

Potential Clinical Significance of a Plasma-Based *KRAS* Mutation Analysis in Patients with Advanced Non–Small Cell Lung Cancer

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Abstract

Purpose: Non–small cell lung cancer (NSCLC) with *KRAS* mutation may be resistant to epidermal growth factor receptor tyrosine kinase inhibitors (*EGFR*-TKI). This study aims to evaluate a plasma-based *KRAS* mutation analysis and the clinical significance of plasma *KRAS* mutation as a predictive marker for tumor resistance to *EGFR*-TKIs in patients with NSCLC.

Experimental Design: DNA extracted from plasma and matched tumor tissues were obtained from 273 patients with advanced stage NSCLC. Patients were followed up prospectively for treatment outcomes. *KRAS* mutations in codon 12 and 13 were detected using PCR-restriction fragment length polymorphism. Mutations in plasma and matched tumors were compared. Associations between *KRAS* mutation status and patients' clinical outcomes were analyzed.

Results: *KRAS* mutation was found in 35 (12.8%) plasma samples and 30 (11.0%) matched tumor tissues. The consistency of *KRAS* mutations between plasma and tumors is 76.7% (23 of 30; $\kappa = 0.668$; $P < 0.001$). Among 120 patients who received *EGFR*-TKI treatment, the response rate was only 5.3% (1 of 19) for patients with plasma *KRAS* mutation compared with 29.7% for patients with no *KRAS* mutation in plasma DNA ($P = 0.024$). The median progression-free survival time of patients with plasma *KRAS* mutation was 2.5 months compared with 8.8 months for patients with wild-type *KRAS* ($P < 0.001$).

Conclusions: *KRAS* mutation in plasma DNA correlates with the mutation status in the matched tumor tissues of patients with NSCLC. Plasma *KRAS* mutation status is associated with a poor tumor response to *EGFR*-TKIs in NSCLC patients and may be used as a predictive marker in selecting patients for such treatment. *Clin Cancer Res*; 16(4): 1324–30. ©2010 AACR.

Epidermal growth factor receptor tyrosine kinase inhibitors (*EGFR*-TKI) such as gefitinib and erlotinib are selective TKIs that can block the intracellular receptor binding sites of ATP, thus inhibiting the downstream signaling transmission. Several *EGFR*-TKIs have been approved as second- or third-line agents for advanced non–small cell lung cancer (NSCLC) patients who failed in platinum-based chemotherapy (1, 2).

The discovery that *EGFR* tyrosine kinase domain mutations were strongly associated with greater sensitivity of NSCLC to *EGFR*-TKIs *in vitro* and higher response rates in clinical studies provided rationale for using molecular markers to identify patients who are most likely to benefit

from *EGFR*-TKI therapy. Subsequent prospective studies focusing on exploring the possibility of *EGFR*-TKIs as first-line therapy, such as IPASS (IRESSA Pan-Asia Study, a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced NSCLC in Asia) and the Spanish Lung Cancer Group trial (a multicenter prospective phase II trial of customized erlotinib for advanced NSCLC patients with *EGFR* mutations), have shown an outstanding survival benefit for patients with *EGFR* mutant tumors who received first-line *EGFR*-TKI therapy, which is superior to the outcomes of first-line chemotherapies, suggesting that *EGFR* mutation is a useful marker for selecting patients who may benefit from first-line *EGFR*-TKI treatment (3, 4).

KRAS is an important molecule in the downstream signaling network of *EGFR*. The mutation rate is about 15% to 30% in NSCLC (5–8). *KRAS* mutations are mostly on codon 12, 13, and 61 and result in the inhibition of GTPase activity, leading to the constitutive activation of Ras protein, which may render tumor cells independent of *EGFR* signaling. Occurrences of *KRAS* and *EGFR* mutations are often mutually exclusive and patients with *KRAS* mutations are nonresponsive to *EGFR*-TKIs. Several studies testified that *KRAS* mutations are associated with primary

