Original Article

A comparison of *EGFR* mutation status in tissue and plasma cell-free DNA detected by ADx-ARMS in advanced lung adenocarcinoma patients

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Background: Previous studies have shown that there are different methods used to detect the epidermal growth factor receptor (*EGFR*) mutation status in plasma cell-free DNA (cfDNA) for advanced lung adenocarcinoma patients including the ADx-Amplification Refractory Mutation System (ADx-ARMS). We explored the performance of the ADx-ARMS in detecting the *EGFR* mutations in cfDNA.

Methods: This prospective cohort study enrolled patients who presented with advanced (stage IIIb/IV) lung adenocarcinoma. *EGFR* mutations in plasma cfDNA and tumor tissues by ADx-ARMS were detected. Next-generation sequencing (NGS) in plasma was performed in patients with inconsistent gene region mutations in the plasma and matched tissue samples. We calculated the clinical parameters of the ADx-ARMS for *EGFR* mutation status in the plasma of cfDNA, using the tumor tissues as the standard for measurement. The objective response rate (ORR) and progression-free survival (PFS) were also calculated for patients receiving first-generation EGFR-tyrosine kinase inhibitors (TKIs) therapy.

Results: In total, 203 patients were included in the final analysis. Mutations were discovered in 58.6% (119/203) of the tumor tissues and 31.0% (63/203) were detected *EGFR* mutations in both tumor tissues and matched plasma. The sensitivity and the specificity setting for detecting the *EGFR* mutations in the plasma using the ADx-ARMS were configured to 52.9% and 98.8%. An ORR of 64.8% was observed among the 71 patients who were identified as being *EGFR*-positive in their tumor tissues, who had received treatments using Gefitinib or Icotinib. Next, the ORR was observed to be 69.0% among the 42 patients with an *EGFR* mutation in their plasma. The median PFS of the patients with an *EGFR* mutation in tumor tissues and plasma were 10.0 *vs.* 11.0 months (P=0.175). The median PFS of the patients with an *EGFR* wild-type in the plasma was 8.7 months, which was significantly shorter than the *EGFR* mutant-type in plasma (P=0.001).

Conclusions: Using ADx-ARMS as an approach with high specificity but moderate sensitivity to detect the *EGFR* mutations in plasma cfDNA and *EGFR* mutation status in plasma cfDNA using the ADx-ARMS can predict the tumor response for EGFR-TKIs.

Keywords: Epidermal growth factor receptor (EGFR); cfDNA; lung adenocarcinoma

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Introduction

Lung cancer is one of the leading causes of cancer-related deaths worldwide. Recently, the clinical management of patients with advanced non-small cell lung cancer (NSCLC) has shifted from a histology-based to a molecularly-driven approach because of the identification of actionable genetic alterations and the subsequent development of highly effective targeted therapies. Genomic analysis has become routine in clinical practice to identify the molecular predictors of targeted therapies efficacy, such as somatic epidermal growth factor receptor (*EGFR*) mutations (1-3). The EGFR-tyrosine kinase inhibitors (EGFR-TKIs) have been successfully developed, and demonstrated a much higher objective response rate (ORR), a prolonged progression-free survival (PFS), better quality of life (QoL), and to have fewer side effects compared to first-line of chemotherapy for the advanced NSCLC patients with sensitizing *EGFR* mutations in large randomized phase III clinical trials (4-6). Although tumor tissue is still recognized to be the preferred standard sample for *EGFR* mutation detection, it is not readily available from every patient and may suffer from spatial and temporal heterogeneity (7-10). Therefore, extensive research has explored *EGFR* mutation detection in the plasma cell-free plasma DNA (cfDNA), with the primary aims of developing a novel, non-invasive, dynamic diagnostic method, with the potential advantage of providing a longitudinal evaluation of patients response and resistance to treatment (11). However, the concordance rate of the *EGFR* mutations status between plasma and tumor tissue varies (12,13).

Previous studies have shown that there are different methods used to detect the *EGFR* mutation status in cfDNA including direct sequencing, amplification-refractory mutation system (ARMS), next-generation sequencing (NGS), droplet digital PCR (ddPCR) and others (9,14-16).

