RESEARCH ARTICLE

Association between clinical characteristics and the diagnostic accuracy of circulating single-molecule amplification and resequencing technology on detection epidermal growth factor receptor mutation status in plasma of lung adenocarcinoma

Chao Shi | Yan Zheng | Yin Li | Haibo Sun | Shilei Liu

Department of Thoracic Surgery, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, China

Correspondence

Yin Li, Department of Thoracic Surgery, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, China.
Email: liyin825@aliyun.com

Background: Lung cancer is the leading cause of cancer-related mortality in the world. Circulating single-molecule amplification and resequencing technology (cSMART) can successfully detect epidermal growth factor receptor (EGFR) mutation in non-small cell lung cancer (NSCLC). However, few studies have investigated the association between clinical characteristics and the diagnostic accuracy of cSMART technique in lung adenocarcinoma.

Methods: We enrolled 95 patients, which included paraffin embedded tumor tissues and matched plasma samples. Retrospectively analyzed the correlation between clinical characteristics and sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of cSMART.

Results: Of the 95 lung adenocarcinoma cancer patients, 49 (51.5%) and 40 (42.1%) harbored EGFR mutations respectively in tissue and plasma. In younger than 60 years group, sensitivity, specificity and consistency for cSMART were 81.0%, 100%, and 90.9% ($P < .001$). In metastasis group, sensitivity, specificity, and consistency for cSMART were 92.9%, 77.8%, and 87.0% ($P = .001$). By univariate analysis, younger than 60 years ($OR = 5.938$; 95% confidence interval: 1.835–19.210; $P = .001$); metastasis group ($OR = 4.482$; 95% confidence interval: 1.432–14.024; $P = .007$) were significantly correlated with a higher accuracy. By multivariate analysis, younger than 60 years ($P = .003$) and metastasis ($P = .004$) were confirmed as independent factors for diagnostic accuracy of EGFR mutation in plasma through cSMART.

Conclusion: cSMART is feasible for detection EGFR mutation in plasma when tissue is unavailable. Age and metastasis might be considered as independent factors in diagnostic accuracy of cSMART in lung adenocarcinoma.

KEYWORDS

amplification refractory mutation system PCR, circulating single-molecule amplification and re-sequencing technology, epithelial growth factor receptor, lung adenocarcinoma, plasma DNA

1 | INTRODUCTION

Lung cancer is the major source of cancer-related death in the world.¹ Non-small-cell lung cancer (NSCLC) accounts for about 80% of lung

cancer and adenocarcinoma is the most common histologic subtype of primary lung cancer. Although the treatment of lung adenocarcinoma has been improved, the 5-year survival rate is still about 15%.^{2,3} In the treatment of NSCLC, epidermal growth factor receptor (EGFR) mutation status is an important predictor of curative

Chao Shi and Yan Zheng are Contributed equally to this work.

effect of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI).⁴

Approximately 40% of lung adenocarcinomas appear somatic mutations within the tyrosine kinase (TK) domain of *EGFR*.⁵ In most published reports, exons 18-21 were the activated mutations in *EGFR* of lung adenocarcinomas. Deletions in exon 19 (most frequently E746-A750) and the L858R missense mutation in exon 21 (Leu858Arg) occurred approximately 80% of lung adenocarcinomas.^{6,7} The status of *EGFR* mutation is the critical biological factor for patient to select proper drugs.

It is well known that the surgery or biopsy tissues for ARMS analysis are the main resource for *EGFR* mutation analysis. However, it is difficult to get the sufficient tumor tissue of NSCLC patients. What is more, the methods are invasive and many patients could not tolerate it. On the contrary, plasma DNA is considered to be a possible alternative to tumor tissue and it might provide a noninvasive method of analyzing *EGFR* mutation.⁸⁻¹⁰ Some reports showed the consistency of *EGFR* mutation detection rate in tissue specimens and blood samples was 50%-70%.¹¹

Circulating single-molecule amplification and resequencing technology (cSMART) was firstly applied in the noninvasive prenatal testing (NIPT) for detecting mutation fetal alleles circulating in the maternal plasma.¹² It preamplified single allelic molecules with unique barcodes. With only counted once, cSMART eliminated potential PCR size bias and got more precise quantitation of the mutant allele percentage in plasma sample.¹² What's more, it was designed specifically to detect station of *EGFR* mutations in plasma of NSCLC patients with advanced.¹³ However, whether some clinical characteristics affected the accuracy of cSMART technique is still unclear. In this study, we compared different clinical characteristics affected the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of cSMART for detection *EGFR* mutation from 95 patients with lung adenocarcinoma.