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Translational Relevance

KRAS mutation in patients with non-small cell lung cancer (NSCLC) is a negative predictive biomarker for epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKI) treatment. However, obtaining tumor tissues for mutation analysis is challenging. Our study showed a high consistency of KRAS mutation between plasma DNA and matched tumor tissues for Chinese patients with NSCLC. Further, plasma KRAS mutation status is associated with a poor response to EGFR-TKIs and with a shorter progression-free survival in patients with stage IIIB/IV NSCLC. This study represents KRAS mutation in large samples of plasma DNA in Chinese patients and suggests that KRAS mutation in plasma DNA may be an available biomarker to select suitable patients for EGFR-TKI therapy for advanced NSCLC when their tissue samples were not obtained to perform detection.

drug resistance to EGFR-TKIs (9–11). In a phase III trial (BR21.) comparing the effects of erlotinib and the best supportive care for advanced NSCLC patients who failed in platinum-based chemotherapy, patients with tumors carried KRAS mutation had lower response rate and poorer median survival time (MST) to erlotinib compared with patients without tumors KRAS mutation (12).

Although KRAS mutations can be detected in tumor tissues, obtaining such tissues is clinically difficult, particularly for patients with recurrent and refractory NSCLC. Even in prospectively conducted clinical trials, <40% of the patients had available tumor tissues for the mutation analysis. In our previous study, we have shown that DNA abnormalities such as EGFR mutations can be reliably detected in plasma samples of patients with stages IIIB to IV NSCLC and can be used as a biomarker to predict tumor response to EGFR-TKIs and progression-free survival (PFS; ref. 13), suggesting that plasma may be an alternative sample source for such genetic evaluation.

In this study, we compared KRAS mutation status between plasma DNA and the matched tumor tissues in 273 consecutive Chinese patients with stage IIIB/IV NSCLC in a hope to use plasma as an alternative source for KRAS mutation analysis.

Materials and Methods

Patients population. All patients had pathologically confirmed stage IIIB or stage IV NSCLC, an Eastern Cooperative Oncology Group performance status of 0 to 2, has available plasma and primary tumor tissue, and received either chemotherapy or EGFR-TKI treatment. Two hundred and seventy-three consecutive patients who met the above criteria were identified in the Beijing University School of Oncology, Beijing Cancer Hospital from June 2005 to

June 2008. The patient population consists of 115 females and 158 males. There were 198 cases of adenocarcinoma and 67 cases of squamous cell carcinoma and 8 other tumor types. There were 74 and 199 cases of stage IIIB and IV, respectively, with 113 cases of smokers (≥ 10 pack-year), 22 cases of exsmokers (100 cigarettes in a life time \leq cigarettes < 10 pack-year), and 138 nonsmokers (< 100 cigarettes in a lifetime). The laboratory data were obtained and recorded independently and blinded from clinical data until analyses by a biostatistician. The study was reviewed and approved by the Institutional Ethic Committee. All the patients signed an informed consent for participation of the study and the use of their biological tissues.

Evaluation of treatment response. Objective tumor response was determined using the Response Evaluation Criteria in Solid Tumors. The patients had computed tomography scan covering target lesions every 6 wk (two cycles) during the course of chemotherapy and every 8 wk in the EGFR-TKI treatment phase. Brain or bone metastases were evaluated every 3 or 6 mo by magnetic resonance imaging or bone scintigraphy.

According to the Response Evaluation Criteria in Solid Tumors guideline, response to therapy was categorized into four group: complete response, partial response, stable disease, and progression of disease. Overall survival (OS) was calculated as the time from the beginning of therapy to death or last follow-up. The PFS of disease was calculated from the date of the beginning of chemotherapy to the date of tumor progression or death.

Specimen collection and DNA extraction. Each enrolled patient contributed one blood sample and one tumor tissue sample. The 4 mL anticoagulated venous blood from each patient was collected before TKI or second-line chemotherapy and placed at 4°C for 4 h, then centrifugalized at 2,500 rpm for 10 min at low temperature. The plasma was aspirated to a new centrifuge tube and then high-speed centrifugation was carried out again. Plasma (1.5 mL) was obtained, and then added 15 μ L protease K (20 mg/mL), 50 μ L SDS (20%), and DNA was extracted by phenol/chloroform/isopentanol after water bath at 60°C for 2 h. Then, it was precipitated in alcohol and centrifugalized the next day. After salt washing, the sediment was dissolved in quantity-sufficient Tris-EDTA buffer and then preserved at -20°C for spare use.

