

A Case-control Study Supporting the Use of Liquid Biopsy in the Targeted Therapy for Lung Cancer

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Abstract

Backgrounds: Targeted therapy for lung cancer depends on the genetic testing. Liquid biopsy provides a valuable source for the genetic testing. However, direct evidence was lacking for whether liquid biopsy could guide the targeted therapy. **Methods:** In this retrospective study, the admitted patients from Jan 2015 to Feb 2016 were screened through a pre-established database. Patients with metastatic, pathologically-confirmed, and treatment naïve non-small cell lung cancer who were prescribed with epithelial growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) from the guidance of liquid biopsy were enrolled (Liquid group). The mutation status in tumors was not mandatory. During the same period, patients medicated with TKI based on tumor samples were included in the Control group. They were enrolled in an age-, gender-, performance-, smoking-, and histology-matched manner. **Results:** We screened 536 patients and enrolled 26 patients in the Liquid group. Another 26 patients were enrolled in a 1:1 ratio in the Control group. In the Liquid group, a high consistence (84.6%) in EGFR mutation status between liquid and tumor was observed. The best response was partial response in 19 patients (73.1 %), and followed by stable disease in 6 patients (23.1 %). The median progression-free survival was 10.0 months (95%CI: 4.2-15.8 months). In the Control group, a similar disease control rate (88.4%, P=0.603) and comparable PFS (8.6 months, 95% CI: 7.6-10.4 months, P=0.714, HR=0.657, 95% CI: 0.309-1.396) was found. In the Liquid group, 3 of 4 patients with discordant results between tumor and liquid biopsy showed treatment responses favoring the liquid biopsy. **Conclusion:** This study provided direct evidence supporting the liquid biopsy for guiding the targeted therapy for lung cancer.

Keywords: Epithelial growth factor receptor (EGFR)-liquid biopsy-lung cancer (NSCLC)-progression-free survival

Asian Pac J Cancer Prev, **19** (7), 1761-1766

Introduction

Lung cancer is a major cause of cancer-related mortality worldwide (Siegel et al., 2013). 80% of all cases are non-small cell lung cancer (NSCLC). More than half patients were diagnosed as advanced stage, with median overall survival (OS) of merely 10-12 months with standard platinum-based chemotherapy (Reck et al., 2013). Targeting the epidermal growth factor receptor (EGFR) is an appealing strategy, as the superiority of EGFR tyrosine kinase inhibitors (TKI) such as gefitinib, erlotinib or afatinib have been proved in patients harboring EGFR mutation (Maemondo et al., 2010; Mok et al., 2009; Rosell et al., 2012; Sequist et al., 2013; Wu et al., 2014; Zhou et al., 2011). Thus, determining EGFR mutation status is critical in the successful management of NSCLC.

To facilitate the genetic testing of EGFR mutation, more sensitive methods are developed besides Sanger sequencing. Some of these novel methods such as amplification refractory mutation system (ARMS), digital polymerase chain reaction (dPCR), and next-generation

sequencing (NGS) are being used in clinical practice (Couraud et al., 2014; Li et al., 2014; Wang et al., 2014). The genetic testing is restricted by the availability of tumor tissue, as the small biopsy technique such as fine needle aspiration is widely adapted nowadays. Facing this issue of tissue, the plasma containing circulating free DNA (cfDNA) is suggested as an alternate sample source. Early attempts comparing the paired tumor tissue and liquid biopsy gave about 70% of consistency (Mok et al., 2012). However, direct evidence was lacking for whether liquid biopsy could guide the targeted therapy.

Here, a case-control study was conducted where patients prescribed with EGFR TKI guided by either tumor samples or liquid biopsy were compared in parallel to explore the value of liquid biopsy in the targeted therapy.

