



# Effect of icotinib on advanced lung adenocarcinoma patients with sensitive *EGFR* mutation detected in ctDNA by ddPCR

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**Background:** Whether or not *EGFR* mutation status detected by ddPCR in plasma predicts the effect of icotinib on patients with advanced lung adenocarcinoma was determined.

**Methods:** Plasma and matched tissue specimens from patients with advanced lung adenocarcinoma were collected prior to icotinib treatment. The ARMS method was used to detect *EGFR* mutation status in DNA extracted from tissue specimens, while the *EGFR* mutation status in ctDNA extracted from plasma specimens was determined by ddPCR. The therapeutic effects of icotinib were compared between patients with *EGFR*-activating mutations detected by ddPCR in ctDNA and ARMS in tissue DNA.

**Results:** *EGFR* mutation status was detected in 96 tissue and 100 plasma specimens. The sensitivity and positive predictive value of 19del detected in ctDNA by ddPCR was 70.97% (22/31) and 44.90% (22/49), respectively. The positive predictive value was 84.62% (22/26) and the sensitivity was 53.66% (22/41) for the L858R mutation. For the common sensitive *EGFR* mutations, ddPCR had a positive predictive value of 77.19% (44/57) and a sensitivity of 48.89% (44/90). Patients with sensitive *EGFR* mutations in ctDNA had objective response and disease control rates (DCR) similar to patients who had sensitive *EGFR* mutations in tissues detected by ARMS when treated with icotinib (57.14% vs. 51.51% and 92.86% vs. 90.91%, respectively).

**Conclusions:** Patients with sensitive *EGFR* mutations in plasma specimens detected with ddPCR had a higher ORR and DCR compared with patients with sensitive *EGFR* mutations in tissue detected with the ARMS method.

**Keywords:** NSCLC; EGFR-tyrosine kinase inhibitors (EGFR-TKIs); T790M; ddPCR

Submitted Jun 28, 2019. Accepted for publication Oct 10, 2019.

doi: 10.21037/tcr.2019.10.48

View this article at: <http://dx.doi.org/10.21037/tcr.2019.10.48>

## Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide and morbidity is expected to increase in the coming decades (1). Adenocarcinoma is the most common histologic type of lung cancer (2). Radical surgery is still the most effective method for treating early lung adenocarcinoma, but post-operative relapse occurs with or without tumor metastasis, and most patients with lung adenocarcinoma are diagnosed with advanced-stage disease. The response rate of platinum-based chemotherapy for advanced or recurrent patients is only 20–35%, and the median survival time is only 8–11 months (3,4). In recent years, it has been confirmed that EGFR-tyrosine kinase inhibitors (TKIs) prolong the median progression-free survival time to >8 months in lung adenocarcinoma patients with activating *EGFR* mutations, and the response rate has increased to 56–74% (5-9). Indeed, EGFR-TKIs are the first-line standard treatment for lung adenocarcinoma patients with activating *EGFR* mutations.

The relationship between *EGFR* mutation status and the therapeutic efficacy of EGFR-TKIs has been verified. Assessing the presence of *EGFR* mutations is a critical step in therapeutic decision-making for lung adenocarcinoma patients (10). Tumor tissue is still the recommended source for detecting *EGFR*, but because quality tissue samples are not always available it is not possible to conduct *EGFR* testing for every patient in need (11,12). Thus, alternative, easily acquired specimens from patients and feasible methods of detection are essential. Recently, circulating tumor DNA (ctDNA) has been shown to be a suitable alternative sample because ctDNA can be obtained non-invasively (13). Several technologies have been developed for *EGFR* detection using ctDNA (14-19). Of these technologies, the droplet digital polymerase chain reaction (ddPCR) is a method with the following characteristics: high sensitivity; quantitative analysis; and easy detection of gene mutations.