A total of six to eight pieces of 5- μ m-thick slices were cut from paraffin-embedded tissues. The tumors were macrodissected and tumor contents were recorded for each sample by using immediately adjacent sections. About 80% of the samples had >30% tumor contents, whereas in 20% of the tumor samples, the tumor content was between 20% and 30%. After xylene dewaxing, we added lysate and protease K, then placed samples overnight in 60°C water bath. DNA was extracted by phenol/chloroform/isopentanol the next day, and the following steps were the same as the above process of plasma.

KRAS mutation analysis. Plasma KRAS gene mutation was detected using PCR-restriction fragment length polymorphism in 120 NSCLC patients who were treated by

Table 1. Sequences of *KRAS* primers

	Sequences of primers
<i>KRAS</i> -A	5_-ACTGAATATAAACTTGTGGTCCATGGAGCT-3'
<i>KRAS</i> -B	5_-TTATCTGTATCAAAGAATGGTCCTGCACCA-3'
<i>KRAS</i> -C	5_-GGATGGTCCCTCCACCAGTAATATGGATATTA-3'

EGFR-TKIs. The 10- μ L PCR system of the first turn included 20 ng DNA, 1 \times PCR buffer, 1.5 mmol/L MgCl₂, 200 nmol/L PCR primers (*KRAS*-A, *KRAS*-B; Table 1), 200 nmol deoxynucleotide triphosphates (Promega Corp.), and 0.1 unit of Go-Tag DNA polymerase (Promega Corp.). This step was to introduce restriction sites of incision enzyme BstXI (5'-CCANNNNNNTGG-3') and XcmI (5'-CCANNNNNNNNNTGG-3') by primer *KRAS*-A. PCR instrument (2720 Thermal cycler, Applied Biosystems) was used for amplification. The reaction condition was as follows: predegeneration at 95°C for 5 min, degeneration at 95°C for 30 s, renaturation at 55°C for 30 s, and extension at 72°C for 30 s. There were a total of 38 circulations and a final extension for 5 min at 72°C. The product length of the first PCR was 166 bp. PCR product (2 μ L) containing ~150 ng DNA was digested by restriction enzymes BstXI (MBI Fermentas) and XcmI (New England Biolabs) at 37°C for 2 h. The total volume was 20 μ L, and the operations were based on the instruction provided by the manufacturer. Then, 2 μ L digested product was used for the second amplification, and the primers were *KRAS*-A and *KRAS*-C (Table 1), with the same reaction system as the first PCR amplification. Ten microliters of PCR product were then digested by restriction enzymes BstXI and XcmI again under the same condition. The digested products were examined by 18 μ g agarose gel electrophoresis and stained by ethidium bromide, then the results were analyzed by a UV imaging system. The digested fragments of wild-type DNA included 106, 28, and 18 bp, and the digested fragments of mutant DNA included 134 and 18 bp. SW-480 and MDA-MB231 cell line containing mutant codon 12 and 13 were used as positive controls, and RKO and HT-29 containing wild-type codon 12 and 13 were used as negative controls. Meanwhile, the peripheral blood samples from normal subjects and patients with benign pulmonary diseases and autoclaving double-distilled water were used as negative controls.

EGFR mutation detection by denaturing high-performance liquid chromatography. Denaturing high-performance liquid chromatography was done to detect *EGFR* mutation by the TransgenomicWave Nucleic Acid 119 Fragment Analysis System with a DNasep column (Transgenomic) according to method described by Hua Bai and coworkers (13).

Statistical analyses. The SPSS 13.0 software was used for statistical analyses. The relationship between *KRAS* mutation and relevant factors such as sex, age, stage, histologic type, and smoking was examined by χ^2 test, with $P < 0.05$ as bilateral significant difference. The consistency between *KRAS* mutation in peripheral blood and matched tumor

tissue, as well as the consistency between PCR-restriction fragment length polymorphism and sequencing, was analyzed by χ^2 test; κ was used as the consistency testing. The relationship between *KRAS* mutation and response of *EGFR*-TKIs was analyzed by χ^2 test, and the relationships between *KRAS* mutation and PFS as well as OS were analyzed by Kaplan-Meier survival curve. The prognostic factors on responses of *EGFR*-TKIs were discussed by logistic analysis, including age, sex, smoking history, tumor stage, and *KRAS* mutation. The prognostic influencing factors of PFS and OS after *EGFR*-TKIs treatment were analyzed by COX regression analysis.