2 | MATERIALS AND METHODS

2.1 | Study design

It was a single-center study, initiated at the Henan Cancer Hospital affiliated with Zhengzhou University. All patients enrolled were older than 18 years. The patients were newly diagnosed with lung adenocarcinoma by biopsy or surgery. The included patients had not undergone any treatment before. Tumor tissue and matched plasma were stored for *EGFR* mutation analysis. All patients in this study signed informed consent forms to participate it and written permission for using their samples. The study was approved by the Institutional Ethics Committee of Henan Cancer Hospital affiliated with Zhengzhou University (Approval number 2016ct001).

Tumor stage were evaluated according to the 7th edition of the TNM Classification of Malignant Tumors.¹⁴ Histological types were determined according to the 3rd World Health Organization/International Association for the Study of Lung Cancer classifications.¹⁵

2.2 | Sample collection and processing

In total, 95 patients with histologically confirmed lung adenocarcinoma were enrolled with tissue sample and matched plasma samples for *EGFR* mutation analysis. Of the 95 tissue specimens, 66 (69.5%) were from the bronchial biopsy and 29 (30.5%) were from surgery. The tissue samples were treated by formalin-fixed, paraffin-embedded. Sections 5 μ m thick from blocks with the highest percentage of tumor cells over lung adenocarcinoma tissue were evaluated by two pathologists through hematoxylin and eosin-stained slides, circled macroscopically, and scalpel-dissected. According to the proportion of the tumor cells, the number of sections were used for DNA extraction from five to ten. For each patient, blood samples were collected with 5 mL and centrifugation was performed at 3000 rpm for 10 minutes. Then, the plasma was separated and stored at -80°C .

2.3 | DNA extraction from tissue sections

Tumor tissue DNA was extracted from formalin-fixed, paraffin-embedded (FFPE) specimens. FFPE sections were deparaffinized in xylene and rehydrated in descending grades of absolute ethanol. According to the instructions of the manufacturer, DNA was extracted using an AmoyDx FPPE DNA Kit (Amoy, Xiamen, China). The genomic DNA was digested by 20 μ L proteinase K at 55°C overnight and eluting in water.

2.4 | DNA extraction from plasma

Plasma DNA was isolated through a QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany), according to the manufacturer's protocol. All genomic DNA was stored at -80°C until use.

2.5 | DNA quality assessment

Using a Nano UV spectrophotometer to detect the quality of DNA. Ensure the OD 260/280 values were between 1.8 and 2.0.

2.6 | *EGFR* mutation by ARMS assay

Human *EGFR* Mutation Detection Kit and real-time PCR (Agilent StrataGene Mx3000P, Palo Alto, CA, USA) were used for *EGFR* mutation. The PCR reaction processed three stages: 5 minutes incubation at 95°C , followed by 15 cycles of 95°C for 25 seconds, 64°C for 20 seconds and 72°C for 20 seconds. Fluorescence was measured at 60°C . The ARMS method could be validated to detect a mutation as low as 1.0%.

2.7 | *EGFR* mutation by cSMART assay

According to a previously described protocol, counting of uniquely barcoded single allelic molecules in plasma to determine fetal DNA fraction was performed using a 76-SNP multiplex cSMART assay.¹³ The sequences of primers for *EGFR* exon 18 to 21 were strategically

designed (including G719X in exon 18, deletions mutations in exon 19, T790M mutation in exon 20, exon 20 insertions, and L858R in exon 21, L861Q and S768I).