The ARMS has been widely used in the clinical setting to guide the use of targeted therapy, with the most significant feature of being simple to operate and having a short turn-around time (8,17). However, the accuracy of its diagnostic ability remains inconsistent. For example, one study demonstrated a sensitivity of 60% and a specificity of 97% (18), while another study reported an inconsistent value of only 48.2% and 95.4%, respectively (19).

The purpose of this study is to evaluate the performance of ADx-Amplification Refractory Mutation System (ADx-ARMS) for the detection of *EGFR* mutations in cfDNA plasma as well as the consistency between the *EGFR* mutations of the tumor tissues and plasma. Moreover, we also evaluated the clinical treatment outcomes according to the *EGFR* mutation status in advanced lung adenocarcinoma patients.

Methods

Study population and inclusion criteria

This prospective study was performed in the Department of Pulmonary and Critical Care Medicine, the First Affiliated Hospital of Wenzhou Medical University, a tertiary teaching hospital in Zhejiang, China. Patients with histologically confirmed advanced (stage IIIB/IV) lung adenocarcinoma according to the seventh edition of the TNM classification of the malignant tumors system (20) from 01 April 2016 to 01 January 2017 were enrolled in this study. Criteria included newly diagnosed or having a progressive disease (PD) after EGFR-TKIs or recurrence after surgery without subsequent treatment. Patients without valid tissue or plasma samples or with an interval between the collection of tissue and the paired plasma sample longer than 14 days were excluded.

This study was approved by the institutional review board of the First Affiliated Hospital of Wenzhou Medical University (No. 2016017). All patients enrolled in this study signed an informed consent form.

Specimen collection and DNA extraction

The tumor tissues and paired peripheral blood (10 mL) from each patient were collected before subsequent treatment. Four to eight sections (5 mm thickness) of formalin-fixed, paraffin-embedded (FFPE) diagnostic tumor tissue samples were used for the DNA extraction. Peripheral blood samples were collected into EDTA tubes and centrifuged at 2,000 g

for 10 min and then again for 8,000 g at 20 ℃ for 10 min. Plasma was separated within 1 hour and stored at a temperature of –80 ℃ until the cfDNA extraction time. The tumor tissue from the DNA was isolated from the FFPE slides using a QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Plasma cfDNA was extracted from 2 mL of the plasma from each patient using the QIAamp Circulating Nucleic Acid kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *EGFR* mutations were immediately detected using the eluted DNA.

EGFR-sensitive mutation detection by ADx-ARMS EGFR kit

Complying with the manufacturer's instructions, the *EGFR* mutations of the tumor tissues and plasma cfDNA samples were detected by using an ADx-ARMS kit (Amoy Diagnostics, Xiamen, China) (21) which had been approved by the Chinese Food and Drug Administration in 2010, to detect the 29 most-common *EGFR* mutations described in lung cancer (*Table S1*). The data analysis followed the manufacturer's instructions as well.

The NGS analysis for genomic DNA from plasma was performed in patients with an inconsistent gene region mutation in the paired plasma and tissues by the ADx-ARMS. DNA was processed using an AmoyDx Panel of 10 genes (Amoy Diagnostics, Xiamen, China) that are of clinical interest for lung cancer. Genomic DNA NGS library was captured for target enrichment and sequenced on the Illumina Nextseq 500 system (Illumina, San Diego, CA, USA) using the Nextseq 500 High Output kit v2 (300 cycles). Experimental procedure and data analysis followed the manufacturer's instructions in detail.

Statistical analysis

Data were analyzed by SPSS Statistics version 23.0. *EGFR* mutations in the tissue samples were recognized as the standard when calculating the sensitivity and specificity for comparing plasma, while the consistency was measured using a Chi-squared test. We assessed the predictive value of the plasma cfDNA *EGFR* mutations for treatment outcomes with the ORR and PFS according to the Response Evaluation Criteria in Solid Tumors version 1.1 (22) and using the Kaplan-Meier method and the log-rank test, respectively. The data cutoff date used for survival analysis is 31 January 2018. All P values are two-sided and confidence intervals are at the 95% level, with statistical significance defined as $P \leq 0.05$.