Results

KRAS mutations in plasma is correlated with mutations detected in the matched tumors. Thirty (11.0%) *KRAS* mutations were detected in the 273 tumor tissues whereas 35 (12.8%) *KRAS* mutations were found in the 273 plasma samples. Identical mutations were observed in 23 (76.7%) of the 30 cases with *KRAS* mutations in the tumor tissues, which indicated a high correlation between the mutations detected in plasma DNA and the mutations detected in the corresponding tumor DNA ($P < .001$; correlation index, 0.668). Interestingly, 12 (4.4%) patients with plasma DNA mutations had no detectable *KRAS* mutations in the corresponding primary tumors. Similarly, 7 (2.6%) patients with tumor DNA mutations had no detectable *KRAS* mutation in the corresponding plasma DNA samples. The correlation between mutations detected in plasma DNA and tumor DNA is summarized in Table 2.

KRAS mutation status and clinicopathologic parameters. The relationships between *KRAS* mutations (in plasma

Table 2. Consistency of *KRAS* mutation in codon 12 and 13 between plasma and matched tumor tissues

Item	Tissue		Total
	<i>KRAS</i> -	<i>KRAS</i> +	
Plasma			
<i>KRAS</i> -	231	7	238
<i>KRAS</i> +	12	23	35
Total	243	30	273
		$\kappa = 0.668$	$P < 0.001$

Table 3. KRAS mutation status and clinicopathologic parameters

Item	Num (%) <i>n</i> = 273	T12+13 <i>n</i> = 30	<i>P</i>	P12+13 <i>n</i> = 35	<i>P</i>	AnyTP <i>n</i> = 40	<i>P</i>
Age							
≤60	126 (46.2)	16	0.441	16	1	16	0.865
>60	147 (53.8)	14		19		28	
Sex							
Female	115 (42.1)	11	0.562	21	0.856	16	0.863
Male	158 (57.9)	19		14		28	
Smoking							
Smokers	138 (50.5)	14	0.815	21	0.328	22	0.687
Exsmokers	22 (8.1)	2		1		2	
Nonsmokers	113 (41.4)	14		13		16	
Pathology							
Ade	198 (72.5)	26	0.082	29	0.161	31	0.716
Nonade	75 (27.5)	4		6		13	
Stage							
IV	199 (72.9)	22	1	24	0.545	34	0.701
I-III	74 (27.1)	8		11		10	

NOTE: Smoking status: nonsmokers (<100 cigarettes in a life time), exsmokers (100 cigarettes in a life time ≤ cigarettes < 10 pack-year), and smokers (≥10 pack-year).

Abbreviations: T, tissue; P, plasma; T12+13, 12 and 13 codon mutation in tissues; P12+13, 12 and 13 codon mutation in plasma DNA; AnyTP, 12 and 13 codon mutation of KRAS in tissue and plasma samples. Ade, adenocarcinoma; nonade, nonadenocarcinoma.

and tissue) and clinical parameters were summarized in Table 3. No association was observed between KRAS mutation and smoking history, age, sex, tumor stage, histopathologic type, and EGFR mutation.

Association between KRAS mutation status and EGFR-TKI response/patients' survival. Among the 273 NSCLC patients, 120 received EGFR-TKI as second-line treatment or more. One hundred three and 17 of 120 patients received gefitinib and erlotinib therapy, respectively. Until July 10th, 2009, 77 of 120 patients died and 2 lost follow-up. The median follow-up time for the patient population was 23.8 mo. We subsequently analyzed the association between KRAS mutation (from plasma and tissues) and clinical outcomes of EGFR-TKI therapy for these 120 patients (Table 4). The responsive rates to EGFR-TKI treatment in patients with KRAS mutation in plasma and tissue were 5.3% and 7.1%, whereas the response rate of the patients

with wild-type were 29.7% and 28.3% ($P = 0.024$, $P = 0.112$), respectively. Similarly, the disease control rate in patients with KRAS mutation in plasma and tissues were 15.8% and 27.3% compared with the corresponding 58.4% and 55.7% in patients with wild-type KRAS ($P = 0.001$, $P = 0.022$). The median PFS time of patients with KRAS mutation were 2.5 and 2.7 months compared with 8.8 and 8.1 months for patients with wild-type KRAS in plasma and tissues ($P < 0.001$, $P = 0.006$; Fig. 1). The MST of patients with KRAS mutation in plasma DNA and tissues were 16.9 and 13.3 months, which were shorter than those of patients with wild-type KRAS (20.3 and 20.6 months, respectively), but the difference did not reach statistical significance ($P = 0.827$, $P = 0.151$).