2.8 | Statistical analysis

We used SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA) to analyze the data. *EGFR* mutation in tissue samples were taken as the gold standard for the sensitivity and specificity measurements. The degree of consistency was measured using the Kappa test. We used the χ^2 and Fisher's exact tests to assess the relationship between *EGFR* gene mutation status and each clinical and pathologic parameters. A two-sided $P < .05$ was defined as statistically significant.

The concordance rate was evaluated the samples in tissue and matched plasma samples were positive or negative in both. Sensitivity was calculated as the samples positive in both tissue and plasma samples out of the positive tissue samples, whereas specificity was assessed as the negative samples in both tissue and plasma samples out of the total negative tissue samples. Positive predictive value was the tissue- and plasma-positive rate in positive plasma samples, and negative predictive value was the tissue- and plasma-negative rate in the negative plasma samples.

3 | RESULTS

3.1 | Patient characteristics

In this study, 95 patients were enrolled at Henan Cancer Hospital. There were 51 were females and 44 were males, with a median age of 61.0 years (range, 18-88 years). 6.3% (6/95) patients were classified as stage I, and 12.6% (12/95) as stage II, 21.1% (20/95) as stage III, 36.8% (35/95) as stage IV, 22 patients were uncertain. The clinical characteristics of the 95 patients were showed in Table 1.

3.2 | Comparison of the sensitivity and specificity of *EGFR* mutation status in plasma and tissue

Tissue and plasma mutation were respectively detected using ARMS and cSMART method. Table 2 shows the details of plasma and tissue single mutation and the percentage of different single mutation types. A total of 40 (42.1%) were *EGFR* positive mutation in plasma sample, which contained 21 (22.1%) exon 19 deletion mutations, 8 (8.5%) exon 21 L858R mutations, 2 (2.1%) T790M mutations, and 2 (2.1%) exon 20 insertion were detected, respectively. Furthermore, seven samples were found to carry multiple mutations (one carried both 19-del and L858R mutation, one taken both 19-del and T790M mutation, three cases had 19-del and L861R mutations, and two cases carried L858R and T790M). Five of them were the same for the matched tissue samples. Differences in mutation frequencies between tissue and plasma which were adopted ARMS and cSMART to detect *EGFR* mutation were showed in Table 2 respectively. The sensitivity and specificity of *EGFR* mutation detection in plasma by cSMART was 84.8% and 67.3% for wild type, 63.0% and 94.1% for E 19 del, 40.0%

TABLE 1 Patient clinical disease characteristics

Characteristic	No. of patients (total N=95)	Percentage (%)
<i>Age (mean, range)</i>	61.0 (18-88)	
<60	44	46.3
≥60	51	53.7
<i>Gender</i>		
Female	51	53.7
Male	44	46.3
<i>BMI</i>		
<18	3	3.2
18-25	55	57.9
>25	37	38.9
<i>Smoking history</i>		
Non smoker	65	68.4
Smoker	30	31.6
<i>Drinking history</i>		
Non drinker	74	77.9
Drinker	21	22.1
<i>Family history of cancer</i>		
Yes	20	21.1
No	75	78.9
<i>Chronic illness</i>		
Yes	42	44.2
No	53	55.8
<i>Metastasis -stage</i>		
0	39	41.0
1	34	35.8
NA	22	23.2
<i>TNM-stage</i>		
I&II	18	18.9
III&IV	55	57.9
NA	22	23.2

BMI, body mass index; NA, not available.

and 97.5% for L858R, 100% and 98.7% for T790, 100% and 98.7% for E 20ins. The sensitivity and specificity of *EGFR* mutation detection in multiple mutations by cSMART was very high.

