

Case Report

Baseline Plasma EGFR Circulating Tumour DNA Levels in a Pilot Cohort of EGFR-Mutant Limited-Stage Lung Adenocarcinoma Patients Undergoing Radical Lung Radiotherapy

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Keywords

Lung adenocarcinoma · Plasma EGFR · Circulating tumour DNA · Biomarker · Prognosis

Abstract

The use of circulating cell-free tumour DNA (ctDNA) is established in metastatic lung adenocarcinoma to detect and monitor sensitising EGFR mutations. In early-stage disease, there is very little data supporting its role as a potential biomarker. We report on a prospective cohort of 9 limited-stage EGFR mutant lung cancer patients who were treated with radical radiotherapy. We looked at baseline plasma EGFR ctDNA and noted the detection rates to be higher in locally advanced disease. At a median follow-up of 13.5 months, an association between a detectable pre-radiotherapy plasma EGFR ctDNA and early tumour relapse (155 days vs. NR, $p = 0.004$) was noted. One patient with persistent plasma EGFR ctDNA predated radiological progression. The role of ctDNA in early-stage lung cancer is developing. Plasma EGFR ctDNA could be a useful biomarker in lung cancer patients undergoing radical treatments for staging, prognostication, and follow-up. These preliminary findings should be explored in larger studies.

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Introduction

Lung cancer is one of the commonest cancers worldwide and the leading cause of cancer deaths. The presence of targetable epidermal growth factor receptor (EGFR) mutations in the tumour has allowed for the use of tailored treatments like tyrosine kinase inhibitors (TKIs)

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to significantly prolong survival in some patients. In East Asia, the prevalence of these mutations is around 38.4% in non-small cell lung carcinoma (NSCLC) [1].

Circulating tumour DNA (ctDNA) are tumour-specific DNA fragments that are shed into the blood stream. Its presence provides an opportunity to use non-invasive blood biomarkers to detect mutations, including EGFR. Multiplex PCR or next-generation sequencing (NGS) based platforms are available to detect ctDNA. Recently, this technology has been approved by regulators for use in diagnosis, guiding treatment, and monitoring emergence of resistant mutations in metastatic NSCLC [2, 3]. Some groups have also proposed monitoring the plasma EGFR ctDNA levels for dynamic changes to predict treatment failure of TKIs [4–6].

In stage I–III NSCLC, the use of ctDNA in these group of patients is still experimental. In surgical series, it has been suggested that ctDNA could be used to detect minimal residual disease and predict early recurrences [7–10]. There is limited evidence that ctDNA could be used to monitor patients who have undergone radiotherapy [8, 11].

After radical lung radiotherapy, a portion of patients may suffer high-grade treatment-related toxicities and a significant proportion will still relapse despite this. The interpretation of radiological changes after radiotherapy is also complicated by infective or inflammatory changes, scarring and fibrosis resulting in delayed diagnosis. There is thus an unmet need for a non-invasive biomarker that could allow clinicians to prognosticate and predict for treatment failures in these patients.

Our primary aim of this study was to evaluate the use of baseline plasma EGFR ctDNA in a cohort of EGFR mutant patients undergoing radical lung radiotherapy. We hypothesised that patients with a detectable pre-treatment plasma EGFR ctDNA would reach a higher disease stage, which may correlate with a poorer survival outcome than in those without.

Case Presentation

Patients

We conducted a pilot prospective cohort study from April 2017 to August 2018 in the National Cancer Centre Singapore for patients referred to the radiotherapy department for radical lung radiotherapy. The protocol and consent forms were reviewed and approved by SingHealth Institution Review Board. All patients were required to sign informed consent prior to enrolment. The participants had to be fully staged with PET/CT, MRI brain, and EBUS before recruitment. Only radically treatable stage I–III lung adenocarcinoma with a biopsy-proven targetable EGFR mutation in exons 18–21 [11] were recruited. Patients were recruited consecutively. Prior use of chemotherapy was not an exclusion criteria.

Plasma EGFR Mutation ctDNA Procedure

Twenty millilitres of blood (4 EDTA tubes) were collected at CT-simulation scans, prior to radiotherapy, for measurement of baseline test. The blood collected was processed within 2 h and stored for ctDNA extraction. Detection of plasma EGFR mutations, including p.E746_A750del, p.L858R, and p.T790M, was conducted by amplification refractory mutation system (ARMS) PCR. Laboratory staff were blinded to patient and tumour details to avoid read-out bias of PCR results. A second test was planned 1 month after radiotherapy. The results of the tests were blinded to the treating physician and patient.