Association between KRAS mutation and EGFR mutation in plasma DNA and its effect on clinical outcomes. In an earlier study of the same cohort of 120 patients who received

Table 4. Association of KRAS mutation in plasma and tissue with outcomes of EGFR-TKIs treatment

KRAS mutation	Status	RR (%)	<i>P</i>	DCR (%)	<i>P</i>	PFS (mo)	<i>P</i>	OS (mo)	<i>P</i>
Plasma	+	5.3	0.024	15.8	0.001	2.5	<0.001	16.9	0.827
	-	29.7		58.4		8.8		20.3	
Tissues	+	7.1	0.112	27.3	0.022	2.7	0.006	13.3	0.151
	-	28.3		55.7		8.1		20.6	

Abbreviations: RR, response rate; DCR, disease control rate; +, mutant type; -, wild-type.

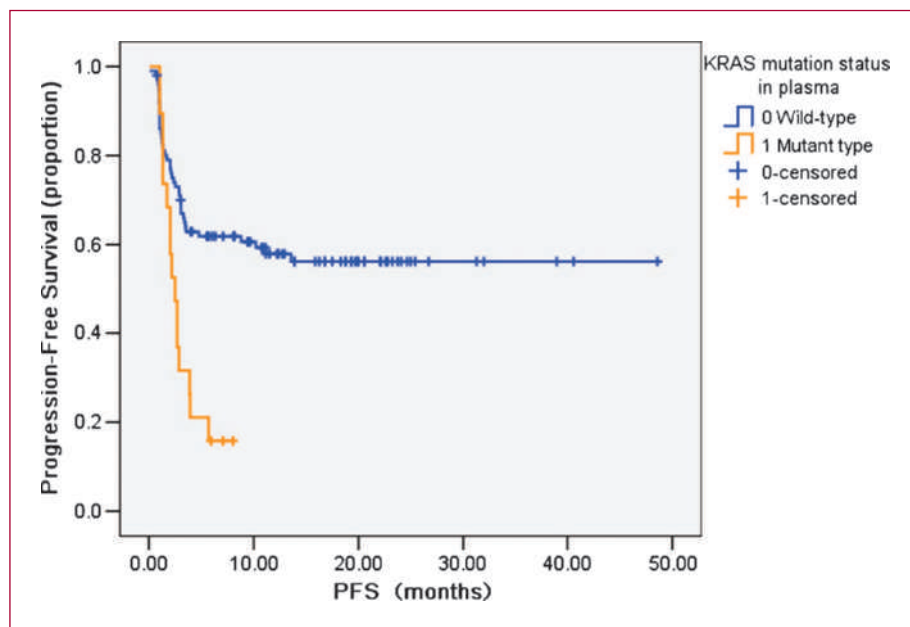


Fig. 1. KRAS mutation in plasma with PFS of EGFR-TKI treatment. The median PFS time of patients with plasma KRAS mutation was 2.5 mo compared with 8.8 mo for patients with wild-type KRAS ($P < 0.001$).

TKI treatment, we analyzed *EGFR* mutations in plasma and determined their clinical implications in patients with advanced NSCLC (13). We subsequently analyzed the association between KRAS mutation and *EGFR* mutation in plasma DNA. Among these patients, there were 5 patients with both KRAS and *EGFR* mutation (group A): 18 patients with KRAS mutation and wild-type *EGFR* (group B), 42 patients with wild-type KRAS and mutant *EGFR* (group C), and 55 patients with wild-type KRAS and *EGFR* (group D). Through comparing the PFS and OS by different KRAS and *EGFR* mutation status in plasma DNA, we found that median PFS in group A, B, C, and D were 2.70, 2.50, 13.25, and 3.53 months ($P < 0.001$; Fig. 2), and MST in group A, B, C, and D were 17.67, 12.70, 26.40, and 16.9 months ($P = 0.103$; Table 5). The effect of factors such as age, sex, histologic type, stage, smoking, KRAS (both in plasma and tumor tissues) and *EGFR* mutation status on PFS and OS were analyzed by Cox regression analysis, which showed that KRAS mutation in plasma, *EGFR* mutation, smoking status, and adenocarcinoma were independent predictive factors of PFS ($P = 0.001$, $P = 0.004$, $P < 0.001$, $P = 0.041$). In addition, only *EGFR* mutation and adenocarcinoma were inde-