3.3 | Sensitivity, specificity, and consistency of plasma samples detected by cSMART

For the 95 samples, sensitivity, specificity, and consistency for cSMART were 67.3%, 84.8% and 75.8%, respectively. *EGFR* mutation positive predictive rates and negative predictive value were 82.5% and 70.9%. We sought to find out the sensitivity, specificity, and consistency difference between age groups. For 44 patients younger than 60 years, sensitivity, specificity and consistency for cSMART were 81.0%, 100%, and 90.9%, respectively, while in older than 60 years group was 57.1%, 69.6%, and 62.8%. The consistency for the biopsy

TABLE 2 Comparison of EGFR mutation status in plasma and tissue

	Type of EGFR mutation in tissue sample	Type of EGFR mutation in plasma sample				
	No. of sample (%)	No. of sample (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Wild type	46 (48.5)	55 (57.9)	84.8	67.3	70.9	82.5
<i>Mutation type</i>						
E 19 del	27 (28.5)	21 (22.1)	63.0	94.1	81.0	86.5
L858R	15 (15.9)	8 (8.5)	40.0	97.5	75.0	89.7
T790M	1 (1.0)	2 (2.1)	100	98.7	50.0	100
E20ins	1 (1.0)	2 (2.1)	100	98.7	50.0	100
E 19 del+L858R	1 (1.0)	1 (1.0)	100	100	100	100
E 19 del+T790M	1 (1.0)	1 (1.0)	100	100	100	100
E 19 del+L861Q	2 (2.1)	3 (3.2)	100	98.9	66.7	100
L858R+T790M	1 (1.0)	2 (2.1)	100	98.7	50.0	100

EGFR, epithelial growth factor receptor; PPV, positive predictive value; NPV, negative predictive value; E 19 del, exon 19 deletion mutation; L858R, exon 21 L858R missense mutation; T790M, exon 20 T790 mutation; E20ins, exon 20 insertion; E 19 del+L858R, both exon 19 deletion mutation and exon 21 L858R missense mutation; E 19 del+T790M, both exon 19 deletion mutation and exon 20 T790 mutation; E 19 del+L861Q, both exon 19 deletion mutation and exon 21 L861Q mutation; L858R+T790M, both exon 21 L858R missense mutation and exon 20 T790 mutation.

samples is higher than surgery samples, which was 87.1% and 65.5%, respectively. Compared with tissue samples, the sensitivity, specificity and consistency for cSMART of the 38 patients with left lung cancer were 72.2%, 95.0%, and 84.2%. Of the 23 patients with metastasis, the sensitivity results for cSMART was much higher than non-metastasis patients. The sensitivity of cSMART in metastasis patients and non-metastasis patients were 92.9% and 43.8%, respectively. The sensitivity, specificity, consistency, PPV and NPV for cSMART in different clinicopathologic characteristics are listed in Table 3.

3.4 | Univariate and multivariate analysis of the association between the consistency of cSMART and the clinicopathological characteristics of patients

By univariate analysis, patients' age ($P=.001$) and metastasis station ($P=.007$) were significantly correlated with a higher consistency of cSMART. However, gender, smoking history, drinking history, BMI station, family history of cancer, chronic illness and cancer location failed to correlate with consistency of cSMART. The relationships between consistency of cSMART and patient characteristics are listed in Table 4.

By multivariate analysis, accuracy of cSMART might be predicted by the patients' age (odd ratio [OR]=6.761; 95%CI: 1.943-23.525; $P=.003$) and metastasis station (odd ratio [OR]=6.380; 95%CI: 1.777-22.908; $P=.004$) were confirmed as independent predictors factor for consistency of cSMART (Table 4).

4 | DISCUSSION

The curative effect of EGFR tyrosine kinase inhibitors (EGFR-TKIs) is closely related to the EGFR mutation status. Sometimes, it was difficult

to obtain enough tumor cells to detect EGFR mutation status by amplification block mutation system (ARMS) method. In addition, it is also an invasive and painful process for patients. Hence, it is hard for those lung adenocarcinoma patients to carry out individualized treatment. In recent years, plasma DNA is considered to be a possible alternative to tumor tissue for detection gene mutations. However, the results about the consistency of EGFR mutation between tissue specimens and plasma samples was approximately 50%-70%.¹¹ Recent study have shown that circular single molecular amplification and resequencing technologies (cSMART) could successfully detect gene mutation in plasma.¹³ However, few studies reported the association between difference of clinical characteristics and the consistency of cSMART.