The protocol was later amended to allow for use of multi gene panel (gene list: *ALK*, *BRAF*, *ERBB2*, *EGFR* [exons 18–21], *KRAS*, *MET*, *NRAS*, *PIK3CA*, *STK11*, and *TP53*) NGS LiquidMARK™ test [12] on the stored frozen plasma samples.

Table 1. Patient and tumour characteristics and plasma EGFR ctDNA results and outcomes

No.	Gender	Age, years	TNM (AJCC 8th ed.)	Histological EGFR	Prior chemo	ARMS PCR	Plasma NGS	GTV, mL	PET SUVmax	Treatment	Post-RT plasma	Outcome
1	Female	56.8	cT2aN2M0 Stage IIIA	Exon 21	No	Exon 21 L858R (1.7%)*	Not done	44.9	17.6	Concurrent CRT	Exon 21 L858R (0.18%)	Relapse, dead
2	Male	77.8	cT1aN0M0 Stage IA	Exon 19	No	Undetectable	TP53 c.780del (1.7%)*	9	8.9	SBRT	Not done	Alive
3	Male	71.1	cT0N2M0 Stage IIIA	Exon 19 c.2235_49del	No	Undetectable	Undetectable	9.2	9.6	Concurrent CRT	Not done	Alive
4	Female	73.9	cT3N0M0 Stage IIB	Exon 19	Yes	Undetectable	Undetectable	124.9	10.8	Concurrent CRT	Not done	Alive
5	Male	69.8	cT3N3M0 Stage IIIC	Exon 21 L858R	No	Exon 21 L858R (0.34%)*	Exon 21 L858R (0.36%)*	Not calculated	16.3	Nil	Not done	Lost to follow-up
6	Female	76.4	cT1bN0M0 Stage IA2	Exon 19 delE746-S752insl	No	Undetectable	Undetectable	11.6	6.5	SBRT	Not done	Alive
7	Male	79.3	cT1bN2M0 Stage IIIA	Exon 19 c.2270A>G; c.2235_49del	Yes	Undetectable	Exon 19 c.2270A>G (48.6%)*	12.4	6.2	Sequential CRT	Not done	Relapse, alive
8	Female	79.0	cT3N3M0 Stage IIIC	Exon 21 L858R	Yes	Undetectable	Undetectable	49.1	16.6	Concurrent CRT	Not done	Alive
9	Male	76.8	cT2aN0M0 Stage IB	Exon 19 c.2235_49del	No	Undetectable	Undetectable	17.0	6.3	SBRT	Not done	Alive

CRT, chemo-radiotherapy; SBRT, stereotactic body radiotherapy; GTV, gross tumour volume. * % of cell-free DNA.

Table 2. Test for factors on detection of plasma EGFR ctDNA

Variable	Plasma EGFR ctDNA		p value
	Detected	Undetected	
Gender			1.00*
Male (n = 5)	2	3	
Female (n = 4)	1	3	
Stage			0.167*
III (n = 5)	3	2	
I/II (n = 4)	0	4	
Pre-ARMS PCR chemotherapy			0.774*
Yes (n = 3)	1	2	
No (n = 6)	2	4	
Mean GTV, mL	28.7	36.8	0.759 [^]
Mean SUVmax	13.4	9.8	0.433 [^]

* Fisher's exact test. [^] Welch t test.

Baseline and Follow-Up Evaluation

The patient's characteristics, tumour details and, treatment plans were recorded at baseline, and treatment outcomes (local or distant relapses) were recorded at the 3–4 monthly follow-up. Survival outcomes were recorded from time of blood taking to event or last follow-up visit. Patients lost to follow-up would be censored at last visit. Patient who did not complete the radical treatment would not be assessed for outcome studies.