Results

Patient characteristics

In total, 268 patients were enrolled in this study. Thirty-nine patients without a paired tumor tissue and 26 patients with more than 14 days interval between the collection of tissue and matched blood specimens were excluded from the analysis. Finally, 203 patients entered the analysis, which consisted of 100 women and 103 men. Sixty-four (31.5%) patients were smokers, 184 (90.6%) patients were newly diagnosed with advanced NSCLC, and 169 (83.3%) patients were classified as stage IV. From these FFPE tissue samples, 119 (58.6%) were collected via computed tomography-guided percutaneous lung biopsies, and 24 (11.8%) were pleural effusion cell blocks. All specimens were confirmed by histopathology with the diagnosis of lung adenocarcinomas. The clinical characteristics of these patients are shown in *Table 1*.

EGFR mutations in tumor tissue and plasma samples

EGFR mutations were detected in 119 (58.6%, 119/203) of the 203 tumor tissue samples, of which 58 (48.7%, 58/119) samples detected a single *EGFR* exon 19 deletion (19-Del), 46 (38.7%, 46/119) samples single EGFR L858R mutations, 2 (1.7%, 2/119) samples *EGFR* G719X mutations, 2 (1.7%, 2/119) samples *EGFR* 20-ins mutations, 2 (1.7%, 2/119) samples *EGFR* L861Q mutations, and 1 (0.8%, 1/119) sample *EGFR* S768I mutations. Eight (6.7%, 8/119) tumor tissue samples had detected multiple mutations (4 carried both 19 E19Del + T790M mutations; 4 carried both L858R + T790M mutations).

EGFR mutations were detected in 64 (31.5%, 64/203) plasma samples using the ADx-ARMS, including 33 (51.5%, 33/64) *EGFR* 19-Del, 27 (42.2%, 27/64) *EGFR* L858R mutations, 2 (3.1%, 2/64) *EGFR* 20-ins mutations, 1 (1.6%, 1/64) *EGFR* L861Q mutation, and 1 (1.6%, 1/64) *EGFR* S768I mutation. The details of the *EGFR* mutation status in tumor tissues and the plasma samples are shown in *Table S2*.

Fifty-six patients with documented *EGFR* mutation in tumor tissue had no detectable *EGFR* mutation in the paired plasma cfDNA test using ADx-ARMS. One patient with plasma *EGFR* mutation had no detectable mutation in the corresponding tumor tissue sample. Three cases carried the

Table 1 Clinical characteristics of 203 lung adenocarcinoma patients

Characteristic	No. of patients (N=203)	Percentage (%)
Age, years		
<60	57	28.1
≥60	146	71.9
Sex		
Male	103	50.7
Female	100	49.3
Stage		
IIIB	34	16.7
IV	169	83.3
Smoking history		
Never smokers	139	68.5
Current or former	64	31.5
Stage of treatment		
Newly diagnosed	184	90.6
PD after EGFR-TKIs	8	3.9
Recurrence after surgery	11	5.4
Tissue		
Biopsy under bronchoscopy	32	15.8
Pulmonary aspiration	119	58.6
Intraoperatively	2	1.0
Metastatic sites		
Lymph nodes	12	5.9
Pleura	12	5.9
Pleural effusion cell blocks	24	11.8
Pericardial effusion cell blocks	2	1.0

PD, progressive disease; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors.

EGFR mutation in both tumor tissues, and matched plasma samples, but were inconsistent in the specific gene regions. One carried both 19-Del + T790M in tumor tissue but carried single 19-Del in plasma, and 2 carried both L858R + T790M but single L858R in plasma. We validated these 4 cases using NGS in plasma. Two of them carried L858R + T790M and one carried 19-Del + T790M, consistent with the result of tumor tissue detected by ADx-ARMS. The other one carried 19-Del, consistent with the result of ADx-ARMS in plasma. The details of the *EGFR* mutation status in tumor tissues and the plasma samples using NGS are listed in *Table 2*.