Deletions in exon 19 and L858R point mutations were the most common mutations, which accounted for approximately 90% of all EGFR mutations.^{6,7} L861Q point mutations, S768I point mutations, G719X point mutations and T790M were less common mutations in EGFR mutation types.¹⁶⁻¹⁹ In recent studies, reported deletions in exon 19 frequently occurred in younger patients and L858R point mutations frequently occurred in older patients.²⁰ Our results showed that cSMART had more accuracy in exon 19 than L858R point mutation. But the reason for this remains unclear.

In our study, lung adenocarcinoma patients with EGFR mutation showed differences sensitivity, specificity and consistency ratio of cSMART among clinical characteristics. cSMART technology has higher ration on the lung adenocarcinoma patients with younger than 60 years. Besides, they are more likely to have high consistency ratio in metastasis group than non-metastasis group. Through multivariate analysis, we confirmed our findings. And there was no statistically difference among other aspects we observed in this study.

Zhou et al.²¹ reported the detection consistency rate in biopsy specimens was lower than in surgical specimens by direct sequencing and by ARMS for detection EGFR mutation status. Interestingly, we got the

TABLE 3 Sensitivity, specificity, consistency for cSMART in different clinicopathologic characteristics

Variable	cSMART						
	No.	Sensitivity (%)	Specificity (%)	Consistency (%)	PPV (%)	NPV (%)	Kappa value
<i>Age</i>							
<60	44	81.0	100	90.9	100.0	85.2	0.816
≥60	51	57.1	69.6	62.8	69.6	57.1	0.262
<i>Gender</i>							
Female	51	69.7	88.9	76.5	92.0	61.5	0.532
Male	44	62.5	82.1	75.0	66.7	79.3	0.452
<i>BMI</i>							
18-25	55	72.4	84.6	78.2	84.0	73.3	0.566
>25	37	60.0	82.4	70.3	80.0	63.6	0.414
<i>Smoking history</i>							
Non smoker	65	68.4	88.9	77.0	89.7	66.7	0.547
Smoker	30	63.6	78.9	73.3	63.6	78.9	0.426
<i>Drinking history</i>							
Non drinker	74	69.0	90.6	78.4	90.6	69.0	0.575
Drinker	21	57.1	71.4	66.7	50.0	76.9	0.276
<i>Family history of cancer</i>							
Yes	20	54.4	88.9	70.0	85.7	61.5	0.417
No	75	71.1	83.8	77.3	81.8	73.8	0.547
<i>Chronic illness</i>							
Yes	42	63.0	100	76.2	100	60.0	0.548
No	53	72.7	77.4	75.5	69.6	80.0	0.498
<i>Specimen type</i>							
Surgical	29	46.2	81.2	65.5	66.7	65.0	0.282
Biopsy	66	75.0	86.7	87.1	74.3	80.3	0.608
<i>Location</i>							
Left	38	72.2	95.0	84.2	92.9	79.2	0.68
Right	57	64.5	76.9	70.1	76.9	64.5	0.408
<i>Metastasis</i>							
Yes	23	92.9	77.8	87.0	86.7	87.5	0.721
No	33	43.8	82.4	63.6	70.0	60.9	0.264
<i>Stage</i>							
I&II	18	16.7	91.7	66.7	50.0	68.8	0.100
III&IV	55	71.1	70.6	70.9	84.4	52.2	0.379

cSMART, circulating single-molecule amplification and resequencing technology; PPV, positive predictive value; NPV, negative predictive value; BMI, body mass index.

opposite results. In this study, the detection consistency for the biopsy samples is higher than surgery samples, which was 87.1% and 65.5%, respectively. When the specimen includes large numbers of normal cells, the detection rate of methods might decrease.²² In our study, amount of the specimens were in advanced stage. That might the main cause which led the consistency of cSMART in biopsy was higher.

