in selecting patients to benefit from gefitinib treatment in NSCLC, especially those with histological subtype of adenocarcinoma.

The HER2 gene, known as human EGFR2 or ERBB2 or NEU, belonging to the ERBB family, was established as a relatively new biomarker for NSCLC, representing an appealing target for anti-cancer strategies [31-33]. The other ERBB family members such as EGFR (HER1/ERB1), HER3 (ERBB3) and HER4 (ERBB4) have their ligands including EGF, epiregulin, betacellulin, TGF and neuregulins targeted to potentiate their activity, but no known ligands proved for HER2.

The prognostic role of HER2 in non-small cell lung cancer remains controversial. The role of HER2 expression was investigated with discordant [12, 32, 34]. One study found that HER2-neu mRNA expression in non-small cell lung cancer correlated with survival [13]. In 2002, Hirsch et al. draw the conclusion that no significant difference in survival was observed among patients with positive (HercepTest 2+/3+) and negative (HercepTest 0/1+) tumors [35]. HER2 overexpression was analyzed as poor prognostic factor in lung cancer in the recent review and meta-analysis [36, 37]. Similar findings have been observed in Uygur population, and we found that the HER2 overexpression positive status was 21.62%, which showed no superior efficacy, Median PFS and OS (response rate: 29.17%, Median PFS: 4.7±1.2 months, OS: 20.5±2.4 months) to gefitinib treatment compared with HER2 negative group (response rate: 13.80%, Median PFS: 3.9±1.6 months, OS: 19.2±2.6).

Also, EGFR mutations combined with HER2 overexpression patients showed no superior efficacy (response rate), PFS and OS to gefitinib treatment compared with EGFR mutations with HER2 negative Jian Ming Xu et al. [14] found that EGFR mutations combined with HER2 overexpression patients had a better outcome than patients with wild type EGFR regardless of HER2 and/or HER3 protein levels, which suggested EGFR mutations play a valuable role in predicting sensitivity to TKIs in Chinese NSCLC patients [14]. In concordance with previous data, in our study, EGFR mutations combined with HER2 overexpression patients showed no superior efficacy and long Median PFS to gefitinib treatment compared with EGFR mutations

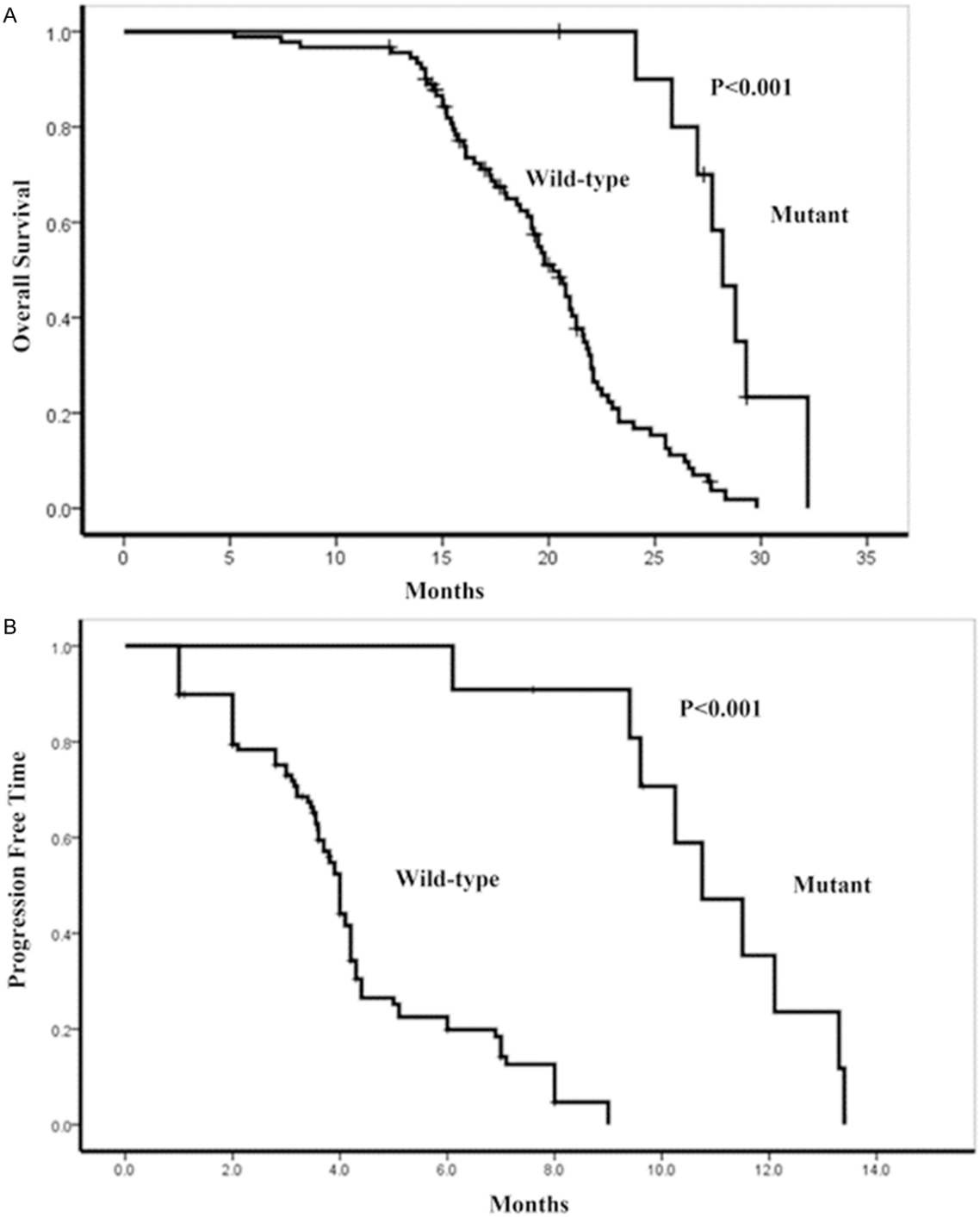

Figure 3. Effect on Kaplan-Meier curves for overall survival (A) and PFS (B) by EGFR mutation status.

combined with HER2 negative. As a consequence of the limited number of patients in our study, efficient assays should be developed to confirm this.

In conclusion, in Uygur population, the prognostic role of EGFR mutations in lung cancer was confirmed in selecting patients sensitive to gefitinib treatment in NSCLC, especially those with histological subtype of adenocarcinoma. HER2 overexperession showed low incidence in Uygur population in this study, and poor prognosis factor in the evaluation of selecting patients sensitive to gefitinib treatment in NSCLC.

Acknowledgements

The protocol of this study was approved by the Research Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University, Urumqi, China. Written informed consent was obtained from all patients participated in this study.

Disclosure of conflict of interest

None.

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