Using *EGFR* mutation-positive cell DNA, ddPCR has expanded to 0.02% sensitivity (20). *EGFR* detection by ddPCR in plasma ctDNA could achieve a sensitivity of 70–81%, specificity of 85–100%, and concordance of 86–94% compared to tumor *EGFR* status (21-23); however more detailed clinical validation is needed to evaluate plasma specimens using ddPCR for *EGFR* detection, especially with respect to the diagnostic and prognostic significance compared to tissue specimens. In this study we compared the *EGFR* detection results and analyzed the clinical

outcomes following icotinib treatment, as determined by ARMS for tissue specimens and ddPCR for matched plasma specimens. The purpose of the current study was to provide clinical evidence to verify the value of predicting *EGFR* mutation status detected by ddPCR in plasma.

## Methods

### *Patients and treatment*

In this study, patients who diagnosed with stage IV lung adenocarcinoma and sensitive *EGFR* mutations based on tissue specimens or ctDNA were enrolled from January 2016 to December 2017 at Zhejiang Rongjun Hospital were enrolled in the current study. The study was reviewed and approved by the Institutional Ethics Committee of this hospital. Informed consent was obtained from each patient prior to testing. Demographic, epidemiologic, and pathologic information were obtained from patient medical records. None of the patients received targeted therapy, radical surgery, or radiation treatment within 28 days prior to sample collection. The enrolled patients were given 125 mg of icotinib three times per day on the basis of the *EGFR* mutation detection results and patient preference. Chest CT scans were performed 1 month after beginning EGFR-TKI treatment. The response to EGFR-TKIs was evaluated according to the Response Evaluation Criteria in the Solid Tumor guideline (version 1.1).

### *DNA extraction*

Plasma was collected from every patient before therapy. Ten milliliters of whole venous blood were collected into tubes with an anti-coagulant which also contained a ctDNA protective agent (Righton, Shanghai, China), stored at 4–25 °C, and centrifuged (1,800 g for 10 min at 4 °C) as soon as possible. A Qiamp® Circulating Nucleic Acid Kit (Qiagen, Duesseldorf, Germany) was used to extract ctDNA from plasma specimens. Tissue DNA was extracted using an AmoyDx® Tissue DNA Kit (Amoydx, Xiamen, China). DNA quality was verified using a nucleic acid quantitative instrument (Thermos City, MA, USA).

### *Detection of EGFR mutations in tissue DNA and ct DNA*

*EGFR* mutation status in tissue DNA was detected using the ARMS method. The tests were carried out according to the manufacturer's protocol using the AmoyDx *EGFR*

29 Mutation Kit (Amoydx) with the MX3000P real-time PCR system (Stratagene, La Jolla, CA, USA). The results were identified according to the criteria defined by the manufacturer's instructions. *EGFR* mutation status in ctDNA was detected using ddPCR. Scientists affiliated with the Righton Testing Institute, who conducted the ddPCR, were blinded to the tissue results.

### Statistical and database analyses

The objective response rate (ORR) and disease control rate (DCR) after TKI treatment were analyzed by chi-square or Fisher's exact tests. The identified mutation status (M+ or M-) in both samples was the basis of comparison of ctDNA versus tumor *EGFR* mutations before treatment. The data were analyzed using GraphPad Prism (version 7.0; GraphPad, city, CA, USA), and statistical significance was indicated by a P value <0.05.

## Results

### Clinical and pathological characteristics of patients

Sensitive *EGFR* mutations were detected in 100 patients from four hospitals. All 100 patients provided sufficient plasma specimens for *EGFR* detection, but tissue specimens were not available or insufficient for *EGFR* detection in four patients prior to treatment. The patient age range was 26–83 years and the median age was 59 years. The clinical and pathologic profiles of the enrolled patients are listed in Table 1.