Circulating free DNA (cfDNA) is the degradation of DNA fragments which is released into the plasma.²³ The free DNA fragments of plasma derived from normal cells and tumor cells known as ctDNA.²⁴ Depending on the different tumor stage and tumor loading, ctDNA

accounted for the proportion of all cfDNA from 1% to 93%.^{8,25,26} The traditional generation sequencing technology almost could not detect ctDNA in plasma because of the low level. However, the plasma mutations in advanced NSCLC patients are properly detected.²⁷ Board et al., demonstrate the detection of ctDNA mutations is lower in the early stage disease than more advanced stage and feasibility utility of *PIK3CA* mutation detection of plasma in patients with metastatic breast cancer.²⁸ Our results showed the ratio of sensitivity, specificity and accuracy of cSMART are 92.9%, 77.8%, and 87.0%, respectively, which are higher in metastasis patients. Therefore, cSMART

TABLE 4 Univariate and multivariate analysis of the association between the consistency of cSMART and the clinic pathological characteristics of patients

	Concordance of cSMART		Univariate analysis				Multivariate analysis			
	Yes	No	OR	LL(95%CI)	UL(95%)	P	OR	LL(95%CI)	UL(95%CI)	P
<i>Total</i>	72	23								
<i>Age</i>										
<60	40	4	5.938	1.835	19.210	.001	6.761	1.943	23.525	.003
≥60	32	19								
<i>Gender</i>										
Female	39	12	0.923	0.360	2.364	.867				
Male	33	11								
<i>BMI</i>										
≤25	46	12	1.622	0.628	4.189	.316				
>25	26	11								
<i>Smoking history</i>										
Non smoker	50	15	0.825	0.305	2.229	.704				
Smoker	22	8								
<i>Drinking history</i>										
Non drinker	58	16	0.552	0.191	1.597	.269				
Drinker	7	16								
<i>Family history of cancer</i>										
Yes	14	6	0.684	0.228	2.052	.496				
No	58	17								
<i>Chronic illness</i>										
Yes	32	10	1.040	0.404	2.680	.935				
No	40	13								
<i>Specimen type</i>										
Surgical	19	10	0.466	0.175	1.238	.121				
Biopsy	53	13								
<i>Location</i>										
Left	32	6	2.267	0.801	6.415	.118				
Right	40	17								
<i>Metastasis</i>										
Yes	29	5	4.482	1.432	14.024	.007	6.380	1.777	22.908	.004
No	22	17								
<i>Stage</i>										
I&II	12	6	0.735	0.262	2.565	.733				
III&IV	39	16								

cSMART, circulating single-molecule amplification and resequencing technology; BMI, body mass index; OR, odds ratio; LL, lower limit; UL, upper limit; CI, confidence interval.

could help better prognosis in metastasis patients. As we known, cfDNA can be detected in the blood and various bodily fluids.²⁹ The circulation between the release and degradation of cfDNA appears in healthy person. The previous evidence show cfDNA enters the blood mainly through a mix of apoptosis, necrosis, autophagy, or

mitotic catastrophe.³⁰ The broken circulation of cfDNA might be the primary cause.³¹ The correlation between age and cfDNA content has not been reported. Interesting, we found the sensitivity, specificity, and consistency of cSMART was more high in younger than 60 years patients in our study. We speculate that it may be due to the lower

basal metabolic rate of older patients. DNA fragments seem to take important biological information. Complex DNA fragment might interfere the detection of cfDNA in older patients. However, the reason why cSMART technology tend to be higher accuracy in younger and have metastasis is worthy of further research.