Materials and Methods

Patients

This was a retrospective study conducted where patients admitted from Jan 2015 to Feb 2016 in the West

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China Hospital (a tertiary referral center) were screened. The data were retrieved through a pre-established database, which was an infrastructure of the National Major Project of China (2011ZX09302-001-01, Li et al., 2015). To be enrolled, patients must have pathological confirmed NSCLC, older than 18 years, ECOG performance of 0 or 1, and have metastatic diseases. EGFR mutation status in plasma was determined before treatment. The detection of EGFR mutation in tumors was not mandatory. Patients prescribed with TKI based on the liquid biopsy were enrolled (liquid group). But those with concomitant other cancer, or target lesion resected were excluded. In the latter situation, the defining of progression was difficult. Patients receiving TKI with the guidance from tumor genetic assay were enrolled in the control group. They were selected at a ratio of 1:1 based on age, gender, ECOG performance, smoking status, and histology. Demographic and clinical data of both groups were collected. The ethical committee of Sichuan University reviewed the study concept and the study was performed in accordance with the Declaration of Helsinki.

Genetic testing

For the tissue specimens, genetic testing was performed by ARMS using a commercially available kit (AmoyDx, Shameng, China) in a College of American Pathologists-certified lab in West China Hospital. The detection is under the authorization of the Chinese Food and Drug Administration. Briefly, tissue blocks were sliced into 5 μ m sections, and tumor content was assessed by board-certified pathologists using hematoxylin and eosin staining. All specimens contained more than 10% of tumor content. DNA was extracted using the QIAamp DNA mini kit (Qiagen).

Blood specimens were collected in ethylenediaminetetraacetic acid tubes (CDRich, Chengdu, China) and subjected to plasma separation by centrifugation. DNA was extracted using QIAamp DNA Blood mini kit (Qiagen, Valencia, CA). Mutation detection was performed domestically by ARMS, as described above, or shipped to NGS testing (Burning Rock Dx, Guangzhou, China), or dPCR (Gezhi Inc, Nanjing, China).

Treatments

TKI was prescribed by the treatment physicians at

their own discretion. EGFR TKIs (gefitinib, erlotinib, and icotinib) were all permitted. In addition, Osimertinib (AZD9291) was also accessible in some way. Therefore, patients taking Osimertinib were also allowed. The tumor response was monitored by radiographic examinations including chest and abdominal enhanced computed tomography, brain magnetic resonance imaging, and bone single-photon emission computed tomography regularly. The response was assessed by the treating physician according to the Response Evaluation Criteria in Solid Tumor 1.1 criteria (Eisenhauer et al., 2009). Briefly, the complete remission of all lesions was defined as Complete Response (CR). The significant shrinkage of target lesions over 30% was defined as Partial Response (PR). The stable status of the tumor was defined as Stable Disease (SD). The sum of the percentage of CR, PR, and SD was defined as Disease Control Rate (DCR).

Statistical Analysis

Overall survival (OS) was defined as the interval between the date of TKI therapy to the date of death or last follow up. Progression free survival (PFS) was defined as the duration of time between the date of TKI and the date of first sign of progression. For survival comparison, a Kaplan-Meier analysis was used. For quantitative and categorized data, t and chi-square tests were used respectively. Hazard Ratio (HR) was obtained by using the Cox proportional hazards model. All statistical analyses were performed using SPSS 19.0 software (IBM Inc., Chicago, IL), and statistical significance was defined as a p value <0.05.

Results

Patients

During this time period, totally 536 patients with treatment naïve metastatic NSCLC were screened. Among them, 216 patients were prescribed with EGFR TKI. Most of them were based on the tumor genetic testing and merely a few were directed by liquid biopsy. These patients were sorted out manually and confirmed by counseling with the treating physician. And a cohort of 26 patients was enrolled. During this time period, another 26 patients with the matched age, gender, ECOG performance, smoking status, and histology were enrolled in the control group.