### EGFR mutation status

Sensitive *EGFR* mutations were detected in tissue specimens from 92 patients prior to treatment. Of the 92 patients, common sensitive mutations were detected in 87 patients; 49 patients had 19del mutations, 41 patients had L858R mutations, and three patients had both 19del and L858R mutations. Five patients had rare sensitive mutations; three patients had G719X mutations and two patients had L861Q mutations (Table 2). T790M mutations existed in two patients who had L858R mutations. An *EML4-ALK* rearrangement was detected in one patient with a 19del mutation. *EGFR* mutations were detected in plasma ctDNA from 100 patients by ddPCR prior to treatment. Fifty-eight patients had common activating *EGFR* mutations. Of the 58 patients, 33 had 19del mutations, 28 had L858R mutations,

**Table 1** Clinical and pathological profiles of the enrolled patients

Variable	N (n=100)	Percentage (%)
Age		
≥60	42	42
<60	58	58
Gender		
Male	39	39
Female	61	61
Smoking history		
Non-smoker	84	84
Smoker	16	16

**Table 2** *EGFR* mutation status in tissue and plasma specimens

Mutation type	Tissue (n=96)	Plasma (n=100)	Overall in this study
19del	49	33	60
L858R	41	28	47
G719X	3	0	3
L861Q	2	0	2
Total	92	58	100

both 19del and L858R mutations existed in three patients, one patient had both T790M and L858R mutations, and two patients were shown to have no other mutations. No patients had rare sensitive mutations (Table 2).

### Comparison of *EGFR* mutation status in plasma and tumor tissue

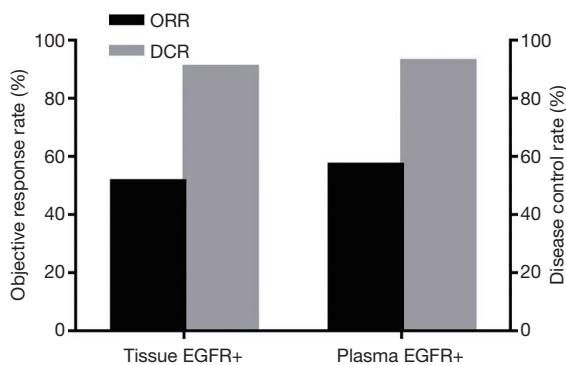
*EGFR* mutation status was determined in the plasma and tissue specimens from 96 patients. For four patients, the *EGFR* status was determined in their plasma specimens, but not in their tissue specimens from four patients. The *EGFR* status was not detected in tissue specimens, but not plasma specimens from any patients.

Thirty-three plasma specimens were positive for 19del mutations. The matched tissue results were lost in two of the 33 cases, nine had false-positive results (nine patients had positive plasma specimen results, but negative tissue specimen results); the positive predictive value for 19del mutations was 70.97% (22/31) and the sensitivity was

**Table 3** Comparison of *EGFR* mutation status in plasma specimens and matched tissue specimens

<i>EGFR</i> mutation status, plasma, n	<i>EGFR</i> mutation status, tissue, n	
	Positive	Negative
19del		
Positive	22	9
Negative	27	NA
Total	49	NA
L858R		
Positive	22	4
Negative	19	NA
Total	41	NA

NA, not available.



**Figure 1** Comparison of ORR and DCR in the plasma mutation group and tissue mutation group. ORR, objective response rate; DCR, disease control rate.

44.90% (22/49). Of 28 plasma specimens with L858R mutations, there were no matched tissue results in two cases and four cases had L858R mutations in plasma specimens, but not in tissue specimens. The positive predictive value for L858R mutations was 84.62% (22/26) and the sensitivity was 53.66% (22/41). For the common sensitive *EGFR* mutations, the positive predictive value was 77.19% (44/57) and the sensitivity was 48.89% (44/90; Table 3).

**Relationship between *EGFR* mutation status in plasma and tissue specimens with *EGFR*-TKI efficacy**

Of the 100 patients in whom plasma or tissue specimens were *EGFR* sensitive mutation-positive, 66 received icotinib treatment. All 66 patients had sensitive *EGFR* mutations

in tissue specimens. Thirty-four patients had partial responses (PR) to icotinib treatment; of these patients, 16 had 19del mutations, 15 patients had L858R mutations, and the other three had both mutations. The total ORR was 51.52% (34/66). The total DCR was 90.91% (60/66). With the exception of patients who had a PR, 26 had disease stabilization (SD); specifically, 14 patients had 19del mutations, nine had L858R mutations, two had G719X mutations, and one patient had a L861Q mutation. Forty-two of 66 patients had sensitive *EGFR* mutations in plasma specimens. The total ORR and DCR was 57.14% (24/42) and 92.86% (39/42), respectively. Twenty-four of the 42 patients had a PR, including 12 patients with 19del mutations, 11 patients with L858R mutations, and one patient with both mutations. Nine patients with 19del mutations and six patients with L858R mutations had SD (Figure 1).