Table 2 Validation using NGS in patients with inconsistent gene regions mutation in the paired plasma and tissue samples by ADx-ARMS

No.	ARMS in plasma	ARMS in tumor tissue	NGS in plasma	
			Mutant-type	Abundance (%)
1	19-Del	19-Del/T790M	19-Del†	1.26
			T790M	2.20
2	L858R	L858R/T790M	L858R	0.48
			T790M	0.27
3	L858R	L858R/T790M	L858R	15.37
			T790M	1.61
4	19-Del	Wild-type	19-Del‡	9.45

†, L747_T751del; ‡, E746_A750del. ADx-ARMS, ADx-Amplification Refractory Mutation System; NGS, next-generation sequencing; 19-Del, exon 19 deletion.

The sensitivity, specificity and consistency of detecting EGFR mutation by ARMS

As compared to the paired tumor tissues, the sensitivity and specificity for *EGFR* mutation detection in plasma by ADx-ARMS was at 52.9% [with a 95% confidence interval (CI), 49.0–53.7%) and 98.8% (95% CI, 93.3–99.9%), respectively]. The PPV was 98.4% (95% CI, 91.2–99.9%) and the NPV was 59.7% (95% CI, 56.4–60.4%). The *EGFR* mutations in the tissue and the paired plasma are summarized in *Table 3*. Of the 203 matched samples, 146 presented consistent *EGFR* mutation status in the plasma and the paired tumor tissues using the ARMS. The overall consistency of the *EGFR* mutation was at 71.9%. Moreover, the kappa test showed a coefficient value of 0.472 revealing the degree of agreement for detecting *EGFR* mutations in tissues and plasma.

EGFR mutations in tumor tissue or plasma for predicting efficacy of EGFR-TKIs treatment

Seventy-one patients of the total 119 patients with the *EGFR* mutation in their tumor tissues had received a Gefitinib treatment [Iressa(R), Astra Zeneca Inc., UK] or an Icotinib treatment (Zhejiang Bata Pharma Ltd., China)

Table 3 Comparison of detection of EGFR mutation in the paired plasma and tissue samples (N=203)

Parameter	Plasma		
	Mut	Wt	Total
Tissue			
Mut	63	56	119
Wt	1	83	84
Total	64	139	203
Sensitivity†	52.9% (49.0–53.7%)		
Specificity†	98.8% (93.3–99.9%)		
PPV	98.4% (91.2–99.9%)		
NPV	59.7% (56.4–60.4%)		
Overall agreement†	71.9% (67.3–72.9%)		

†, percentage [95% confidence interval (CI)]; Mut, mutation; Wt, wild type; PPV, positive prediction value; NPV, negative prediction value.

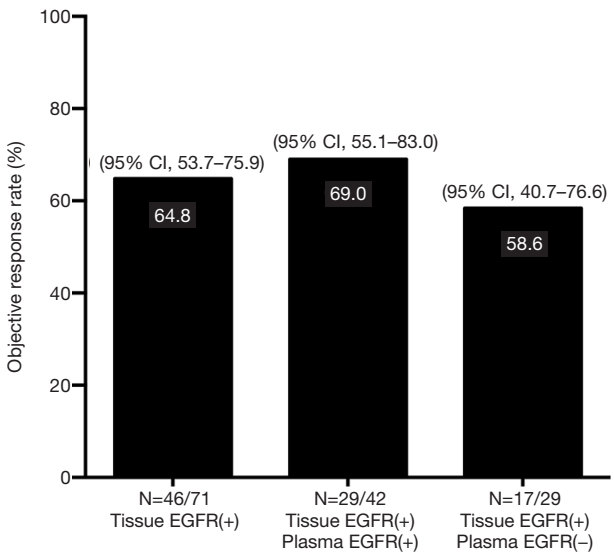

Figure 1 Objective response rate according to EGFR mutation status in patients who were tumor tissue EGFR mutation positive, plasma EGFR mutation positive and plasma EGFR mutation negative. EGFR, epidermal growth factor receptor.