pendent prognostic factors for OS ($P = 0.026$ and 0.009 , respectively). Logistic regression analysis was used to investigate the effects of these eight factors on response. The results showed that KRAS mutation in plasma, *EGFR* mutation, smoking status, and adenocarcinoma are independent predictors of response of *EGFR*-TKI therapy ($P = 0.001$, $P = 0.036$, $P = 0.002$, $P = 0.012$).

Discussion

KRAS mutation has been linked to *EGFR*-TKI resistance in multiple studies (9–11), making KRAS mutation a possible marker of resistance to such treatment. However, it is difficult to test KRAS mutation in NSCLC in a routine basis because of the difficulty to obtain tumor tissues from patients with advanced NSCLC, particularly in a repeated manner. Therefore, to explore whether plasma can be used as an alternative source for KRAS mutation testing has important clinical significance. Our study found a positive correlation of KRAS mutation in plasma DNA and in cancer tissues (consistency of 0.668; $P < 0.001$). Plasma KRAS mutation status is associated with a poor outcome to *EGFR*-TKIs in NSCLC patients and may be used as a negative predictive marker in selecting patients for such treatment.

Recently, several studies have focused on investigating the association of KRAS mutation between plasma or serum-free DNA and tumor tissues, and their clinical significance in the patients with advanced NSCLC and other cancers, regardless of the type of cancer therapy (14–16). Kimura et al. (14) analyzed KRAS gene mutation in 25 plasma specimens from NSCLC patients with paclitaxel/carboplatin chemotherapy, among whom, there were 12 cases of matched tumor tissues after treatment. The results showed that KRAS mutation rate in peripheral blood was 20%, which was consistent with that in matched

Table 5. KRAS and *EGFR* mutation status with PFS and OS in plasma

Group	KRAS mutation	EGFR mutation	PFS (mo)	<i>P</i>	OS (mo)	<i>P</i>
A	+	+	2.7	<0.001	17.67	0.103
B	+	–	2.5		12.7	
C	–	+	13.25		26.4	
D	–	–	3.53		16.9	

tumor tissues. There was no significant difference in MST (3.3 versus 11.4 months; $P = 0.056$) between patients with *KRAS* mutation and wild-type *KRAS*. The study of Ramirez et al. (15) showed that serum *KRAS* mutation rate in NSCLC patients was higher than that of matched tumor tissues (23% versus 18%), but the difference was not significant. A study by Gautschi and his colleagues (17) found matching codon 12 sequences in blood and tumor in 78% evaluable cases, which support that circulating *KRAS* mutations originate from tumors and are prognostically relevant in lung cancer. However, contrasting results were also reported (16, 18, 19). For example, Trombino et al. (16) failed to find a correlation between *KRAS* mutations in serum and the matched tumor tissues. These data together suggest that consistency of *KRAS* mutation in circulating DNA and tissue remains to be of great controversy. Therefore, standardizing the detection method, developing large samples, and testing it in a cohort of patients with specific cancer therapy are impending for learning its clinical application.