**Discussion**

*EGFR* status is a significant prognostic factor for NSCLC patients who receive *EGFR*-TKI treatment. Plasma has been shown to be effective as an addition to tissue specimens for *EGFR* testing (24,25). In this study, *EGFR* status in plasma ctDNA detected by ddPCR was compared to matched tissues detected by the ARMS method, and the efficacy of icotinib on patients with sensitive *EGFR* mutations detected in plasma ctDNA was explored.

In this study, 100 plasma specimens and 98 matched tissue specimens were collected for *EGFR* detection by ddPCR and ARMS, respectively. The sensitivity and positive predictive value of 19del mutations detected in plasma by ddPCR were 44.90% (22/49) and 70.97% (22/31), respectively. We detected L858R mutations in plasma by ddPCR with a positive predictive value of 84.62% (22/26) and sensitivity of 53.66% (22/41). For the common sensitive *EGFR* mutations, ddPCR had a positive predictive value of 77.19% (44/57) and a sensitivity of 48.89% (44/90). The ddPCR method failed to detect rare sensitive mutations. Negative results existed in the five plasma specimens with rare sensitive *EGFR* mutations detected in matched tissue specimens.

In 13 patients, including nine with 19del mutations and four with L858R mutations, *EGFR* mutations were detected in plasma samples, but not in the paired tumor tissues. This finding may reflect tumor heterogeneity or biopsy bias (26,27). In another 46 patients (27 with 19del mutations and 19 with L858R mutations), *EGFR* mutations were present

in tissue specimens, but not in plasma specimens. Tumors with *EGFR* mutations may not or rarely release mutant tumor DNA fragments into the blood; thus, no mutations cannot be detected in plasma (28).

The goal of *EGFR* mutation testing in the clinic is to select patients who might benefit from EGFR-TKIs. Therefore, we examined the efficacy of icotinib on patients who had sensitive *EGFR* mutations in plasma based on ddPCR. Compared with patients who had sensitive *EGFR* mutations in tissue based on the ARMS method, the patients who had sensitive *EGFR* mutations in plasma specimens had a higher ORR and DCR (57.14% vs. 51.51% and 92.86% vs. 90.91%, respectively), but the difference was not statistically significant.

### Acknowledgments

**Funding:** This work was supported by National Natural Science Foundation of China (31871402), Science and Technology Planning project of Zhejiang Province (LGF19H160002), Medical Scientific Research Foundation of Zhejiang Province of China (2019RC027), Scientific research foundation of Zhejiang Medical Association (2018ZYC-B5), Science and Technology Bureau Project of Jiaxing City of Zhejiang Province (2016AY23087, 2017BY1850, 2018AD32163, 2019AD32266, 2019AD32097), and Xisike-Hanson Cancer Research Foundation (Y-HS2019-20).

### Footnote

**Conflicts of Interest:** All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.10.48>). The authors have no conflicts of interest to declare.

**Ethical Statement:** The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was reviewed and approved by the Institutional Ethics Committee of this hospital. Informed consent was obtained from each patient prior to testing.

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**Cite this article as:** Chen HF, Lei L, Wu LX, Li XF, Zhang QX, Pan WW, Min YH, Zhu YC, Du KQ, Wang M, Wang WX, Xu CW. Effect of icotinib on advanced lung adenocarcinoma patients with sensitive *EGFR* mutation detected in ctDNA by ddPCR. *Transl Cancer Res* 2019;8(8):2858-2863. doi: 10.21037/tcr.2019.10.48