as their first-line of treatment. Forty-six (64.8%) patients achieved a partial response (PR), 23 (32.4%) had a sustained disease response (SD), and 2 (2.8%) had PD. At data cut-off, 62 (87.3%) patients experienced PD and none of the patients had died. The ORR was 64.8% (95% CI, 53.7–75.9%). An objective response was seen in 42 patients who were *EGFR* mutation-positive in their plasma as detected by the ADx-ARMS, with an ORR of 69.0% (95% CI, 55.1–83.0%) (29 with PR, 13 with SD). The ORR was 58.6% (95% CI, 40.7–76.6%) for 29 patients with *EGFR* wild-type in their plasma. There were no significant differences between the three subgroups (P=0.664) (*Figure 1*). The median PFS of the patients with *EGFR* mutations detected by the ADx-ARMS in their tumor tissue and plasma samples was 10.0 months (95% CI, 9.3–10.7 months) *vs.* 11.0 months (95% CI, 9.7–12.3 months) (P=0.175), while the median PFS was 8.7 months (95% CI, 7.8–9.5 months) for the patients with an *EGFR* wild-type in their plasma, which was significantly shorter when compared to patients with an *EGFR* mutant type (P=0.001). The Kaplan-Meier curves for the PFS according to the tissue and plasma mutation status are shown in *Figure 2*.

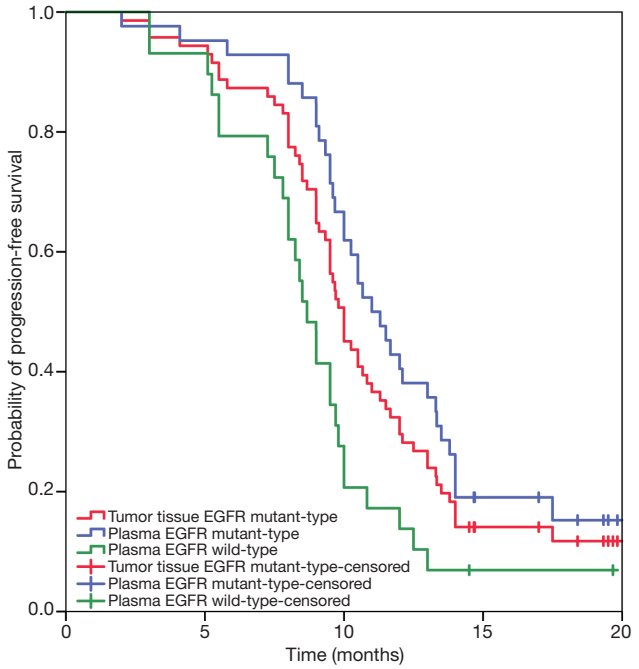

Figure 2 Kaplan-Meier curves for PFS by EGFR mutation status measured in tumor tissue and plasma by ADx-ARMS. †, all patients were tissue-positive for EGFR mutations. EGFR, epidermal growth factor receptor; PFS, progression-free survival; ADx-ARMS, ADx-Amplification Refractory Mutation System.

Discussion

Plasma cfDNA has been proposed as an alternative surrogate

for the detection of the *EGFR* mutation because it is feasible and sensitive (23). In the past decades, many methods have been developed to detect *EGFR* mutations using cfDNA level in plasma samples (16,24-27). A meta-analysis indicated that the pooled sensitivity for cfDNA was 67.4% and the specificity was 93.5% (28). Compared with a long turn-around time, a complicated procedure, and a limitation of the equipment availability of NGS (29), the ARMS assay has been found to be a stable and easy-to-operate method for assessing the *EGFR* mutation status for routine clinical use. Previous studies have reported that the ARMS achieved a 48.2–67.4% sensitivity and a 93.5–100% specificity when testing plasma when compared with the matched tumor tissues for the *EGFR* mutation status (8,18,28,30,31). In this study, we chose the ADx-ARMS method with the analytical sensitivity of 1% to evaluate the paired tumor tissue samples. We found a moderate sensitivity of 52.9%, but a high specificity of 98.8% for detecting *EGFR* mutations in plasma by ADx-ARMS, consistent with the previous studies (18,19) which have suggested that *EGFR* mutations detected in the plasma might be highly predictive of identical mutations in a paired tumor.