Statistics

Baseline characteristics of patients were compared with plasma EGFR ctDNA detection rate, these were also compared against relapse/death outcomes. We used χ^2 test for categorical data and the *t* test for continuous data. Time to event was taken from the start of radiotherapy. Kaplan-Meier method was used to detect the differences between survival outcomes. The impact of plasma EGFR ctDNA test results on survival outcomes was evaluated by log-rank test. *p* values are two-tailed and those below 0.05 were considered statistically significant. Statistical analysis was conducted using IBM SPSS statistics ver. 25.

Results

Patient Characteristics

During the trial enrolment period, 9 patients consented to the study. Patient and tumour characteristics are summarised in Table 1. One patient subsequently declined radiotherapy and was lost to follow-up. This patient was excluded from outcome analysis. Eight underwent radical lung radiotherapy (3 SBRT, 5 chemo-radiotherapy).

Plasma EGFR ctDNA Test Results

Two of 9 (22.2%) participants had detectable results at baseline using ARMS PCR. In combining this with NGS, 4 of 9 (44.4%) participants had detectable ctDNA (3 EGFR and 1 TP53 mutations) at baseline. The 3 patients who had a detectable baseline plasma EGFR ctDNA had stage IIIA/C disease at diagnosis. None of the 4 stage I/II patients had a detectable plasma EGFR ctDNA result. The sensitivity of tests in detecting plasma EGFR ctDNA in stage III was 60% versus 0% in stage I/II patients, although this result was not statistically significant. No patient and tumour variable studied had a significant correlation with baseline plasma EGFR ctDNA detection (Table 2). Only 1 patient did a 1-month post-treatment test.

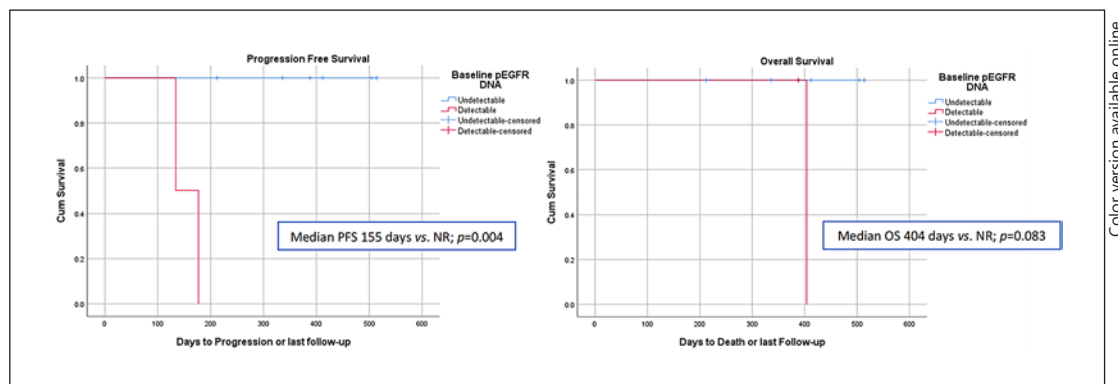


Fig. 1. Progression-free survival and overall survival curves stratified by plasma EGFR ctDNA detection.

Table 3. Characteristics of participants by outcome (relapse/death)

Characteristics	Outcome		p value
	Yes	No	
Age, years (SD)	68.0 (15.9)	75.8 (2.9)	0.2 [^]
Gender, female	1	3	0.6*
Baseline plasma EGFR ctDNA			0.01*
Detected	2	0	
Undetected	0	6	
Stage			0.1*
III	2	2	
I/II	0	4	

* Fisher's exact test. [^] Welch t test.

Survival Data

Of the 8 patients who completed radical radiotherapy, the overall median duration follow-up was 396 days (212–514 days). At last follow-up, 6 patients remained disease free and 2 patients had distant metastatic relapse, of which 1 passed away subsequently. The latter had detectable plasma EGFR ctDNA at 1 month post-treatment and had radiological metastatic disease progression 48 days after.

On analysis, patients with a detectable baseline plasma EGFR ctDNA detection were associated with relapse/death outcome ($p = 0.01$) (Table 3). These patients also had a shorter median PFS (155 days vs. NR, $p = 0.004$), although OS was not significantly different (404 days vs. NR; $p = 0.083$) from the patients with a negative test result (shown in Fig. 1).