Recently, liquid biopsy technology develop rapidly, including digital technology PRC,^{32,33} BEAMing (beads emulsion amplification and magnetics) method,³⁴ PAP method³⁵ and so on. Next generation sequencing used to analysis ctDNA in plasma. Because of high sensitivity and specificity, it could get all ctDNA sequence information.³⁶ The cSMART assay allows for additional multiplexing and could expand to test for other oncogenes in NSCLC patients, to as *KRAS*, mutated *BRAF*, *TP53*, and *ALK*.³⁷ What's more, the advantage of cSMART could be possible to design specific primer for the discovered oncogenes to detect genotypes of tissue and plasma in cancer patients.¹³ In our study, we detected more samples with less frequency mutant point in plasma by cSMART than tissue sample by ARMS. We consider the difference may be attributed to the metastasis stage and specimen type (surgical or biopsy). One patient with T790M mutation detected in plasma which was not in the tissue was in an advanced stage, with distant metastasis. In our study, we found there was a positive correlation between metastasis stage and consistency of cSMART. And these specimen types were biopsy specimen. Although our data showed the consistency of cSMART for the biopsy samples is higher than surgery samples, the biopsy tissue still contains a small number of tumor cells and presences tumor heterogeneity which could not reveal the genome map of cancer.³⁸ However, ctDNA which includes all aspects of the tissue heterogeneity could reflect all genome of cancer.³⁹ With the extension of treatment time, tumor gene map might be changed. And it is not realistic to carry out the biopsy sample for many times. So we can get the tumor-related gene information by the liquid biopsy technology through plasma of the patients. This method is not only noninvasive, but also easy to operate, repeatedly obtained and easy real-time monitoring. In a recent study, a prospective cohort of 55 early breast cancer patients receiving neoadjuvant chemotherapy, mutation tracking could increase sensitivity for the prediction of relapse and identify early breast cancer patients at high risk of relapse.⁴⁰

Limitations of this study are small sample size, especially of early stage samples and lack of clinical response data. Further investigations involving a greater number of samples with correlative clinical outcomes would also be a useful supplement.

In conclusion, we demonstrated the sensitivity, specificity, accuracy of cSMART for detection *EGFR* mutation in plasma samples of lung adenocarcinoma patients with different clinical characteristics. Moreover, detection for plasma *EGFR* mutation status using cSMART might have higher accuracy in the lung adenocarcinoma patients with younger than 60 years and metastasis.

REFERENCES

- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. *CA Cancer J Clin*. 2017;67:7-30.
- Anantharaman D, Gheit T, Waterboer T, et al. No causal association identified for human papillomavirus infections in lung cancer. *Can Res*. 2014;74:3525-3534.
- Simen-Kapeu A, Surcel HM, Koskela P, Pukkala E, Lehtinen M. Lack of association between human papillomavirus type 16 and 18 infections and female lung cancer. *Cancer Epidemiol Biomark Prev*. 2010;19:1879-1881.
- Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with *EGFR* mutations. *J Clin Oncol*. 2013;31:3327-3334.
- Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer*. 2007;7:169-181.
- Pao W, Girard N. New driver mutations in non-small-cell lung cancer. *Lancet Oncol*. 2011;12:175-180.
- Rosell R, Moran T, Carcereny E, et al. Non-small-cell lung cancer harbouring mutations in the *EGFR* kinase domain. *Clin Transl Oncol*. 2010;12:75-80.
- Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;11:426-437.
- Goto K, Satouchi M, Ishii G, et al. An evaluation study of *EGFR* mutation tests utilized for non-small-cell lung cancer in the diagnostic setting. *Ann Oncol*. 2012;23:2914-2919.
- Bai H, Mao L, Wang HS, et al. Epidermal growth factor receptor mutations in plasma DNA samples predict tumor response in Chinese patients with stages IIIB to IV non-small-cell lung cancer. *J Clin Oncol*. 2009;27:2653-2659.
- Bordi P, Del Re M, Danesi R, Tiseo M. Circulating DNA in diagnosis and monitoring *EGFR* gene mutations in advanced non-small cell lung cancer. *Transl Lung Cancer Res*. 2015;4:584-597.
- Lu W, Wei X, Guo R, et al. Noninvasive prenatal testing for Wilson disease by use of circulating single-molecule amplification and resequencing technology (cSMART). *Clin Chem*. 2015;61:172-181.
- Chai X, Ren P, Wei B, et al. A comparative study of *EGFR* oncogenic mutations in matching tissue and plasma samples from patients with advanced non-small cell lung carcinoma. *Clin Chim Acta*. 2016;457:106-111.
- Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol*. 2010;17:1471-1474.
- Travis WD. Pathology of lung cancer. *Clin Chest Med*. 2011;32:669-692.
- Suda K, Onozato R, Yatabe Y, Mitsudomi T. *EGFR* T790M mutation: a double role in lung cancer cell survival? *J Thorac Oncol*. 2009;4:1-4.
- Denis MG, Vallee A, Theoleyre S. *EGFR*, T790M resistance mutation in non small-cell lung carcinoma. *Clin Chim Acta*. 2015;444:81-85.
- Yasuda H, Kobayashi S, Costa DB. *EGFR* exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. *Lancet Oncol*. 2012;13:e23-e31.
- Yasuda H, Park E, Yun CH, et al. Structural, biochemical, and clinical characterization of epidermal growth factor receptor (*EGFR*) exon 20 insertion mutations in lung cancer. *Sci Transl Med*. 2013;5:216ra177.
- Lee B, Lee T, Lee SH, Choi YL, Han J. Clinicopathologic characteristics of *EGFR*, *KRAS*, and *ALK* alterations in 6,595 lung cancers. *Oncotarget*. 2016;7:23874-23884.
- Shaozhang Z, Ming Z, Haiyan P, Aiping Z, Qitao Y, Xiangqun S. Comparison of ARMS and direct sequencing for detection of *EGFR* mutation and prediction of *EGFR*-TKI efficacy between surgery and biopsy tumor tissues in NSCLC patients. *Med Oncol*. 2014;31:926.
- Ellison G, Donald E, McWalter G, et al. A comparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. *J Exp Clin Cancer Res*. 2010;29:132.
- Crowley E, Di Nicolantonio F, Loupakakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol*. 2013;10:472-484.

24. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol*. 2014;32:579-586.
25. Marzese DM, Hirose H, Hoon DS. Diagnostic and prognostic value of circulating tumor-related DNA in cancer patients. *Expert Rev Mol Diagn*. 2013;13:827-844.
26. Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. *Nat Med*. 2008;14:985-990.
27. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6:224ra24.
28. Board RE, Wardley AM, Dixon JM, et al. Detection of PIK3CA mutations in circulating free DNA in patients with breast cancer. *Breast Cancer Res Treat*. 2010;120:461-467.
29. Su YH, Wang M, Brenner DE, Norton PA, Block TM. Detection of mutated K-ras DNA in urine, plasma, and serum of patients with colorectal carcinoma or adenomatous polyps. *Ann N Y Acad Sci*. 2008;1137:197-206.
30. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. *Cell*. 2016;164:57-68.
31. Zonta E, Nizard P, Taly V. Assessment of DNA integrity, applications for cancer research. *Adv Clin Chem*. 2015;70:197-246.
32. Vogelstein B, Kinzler KW. Digital PCR. *Proc Natl Acad Sci USA*. 1999;96:9236-9241.
33. Reinert T, Scholer LV, Thomsen R, et al. Analysis of circulating tumour DNA to monitor disease burden following colorectal cancer surgery. *Gut*. 2016;65:625-634.
34. Li M, Diehl F, Dressman D, Vogelstein B, Kinzler KW. BEAMing up for detection and quantification of rare sequence variants. *Nat Methods*. 2006;3:95-97.
35. Liu Q, Sommer SS. Pyrophosphorolysis-activated polymerization (PAP): application to allele-specific amplification. *Biotechniques*. 2000;29:1072-1076, 8, 80 passim.
36. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med*. 2012;4:136ra68.
37. Guibert N, Pradines A, Casanova A, et al. Detection and monitoring of the BRAF mutation in circulating tumor cells and circulating tumor DNA in BRAF-mutated lung adenocarcinoma. *J Thorac Oncol*. 2016;11:e109-e112.
38. Gerlinger M, Rowan AJ, Horswell S, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012;366:883-892.
39. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. *Science*. 2013;339:1546-1558.
40. Garcia-Murillas I, Schiavon G, Weigelt B, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med*. 2015;7:302ra133.

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