In the present study, only three patients had exhibited inconsistent *EGFR* mutations between their tumor tissue and the corresponding plasma samples. Validated by the NGS, the *EGFR* mutation status of these three patients was validated according to the detection results of the ADx-ARMS for tumor tissue. The relatively low abundance of the T790M mutation (0.27–2.2%) which has been observed by NGS might contribute to the false negative of the plasma T790M detection by the ADx-ARMS whose detection limit for the gene mutation was 1%. Apart from the sensitivity of the method itself, it has been reported that the low concentration of cfDNA extracted from plasma samples could lead to the low input of cfDNA template into the reaction, thus affecting *EGFR* mutation detection (32). Additionally, prolonged storage of blood samples could influence the detection efficiency of the *EGFR* mutation in the plasma as well (19). Generally, a high quantity of T790M predicted an inferior PFS after EGFR-TKI when it was compared with the low ones (33). In our study, two out of three carried *de novo EGFR* T790M mutations and the other one was an acquired resistance to the EGFR-TKIs therapy. All of them did not receive subsequent treatment after EGFR-TKIs and were alive at data cut-off, so we could not perform an overall survival outcome analysis.

In contrast, there was one patient who carried 19-Del in the plasma as detected by ADx-ARMS but was negative in the paired tumor tissue. This result was validated by NGS which was consistent with the result of ARMS testing in the plasma. The intra-tumor heterogeneity of the genetic abnormalities might be a possible reason for this inconsistency. The heterogeneity shows a potential advantage for combining the detection of the *EGFR* status in the plasma with the detection of *EGFR* status in tissue, addressing the issue of intra-tumor heterogeneity (34). Importantly, although the number of patients that tested positive for *EGFR* mutation in plasma but negative in tissues was limited, these subsets of patients might lose the chance of receiving anti-EGFR targeted therapy. Therefore, a combination of the detection of *EGFR* mutation status in both the tissue and the cfDNA might be clinically impactful (32).

Previous studies have indicated that T790M is the most common mechanism of acquired resistance to first- and second-generation EGFR-TKIs, accounting for approximately 60% of cases (35,36). In our cohort we found 8 patients who were T790M mutation positive in tumor tissues (4 with both 19-Del and T790M positive and 4 with both L858R and T790M positive). Among them, 6 carried *de novo EGFR* T790M mutation which was associated with primary resistance to treatment (37) and the other two were acquired resistance to the EGFR-TKIs therapy. However, none were detected for the T790M mutation by the ADx-ARMS in the plasma. As we know, plasma has become a preferred surrogate for tumor tissues, to explore the resistance mechanism and to implement a dynamic monitoring method when it is difficult to perform a re-biopsy. However, unlike the 19-Del or the L858R mutation, the T790M mutation is characterized by a low abundance and a high frequency, especially in the cfDNA (35). Yang *et al.* showed the sensitivity of detecting T790M in cfDNA by CastPCR was 45% (38). Our data might indicate the ADx-ARMS was not sensitive enough to detect the T790M mutation in the cfDNA as well (39-41). Since the majority of the patients enrolled in our study were newly diagnosed, additional clinical studies with PD after EGFR-TKIs and larger sample sizes are necessary to assess the performance of the ADx-ARMS for detecting a T790M mutation in the patients who had acquired resistance to the EGFR-TKIs.

Previous studies have reported significant correlations between the *EGFR* mutation status in plasma and the clinical response to EGFR-TKIs (33,42,43). According to the FASTACT-2 study, the patients with plasma cfDNA *EGFR* mutations had a significantly longer PFS when compared with patients without *EGFR* mutations in the plasma (13.1 *vs.* 6.2 months) (30). Another matched plasma

and tumor samples study with DHPLC showed similar results of a prolonged PFS (11.1 *vs.* 5.9 months, P=0.044) and higher ORR rate of 62.2% with the *EGFR* mutations responding to Gefitinib treatment (43). Similarly, in the IFUM study, the ORRs of the baseline plasma *EGFR* mutation positive patients were better than in the negatives (76.9% *vs.* 59.5%) (8). In our study, the ORR with EGFR-TKIs in the group of patients with an *EGFR* mutation in plasma reported by the ADx-ARMS was 69.0%, higher than the ORR rate of 64.8% and 58.6% for the patients who were *EGFR* mutation-positive in tumor tissue and the patients who were *EGFR* mutation-negative in plasma, respectively. Patients with plasma cfDNA *EGFR* mutations also had longer median PFS than those with tumor tissue *EGFR* mutations and with no *EGFR* mutations in plasma (11.0 *vs.* 10.0 *vs.* 8.7 months). Patients with *EGFR* mutation in cfDNA tended to have a higher ORR and a longer median PFS, suggesting that the *EGFR* mutation in the plasma detected by the ADx-ARMS can predict the patients' benefit from the EGFR-TKIs treatment, which is consistent with previously reported data (44,45). This is an interim analysis, therefore mature data for PFS and OS in the patients treated with EGFR-TKIs are not available yet. It is worth noting that most patients (98.6%, 70/71) included in the analysis of treatment outcomes are with EGFR sensitive mutations.