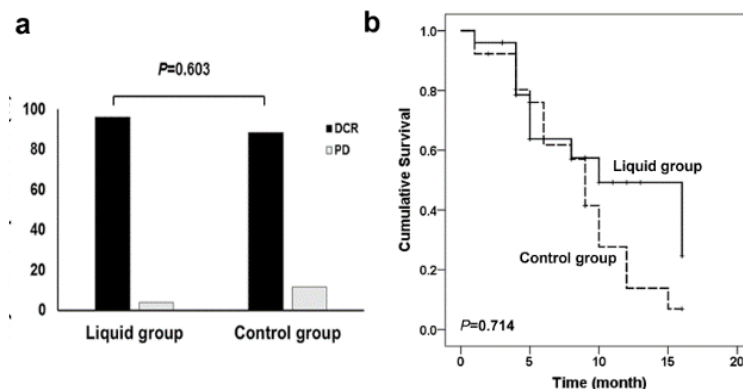


Figure 1. Treatment Response in Patients. The DCR was Similar (a) and PFS was also Comparable (b) in the Liquid Group and Control Group

Table 1. Demographic Features of the Enrolled Patients

Clinical features	N(%)	Liquid group	Control group	P value
Gender				
Male	35 (67.3)	18 (69.2)	17 (65.4)	0.768
Female	17 (32.7)	8 (30.8)	9 (34.6)	
Age (Median, range)	53 (27-79)	53 (27-78)	53 (29-79)	NA
Performance				
0	7 (13.5)	4 (15.4)	3 (11.5)	0.685
1	45 (86.5)	22 (84.6)	23 (88.5)	
Smoking				
Yes	13 (25.0)	8 (30.8)	5 (19.2)	0.525
No	39 (75.0)	18 (69.2)	21 (80.8)	
Histology				
Adeno	49 (94.2)	25 (96.2)	24 (92.3)	0.556
Squamous	3 (5.8)	1 (3.8)	2 (7.7)	
Tumor EGFR				
19DEL	24 (46.2)	11 (42.3)	13 (50)	0.836
L858R	21 (40.4)	11 (42.3)	10 (38.5)	
Other	7 (13.4)	4 (15.4)	3 (11.5)	
Method				
ARMS	43 (82.7)	17 (65.4)	26 (100)	0.004
dPCR	6 (17.3)	6 (23.1)	0	
NGS	3 (5.8)	3 (11.5)	0	
Treatments				
Gefitinib	27 (51.9)	14 (53.8)	13 (50.0)	0.390
Erlotinib	9 (17.3)	1 (3.8)	8 (30.1)	
Icotinib	14 (26.9)	9 (34.6)	5 (19.9)	
Osimertinib	2 (3.9)	2 (7.8)	0	

The study included totally 52 patients and the demographic features were summarized in Table 1.

Genetic profile

As to the EGFR mutation status in the liquid biopsy, the classic exon 19 deletion (19DEL) and exon 21 missense mutation (L858R) were detected in 11 (42.3%) and 13 (50.0%) patients respectively. Another 2 patients harbored “gatekeeper” mutation (L858R+T790M and 19DEL+T790M). The liquid biopsy was confirmed in paired tumor tissues in 22 patients (84.6%). 4 patients had discordant results. Patient 3 had suspected L858R mutation in the tumor which was confirmed in the blood. Patient 4 had unknown EGFR mutation status in tumor due to lack of tissue, while 19DEL was detected in his liquid biopsy. Patient 5 harbored wild-type EGFR in the tumor and L858R in the plasma. Patient 26 had different complex mutation in tumor and plasma, 19DEL+L858R in the tumor and 19DEL+T790M in the plasma. In the control group, tumor mutations were mostly 19DEL (n=15, 57.7%), followed by L858R (n=9, 34.6%). Another 2 patients harbor G719X and S768+G719X respectively.

Treatments

In the liquid group, most patients (n=24, 92.3%)

received the first-generation TKI, either gefitinib (n = 14, 53.8%), or icotinib (n = 9, 34.6%) or erlotinib (n=1, 3.8%). 2 patients harbouring T790M were prescribed with osimertinib (n=2, 8%). The treatments and outcomes of the liquid group were summarized in Table 2. In the control group, gefitinib (n=13, 50%), erlotinib (n=8, 30.1%), and icotinib (n=5, 19.9%) were used, while osimertinib was used in no patient.