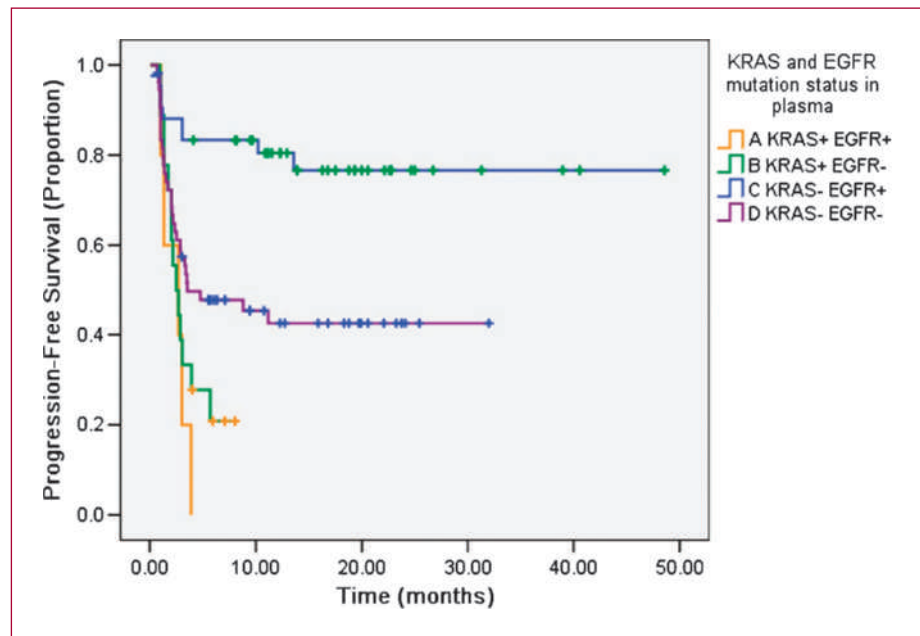
Although high consistency of *KRAS* mutation in tissues and matched plasma DNA was observed in our study, which is consistent with studies by Kimura et al. and Gautschi et al. (14, 17), it is notable that 4.4% *KRAS* mutation was identified in plasma DNA samples only and 2.6% in the tumor DNA samples only. This phenomenon has also been observed in previous studies with smaller sample sizes (15, 16). Possible explanations might include, first, the intratumor heterogeneity of genetic abnormalities. In such case, tumor biopsy specimens might not carry the *EGFR* mutations detected in circulating plasma DNA because these mutations could come from different parts of the tumor. Second, the lower tumor cell content in some of the tumors might also contribute to the lack of detectable mutations in plasma. Third, if the tumor parts

that carried mutations shed less DNA than the other parts of the tumors into plasma, then one may miss such mutations in the plasma DNA. Fourth, the dilution of DNA derived from noncancerous tissues, such as inflamed tissues, might impede the detection of mutations in plasma DNA, despite the presence of mutations in tumors. The real mechanism for the discordant of molecular abnormalities between two kinds of samples remains to be resolved.

We observed that *KRAS* mutation in plasma DNA, but not in tissues, was a predictor factor for PFS and response of TKI therapy in our study, which may be attributed to the different times in obtaining two kinds of samples in a small fraction of our patients. Tissue samples were collected during the time of initial diagnosis, whereas blood samples were gained after the patients failed in one and in multiple regimen therapy and were ready to receive TKI therapy. At the moment, the detection of plasma DNA may more exactly reflect *KRAS* mutation status. In addition, although we have a decent sample size, due to the low mutation rate, the subgroup of patients with *KRAS* mutation was still small, especially for those with mutations in the tissue sample, which may result in bias in data analyzed.

Interestingly, our study found and further confirmed by using direct sequencing of individual clones that 5 of 120 patients with TKI therapy had coexisting *EGFR* mutation and *KRAS* mutation, which is different from previous reports (20–22). Several studies have indeed suggested that the occurrences of *KRAS* and *EGFR* mutation are mutually exclusive; however, occasional tumors with coexisting *EGFR* and *KRAS* mutation have also been reported (8, 23, 24). Moreover, we found that the patients with both *KRAS* and *EGFR* mutation in plasma DNA had very poor survival and PFS, which may suggest that *KRAS* mutation in plasma may possess stronger negative predicting role for TKI therapy.

Fig. 2. *KRAS* and *EGFR* mutation in plasma with PFS of *EGFR*-TKIs treatment. Median PFS in group A, B, C, and D were 2.70, 2.50, 13.25, and 3.53 mo ($P < 0.001$).



There are several limitations from our study. First, this is a retrospective study in a single center and it needs more prospective study to verify the results. Second, samples might still be small and the mutation rate was low, which may result in some bias in the data analyzed. Third, time point to obtain blood and tissues samples was different in most patients in our study; this may also caused bias.

In summary, our study provides an evidence to suggest that circulating plasma DNA may be used as a surrogate tissue for *KRAS* mutation analysis in NSCLC. Plasma *KRAS* mutation status is associated with a poor tumor response to *EGFR-TKIs* in NSCLC patients and may be used as a predictive marker in selecting patients for such treatment, which needs more prospective, multicenter study to validate.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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