In conclusion, in this large prospective study we compared the *EGFR* mutation status in plasma cfDNA and the corresponding tumor tissues using ADx-ARMS in patients with advanced lung adenocarcinoma. *EGFR* mutation status assessment in plasma cfDNA by the ADx-ARMS was confirmed to be a non-invasive, readily accessible and high specific clinically available tool to optimize patients' selection for treatment with EGFR-TKIs. Our data support the routine clinical application of this technology, which could be used as the initial complementary *EGFR* mutation detection along with tumor biopsy (32). ADx-ARMS is undoubtedly limited by a moderate sensitivity, however, higher sensitive technology such as ADx-SuperARMS are expected to further improve *EGFR* mutation status detection in patients with advanced NSCLC (21).

In summary, ADx-ARMS is an approach with a high specificity but a moderate sensitivity for detecting *EGFR* mutations in the plasma cfDNA of patients with advanced lung adenocarcinoma especially with newly-diagnosed NSCLC. *EGFR* mutation status in plasma cfDNA using ADx-ARMS can predict tumor response to EGFR-TKIs. The combination of testing *EGFR* mutation status in the tumor tissue and plasma should be performed to improve the outcome of our patients.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the institutional review board of the First Affiliated Hospital of Wenzhou Medical University (No. 2016017). All patients enrolled in this study signed an informed consent form.

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Supplementary

Table S1 Details of 29 somatic mutations types in *EGFR* gene in the ADx-ARMS kit

Name	Mutation	Exon
Ex 18-mutant-1	G719A	18
Ex 18-mutant-2	G719S	18
Ex 18-mutant-3	G719C	18
Ex 19-mutant-1	E746_A750del(1)	19
Ex 19-mutant-2	E746_A750del(2)	19
Ex 19-mutant-3	L747_P753>S	19
Ex 19-mutant-4	E746_T751>I	19
Ex 19-mutant-5	E746_T751del	19
Ex 19-mutant-6	E746_T751>A	19
Ex 19-mutant-7	E746_S752>A	19
Ex 19-mutant-8	E746_S752>V	19
Ex 19-mutant-9	E746_S752>D	19
Ex 19-mutant-10	L747_A750>P	19
Ex 19-mutant-11	L747_T751>Q	19
Ex 19-mutant-12	L747_E749del	19
Ex 19-mutant-13	L747_T751del	19
Ex 19-mutant-14	L747_S752del	19
Ex 19-mutant-15	L747_A750>P	19
Ex 19-mutant-16	L747_P753>Q	19
Ex 19-mutant-17	L747_T751>S	19
Ex 19-mutant-18	L747_T751del	19
Ex 19-mutant-19	L747_T751>P	19
Ex 20-mutant-1	T790M	20
Ex 20-mutant-2	S768I	20
Ex 20-mutant-3	H773_V774insH	20
Ex 20-mutant-4	D770_N771insG	20
Ex 20-mutant-5	V769_D770insASV	20
Ex 21-mutant-1	L858R	21
Ex 21-mutant-2	L861Q	21

Table S2 EGFR mutation status in plasma and matched tumor tissue samples

Plasma	Tumor tissue									
	E19Dels	L858R	L861Q	S768I	20-ins	G719X	E19Dels + T790M	L858R + T790M	Wild	Total
E19Dels	31	0	0	0	0	0	1	0	1	33
L858R	0	25	0	0	0	0	0	2	0	27
L861Q	0	0	1	0	0	0	0	0	0	1
S768I	0	0	0	1	0	0	0	0	0	1
20-ins	0	0	0	0	2	0	0	0	0	2
Wild	27	21	1	0	0	2	3	2	83	139
Total	58	46	2	1	2	2	4	4	84	203