In the liquid group, the best response was PR in 19 patients (73.1 %), followed by SD with 6 patients (23.1 %) and the DCR was 96.2% (Figure 1a). 1 patient (3.8%) had tumor progression after TKI treatment. The median PFS was 10.0 months (95% CI: 4.2-15.8 months). In the control group, the overall response was 65.4% (n=17, all were PR), and disease control rate was 88.4% (n=23). Both groups had similar DCR (P=0.603, Figure 1a). The PFS (8.6 months, 95% CI: 7.6-10.4 months) was similar to that of liquid group (P=0.714, HR=0.657, 95% CI: 0.309-1.396, Figure 1b). Due to the small sample size of the study and the well-balanced baseline features, multivariate analysis was not performed any more. Median survival was not reached in both groups.

Four patients in the liquid group had discordant mutation between tumor and liquid biopsy. Patient 3 had suspected and definite L858R mutation in the tumor

Table 2. Detailed Information of Each Patient in the Liquid Biopsy

No	Gender	Age	Histology	Tumor		Liquid biopsy		Treatment	Best response
				Mutation	Method	Mutation	Method		
1	F	50	adenocarcinoma	L858R	ARMS	L858R	dPCR	Gefitinib	PR
2	F	57	adenocarcinoma	L858R	ARMS	L858R	dPCR	Gefitinib	PR
3	F	42	adenocarcinoma	L858R±	ARMS	L858R	NGS	Gefitinib	PR
4	F	43	adenocarcinoma	Unknown	NA	L858R+T790M	NGS	Osimertinib	PR
5	M	45	adenocarcinoma	WT	ARMS	L858R	NGS	Gefitinib	PR
6	M	54	adenocarcinoma	L858R	ARMS	L858R	dPCR	Gefitinib	PR
7	M	68	adenocarcinoma	L858R	ARMS	L858R	dPCR	Gefitinib	SD
8	F	54	adenocarcinoma	19DEL	ARMS	19DEL	dPCR	Icotinib	SD
9	M	52	adenocarcinoma	L858R	ARMS	L858R	dPCR	Gefitinib	SD
10	F	64	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Icotinib	PR
11	F	78	adenocarcinoma	L858R	ARMS	L858R	ARMS	Gefitinib	PR
12	M	58	Squamous	L858R	ARMS	L858R	ARMS	Icotinib	PR
13	F	43	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Icotinib	PR
14	F	57	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Gefitinib	PR
15	M	62	adenocarcinoma	L858R	ARMS	L858R	ARMS	Erlotinib	PR
16	F	67	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Icotinib	PR
17	F	44	adenocarcinoma	L858R	ARMS	L858R	ARMS	Gefitinib	PR
18	F	61	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Gefitinib	PR
19	F	50	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Gefitinib	PR
20	F	21	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Gefitinib	PR
21	F	60	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Icotinib	SD
22	F	64	adenocarcinoma	L858R	ARMS	L858R	ARMS	Gefitinib	SD
23	F	54	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Icotinib	SD
24	M	43	adenocarcinoma	L858R	ARMS	L858R	ARMS	Icotinib	PR
25	F	49	adenocarcinoma	19DEL	ARMS	19DEL	ARMS	Icotinib	PR
26	M	45	adenocarcinoma	19DEL+L858R	ARMS	19DEL+T790M	ARMS	Osimertinib	PD

and liquid biopsy respectively. Her best response was determined as PR by her treating physician. For patient 4 who had no tissue specimen, and targeted therapy was directed only by liquid biopsy. Osimertinib was prescribed for the complex mutation (L858R+T790M) and achieved a PR in the tumor burden. Patient 5 had a PR in his tumor, which was highly consistent with his liquid biopsy results, unsupported by his wild-type EGFR in the tumor (Figure 2). Patient 26 harbored sensitive complex sensitive mutation (19DEL+L858R) in the tumor and resistant genotype (19DEL+T790M) in the liquid biopsy. He had unsatisfactory response to osimertinib. The clinical

outcome of these 3 patients favored the liquid biopsy against tumor tissues.