Discussion

Our study was conducted as a prospective pilot study to test for plasma EGFR ctDNA in patients with early and locally advanced staged lung adenocarcinoma with biopsy-proven EGFR mutations. These patients underwent initial plasma EGFR ctDNA testing with ARMS PCR followed by radical radiotherapy. The trial was terminated as the investigators felt it was futile to continue due to poor recruitment and low plasma EGFR ctDNA detection rates. The protocol was updated after recruitment was stopped to allow for the retrospective use of NGS

for better sensitivity [13] and detection of other ctDNA. This improved the overall detection rate of ctDNA from 22.2 to 44.4%.

The ctDNA studies performed in early-stage NSCLC have reported poor detection rates [7, 9, 10, 14, 15]. Similarly, our detection rate in this early-stage series was poor. We noted that detection rate of plasma EGFR ctDNA was higher in stage III than in stage I/II (60 vs. 0%) disease. Although this difference was not statistically significant, ctDNA detection rates have also been noted to be higher in patients with greater tumour burden [7, 16–18]. We also studied the difference in mean GTV and SUVmax values between detectable and undetectable patients as surrogates for tumour load and activity, respectively. The results were, however, not significant. We thus would not recommend its use in early-stage disease outside of clinical trials.

In our study, only 1 patient agreed to post-radiotherapy blood test. This patient had completed 33 fractions of concurrent chemo-radiotherapy and had a subsequent decrease in plasma EGFR ctDNA allele frequency at 1 month post-treatment from 1.7 to 0.8%. Unfortunately, he relapsed radiologically at a distal site shortly after the blood test. From previous surgical series, a detectable plasma EGFR ctDNA after radical surgery suggested the presence of molecular/minimal residual disease (MRD). In these series, the persistence of plasma ctDNA identified patients at high risk of relapse and preceded radiological progression by a median of 70 days to 5.2 months [7, 8, 10, 19, 20]. This association between post-treatment ctDNA and MRD may apply to radiotherapy patients as well [8, 11] and may be useful to complement follow-up radiological scans for diagnosis of relapse.

Univariate analyses of our results showed a negative correlation between a detectable baseline plasma EGFR ctDNA and PFS. This result is consistent with some reports performed in metastatic NSCLC patients [21–25]. Given that ctDNA values are correlated with tumour burden, we speculate that these patients with radiological local disease but detectable baseline plasma EGFR ctDNA may harbour a higher systemic burden and do poorly despite local radical treatment.

Our study is limited due to the small sample size, heterogenous patient cohort and short follow-up period. The interpretation of the NGS technique added later in the protocol could have also resulted in increased false negative results due to ctDNA degradation. However, despite these shortcomings, there are very few studies reporting the use of plasma EGFR ctDNA in non-metastatic NSCLC. Given the poor outcomes of lung cancer patients and difficulties of CT-scan interpretation of post-radical lung radiotherapy, the use of plasma ctDNA could serve as an important biomarker in treatable NSCLC for staging, prognostication, and follow-up.

In conclusion, the clinical applications of ctDNA including plasma EGFR ctDNA in NSCLC patients is evolving. With improved technology and access, the use of non-invasive tests like plasma EGFR ctDNA earlier in the course of disease may be useful to guide future clinical management. The findings of our study suggest that plasma EGFR ctDNA was associated with later-stage disease and may have a negative prognostic value in non-metastatic patients. One patient who had a persistent post-treatment plasma EGFR ctDNA had radiological progression shortly after, possibly indicating some utility for monitoring patient outcomes. These results should be explored in future trials.

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Statement of Ethics

This study protocol was approved by the Ethics Committee of SingHealth Centralised Institutional Review Board. All patients provided written informed consent before recruitment and publication of case. The study was conducted in conformance with the guidelines for Good Clinical Practice and the Declaration of Helsinki as well as the Human Biomedical Research Act.

Conflict of Interest Statement

Min-Han Tan is the founding CEO and Medical Director of Lucence Diagnostics. All remaining authors have declared no conflicts of interest.

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Author Contributions

Study concept and design: B.S.H.C., W.L.N., M.-H.T. Laboratory contribution: M.-H.T. Statistical analysis: B.S.H.C., S.C. Data interpretation: B.S.H.C., W.L.N., M.-H.T. Drafting and revision of manuscript: B.S.H.C., W.L.N., S.C., K.W.F., M.-H.T. Study supervision: M.-H.T., K.W.F. All authors approved the final revision.

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