Discussion

Targeted therapy improves the response rate and helps to lengthen the OS in NSCLC patients. Successful targeted therapy relies on the determination of mutation status, which is restricted by the availability of the tumor tissue. Therefore, liquid biopsy provides an invaluable alternative source for the biomarker analysis. Previous studies compared the sensitivity and specificity of liquid biopsy with tumor tissues. A pilot study by Bai et al reported a sensitivity of 82% and a specificity of 90% in their series of 230 patients by high-performance liquid chromatography (Bai et al., 2009). The high sensitive ARMS method achieved a sensitivity about 70% and a specificity near 100% were reported (Liu et al., 2013; Douillard et al., 2014). The application of dPCR achieved a high consistency of over 80% (Yung et al., 2009). In this study, a high consistency of EGFR mutation between liquid biopsy and tumor tissues (88.5%) was also observed. The extraordinary high consistency was explained several aspects. In this study, high sensitive methods (ARMS, dPCR, or NGS) were used. This cohort consisted of only patients with metastatic diseases where high abundance of cfDNA was present. The sufficient



Figure 2. Radiographic Response of Patient 5. He had reduced tumor size after gefitinib treatment, supporting the liquid biopsy (L858R) irrespective of the wild type EGFR in the tumor tissue.

tumor tissue also helped to reduce the inaccuracy of results.

Even with the success of liquid biopsy in these bench-based studies, direct evidence supporting its value in real world was lacking. This study included 2 cohorts of lung cancer patients taking TKI as the first-line therapy, based on either liquid biopsy or tumor tissues. Similar response rates and comparable PFS were achieved in both cohorts. These results strongly supported the liquid biopsy in selecting appropriate patients for TKI therapy. This notion was in good accordance with previous studies. In the companion study of the milestone IPASS study, liquid biopsy clearly identified those benefited from gefitinib (Goto et al., 2012). In the pioneering FACTACTII study, the liquid biopsy definitely showed EGFR mutant patients benefited from the intercalating chemotherapy and targeted therapy (Mok et al., 2015). However both studies were based on post-hoc analysis. In contrast, the current study provides the direct evidence for the application of liquid biopsy in the guidance of targeted therapy.

Liquid biopsy provided invaluable sources for the genetic analysis, esp. for those unsuitable for biopsy. The latter cohorts might include but were not limited to those relapse after surgery, or those with poor performance status (Eastern Cooperative Oncology Group performance over 2), or those with unsatisfied tissue sampling. Even more, liquid biopsy was proposed as a front-line genetic test prior to tumor biopsy to avoid unnecessary damage to the patient (Oxnard et al., 2016). Thus, the important role of liquid biopsy is now establishing and widely accepted.

The reasons underlying the discrepancy between the liquid biopsy and tumor tissues remained elusive. It could be attributed to several factors. First of all, the tumor tissues had intrinsic heterogeneity, both temporally and spatially (Zhang et al, 2014). Additionally, inconsistent EGFR mutation status was found among different regions inside tumors (Bai et al., 2013). Therefore, the discrepancy between liquid biopsy and tumor tissues might be a sequela of the tumor heterogeneity. Secondly, the detection of cfDNA was a challenge to the sensitivity of the liquid biopsy due to the scarcity of cfDNA. Thirdly, the dynamic variation of cfDNA might influence the results of the liquid biopsy. The notion was supported by our unpublished observation where the amplitude of EGFR mutation in plasma varied longitudinally, as monitored by dPCR.

In summary, this study retrospectively analyzed advanced lung cancer patients whose targeted therapy was guided by liquid biopsy. The high response rate and comparable PFS duration strongly supported the value of liquid biopsy in clinical settings. It was of notice the current study was a retrospective one conducted in a single center. The results should be considered provocative but not definitive. The results should be confirmed in the undergoing large scale prospective study (BENEFIT, CTONG1405).

Acknowledgements

This work was supported by National Natural Science

Foundation of China (no. 82172684).

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